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Kazalo / Table of content

1 SUMMARY 4

2 INTRODUCTION 5

3 CYANOBACTERIAL BLOOMS 7

4 TOXICITY OF CYANOBACTERIA 12

5 ALGAL BLOOM OCCURRENCE 15

6 ECONOMIC CONSEQUENCES OF ALGAL BLOOMS 16

6.1 HEALTH IMPACTS 16
6.2 COMMERCIAL FISHERY IMPACTS 17
6.2.1 Case study for Serbia fish ponds 17
6.3 TOURISM AND RECREATION IMPACTS 18
6.4 MONITORING AND MANAGEMENT IMPACTS 18

7 PHYTOPLANKTON AND BACTERIOPLANKTON MONITORING OPTIONS 19

7.1 IMPORTANCE OF PHYTOPLANKTON AND BACTERIOPLANKTON IN WATER BODIES 19
7.2 PHYTOPLANKTON AS AN INDICATOR OF WATER QUALITY 19
7.3 TRADITIONAL APPROACHES TO PHYTOPLANKTON MONITORING AND ITS LIMITATIONS 20
7.4 FLUOROMETRY AS A RAPID AND STRAIGHTFORWARD TOOL FOR DETECTION AND QUANTIFICATION OF CYANOBACTERIA 20

8 IN-LAKE CYANOBACTERIA CONTROL OPTIONS 23

8.1 CATCHMENT MANAGEMENT AS PRIMARY MEASURE FOR CYANOBACTERIA GROWTH CONTROL 23
8.2 COMPARING LIFE STOP CYANOBLOOM SOLUTION WITH EXISTING IN-LAKE METHODS OF CYANOBACTERIAL GROWTH CONTROL 23
8.3 NEW TRENDS IN CONTROLLING CYANOBACTERIAL POPULATIONS: PHAGE INDUCED CELL LYSIS AND STIMULATION WITH REACTIVE OXYGEN SPECIES 25

9 IN-LAKE CYANOTOXIN CONTROL OPTIONS 38

10 LIFE STOP CYANOBLOOM CYANOBACTERIA DETECTION AND IN-LAKE GROWTH CONTROL 39

10.1 LIFE STOP CYANOBLOOM ROBOTIC VESSELS 39
10.2 IN-VIVO CYANOBACTERIA SENSING (DETECTION AND QUANTIFICATION) BY LIFE STOP CYANOBLOOM VESSEL 40
10.2.1 Advantages of the continuous flow monitoring chamber of the LIFE Stop CyanoBloom robotic vessel 41
10.3 PRINCIPLES OF ELECTROCHEMICAL OXIDATION IN ELECTROLYTIC CELL 42
10.4 DESCRIPTION OF THE MAIN LIFE STOP CYANOBLOOM ROBOTIC VESSEL FUNCTIONAL COMPONENTS 43
10.4.1 Combined smart water measurements, treatment and storage 43
10.4.2 Water quality continuous flow measuring chamber 44
10.4.3 Electrolytic cell for prevention of cyanobacteria proliferation and degradation of cyanotoxins 45
10.4.4 Automatic water sampler with a winch 45
10.4.5 Water flow regulation with smart valves 46
10.4.6 Information system for transfer and presentation of measured data 47
10.4.7 Automatic docking 48

11 COST EVALUATION OF LIFE STOP CYANOBLOOM MONITORING AND IN-LAKE CYANOBACTERIAL CONTROL APPROACH 49

12 REPLICABILITY AND TRANSFERABILITY POTENTIAL OF LIFE STOP CYANOBLOOM ROBOTIC VESSEL AND ITS COMPONENTS 52

12.1 LIFE STOP CYANOBLOOM COMPETITIVE ADVANTAGES 52
12.2 REPLICATION POTENTIAL IN AQUACULTURES 53
12.3 REPLICATION POTENTIAL IN NATURAL AND ARTIFICIAL LAKES FOR RECREATION 53
12.4 REPLICATION POTENTIAL IN DRINKING WATER AND IRRIGATION RESERVOIRS 54
12.5 IDENTIFIED END-USERS OF LIFE STOP CYANOBLOOM ROBOTIC VESSEL AND ITS COMPONENTS 54
12.6 LIFE STOP CYANOBLOOM TRANSFERABILITY POTENTIAL 56

13 OPEN ISSUES AND PROPOSALS FOR FURTHER WORK 57

14 LITERATURE 59

List of tables

TABLE 1: TOXINS PRODUCED BY CYANOBACTERIA: THEIR EFFECTS AND PRIMARY TARGETS (NEWCOMBE, 2009; SEDMAK, 2012; SANSEVERINO ET AL., 2016): 13
TABLE 2: REVIEW OF EXISTING IN-LAKE CYANOBACTERIAL GROWTH CONTROL OPTIONS (ADOPTED BY DRABKOVA, MARSALEK, 2007). 27
TABLE 3: ROBOTIC VESSEL WITH MONITORING AND CYANOBACTERIAL CONTROL EQUIPMENT INVESTMENT AND OPERATION COSTS 50

List of Figures

FIGURE 1: BLOOMING OF *MICROCYSTIS AERUGINOSA* ON A FISH POND IN THE NORTH-EASTERN PART OF SLOVENIA. 11
FIGURE 2: LIFE STOP CYANOBLOOM ROBOTIC VESSEL MAIN COMPONENTS 43
FIGURE 3: COMBINED SMART WATER MEASUREMENTS, TREATMENT AND STORAGE 44
FIGURE 4: WATER QUALITY CONTINUOUS FLOW MEASURING CHAMBER WITH ATTACHED ELECTRONICS 44
FIGURE 5: ELECTROLYTIC CELL FOR PREVENTION OF CYANOBACTERIA PROLIFERATION AND DEGRADATION OF CYANOTOXINS 45
FIGURE 6: AUTOMATIC WATER SAMPLER WITH A WINCH 46
FIGURE 7: WATER FLOW REGULATION WITH SMART VALVES 47
FIGURE 8: INFORMATION SYSTEM FOR TRANSFER AND PRESENTATION OF MEASURED DATA 48
FIGURE 9: AUTOMATIC DOCKING 48

Abbreviations

CB	Cyanobacteria
HCB	Harmful Cyanobacterial Blooms
HABs	Harmful algal blooms
Phy	Phycocyanin
Chl *a*	Chlorophlly *a*
AOP	Advanced Oxidation Processes

1 Summary

The Annexe 17 of the Final Report of the project LIFE Stop CyanoBloom, presenting the "Report on economic justification of the project" is composed of 14 chapters. The first part of the report gives a general overview of the cyanobacteria and their blooms, and species and toxicity of cyanobacterial toxins. With a literature review, economic consequences of cyanobacterial blooms on human health, commercial fishery, tourism and recreation, and lake monitoring and management have been evaluated. The chapter on phytoplankton and bacterioplankton monitoring options presents the importance of the phytoplankton for the lake ecology and evaluation of the water quality status. Traditional phytoplankton monitoring approaches are compared to the new monitoring options applied in the project. The chapter in-lake cyanobacterial control options give a broad review of available in-lake rehabilitation and remediation options, which can be compared with the electrochemical oxidation technique presented in the following chapter and applied in the project. According to the results obtained during the pilot testing activities, a cost evaluation of the approach has been performed and presented in the report. The last chapter gathers open issues and proposals for the further work in this field.

2 Introduction

Occurrence of cyanobacteria is an unwanted consequence of water eutrophication
Prevention of accelerated anthropogenic eutrophication of water bodies, by preventing of point and dispersed discharges, is the first measure to achieve good water status. Despite the efforts invested into preventive measures, the eutrophication still occurs. One of the unwanted consequences of eutrophic water state is the occurrence of cyanobacterial blooms.

Cyanobacteria population expansion can result in harmful cyanobacterial blooms
Cyanobacteria (CB) are present in all freshwater environments. They are organisms that share characteristics with both bacteria and algae. They can actively regulate their position in the water column through buoyancy control, using intracellular gas vesicles, to make maximum use of sunlight and nutrients at optimal times and thus pose an ecological advantage over algae. CB settle in the sediment bottom of the lake as spores to endure unfavourable conditions and as such serve as a source of new CB bloom and dominance when conditions become appropriate (Ståhl-Delbanco et al., 2003). During summer months, CB rapidly expands their population in warm, slow-running waters and can completely predominate in lakes and reservoirs. Episodes of excessive CB population growth are referred to as CB blooms, and when they result in harmful effects, they are referred as harmful cyanobacterial blooms (HCB). The main groups of organisms generating blooms are diatoms, dinoflagellates and cyanobacteria. While diatoms and dinoflagellates are principally associated with blooms in seawater, cyanobacteria are most common in freshwater. In the literature a common expression Harmful algal blooms (HABs) is used, representing natural phenomena caused by a mass proliferation of phytoplankton in waterbodies (Sanseverino et al., 2016).

Cyanobacteria produce cyanotoxins
Many CB (i.e., *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Planktothrix*) produce multiple types of potent cyanotoxins (besides irritant compounds and compounds of unknown biological activities). The toxins cause a threat during the mass occurrence and are released into the aquatic environment during the collapse of the bloom. Cyanotoxins are hepatotoxic, neurotoxic, dermatotoxic and/or genotoxic for higher organisms. The greatest risk for human health is acute and chronic hepatotoxicity of microcystins.

Cyanotoxins pose a danger to human and animal health
Natural causes lead to a gradual collapse of the blooms. The cells are lysed and release their toxins into the water. Bloom collapses are therefore often associated with massive fish mortality and other animals' death (EU-US HAB Initiative, 2002). Humans and domestic and wild animals are exposed to cyanotoxins through ingestion of drinking water or food (e.g. fish, shellfish, nutrient supplements), inhalation and during recreation (Funari and Testai, 2008; Backer et al., 2010). Acute health effects range

from gastrointestinal and flulike illnesses to death, whereas repeated lower level chronic exposures are associated with cancers and neurodegeneration (Svircev et al., 2014). Exposure to cyanotoxins can also occur through haemodialysis (Azevedo et al., 2002).

The intensity and frequency of cyanobacterial blooms are increasing worldwide.
The occurrence of HCB is rapidly increasing worldwide because of increasing nutrient input into water bodies and rising temperatures due to the climate change. Lately, many countries are faced with an array of toxic or harmful species and impacts, as well as increasing bloom incidence, more impacted resources, larger areas affected, and higher economic losses (EU-US HAB Initiative, 2002). There is a consensus among experts that the incidence of HCBs has been increasing worldwide in recent decades (Cheung et al., 2013), reflected in the increasing number of published reports and studies (250 reports during 1988-1992 to nearly 2500 during 2008-2012).Today, the mass occurrence of CB is a major health risk related to surface waters in EU and worldwide.

HABs are responsible for substantial economic losses
Visible algal blooms cause an increase in the turbidity of water and can create taste and odours problems. During blooms, algae can also produce toxins that can render water unsafe and cause fish mortality or can influence human health through the consumption of contaminated seafood, skin contact and swallow water during recreational activities. Sectors such as cyprinid aquaculture, commercial fishery, tourism and recreation can be severely impacted by blooms. Surface drinking water accumulation can become unusable. For this reason, globally, many countries have adopted standard measurements for limiting the presence of Microcystin-LR in drinking water, the most toxic and widespread hepatotoxin produced by cyanobacteria, and adopted new management plans to reduce blooms. All mentioned represents a substantial economic loss of HABs (Sanseverino et al., 2016). It is, therefore, necessary to find a solution for rapid detection and prevention of CB bloom occurrence.

This report gathers an overview of HABs economic impacts and possible in-lake cyanobacteria control measures with evaluation and comparison of costs
In the report, we collected the literature review data about the economic impacts of HABs in sectors such as fishery and aquaculture, tourism and recreation, human health, and monitoring and management activities. In the next part of the report, data on possible phytoplankton monitoring approaches are gathered, and the data on different in-lake cyanobacteria control measures. A separate paragraph is dedicated to the economic evaluation of **LIFE Stop CyanoBloom robotic vessel** operation and its operational units, that can be used individually in cyanobacteria monitoring and in-lake control.

3 Cyanobacterial blooms

Cyanobacteria are ubiquitous and highly adopted organisms

Cyanobacteria are present in all water environments. They are organisms that share characteristics with both bacteria and true algae. Like algae, cyanobacteria contain chlorophyll and other pigments and can perform photosynthesis. They can actively regulate their position in the water column through buoyancy control, using specialised intracellular gas vesicles, to make maximum use of sunlight and nutrients at optimal times and thus have an ecological advantage over other algae. Cyanobacteria settle in the sediment bottom of the lake as akinetes and spores to endure unfavourable conditions and as such serve as a source of new cyanobacterial bloom and dominance when conditions become appropriate (StåhlDelbanco et al., 2003).

Predominance of algae and cyanobacteria are referred as algal and cyanobacterial blooms

During summer months, cyanobacteria rapidly expand their population in warm, slow running waters and can completely predominate in lakes and reservoirs. Episodes of excessive cyanobacterial population growth are referred to as algal blooms, and when they result in harmful effects, they are referred as harmful algal blooms (HAB). Blooms can have a wide variety of appearance, forming colonies, mats, and scums that can range from blue-green to black in colour.

Cyanobacteria are highly adapted to changing environmental nitrogen and phosphorous conditions

Cyanobacteria can exploit anthropogenic modification of aquatic environment as evidenced by their higher affinity for nitrogen or phosphorus compared to other photosynthetic organisms (Sanseverino et al., 2016). Many cyanobacteria have the ability to fix atmospheric nitrogen, and they have a substantial storage capacity for phosphorus. Also, they can sequester iron and a range of trace metals. Therefore, they can out-compete other phytoplankton organisms under nitrogen or phosphorus limitation. Phosphorus has traditionally been considered the major nutrient controlling the manifestation of cyanobacterial blooms in a freshwater ecosystem. Nevertheless, it took times to recognise that phosphorus reduction programs did not always improve water quality and that the nitrogen has the key role of in control of coastal water productivity (Sanseverino et al., 2016). A reason to warrant also the reduction in nitrogen, is the evidence that in freshwater and oligohaline ecosystems characterised by excessive nitrogen inputs, phytoplankton may be dominated by cyanobacteria, in particular, non-dinitrogen-fixing cyanobacteria such as *Microcystis*, *Planktothrix*, *Aphanocapsa*, *Raphidiopsis* and *Woronichinia* (Sanseverino et al., 2016). The ammonium seems to be the favourite nitrogen source for non-atmospheric nitrogen-fixing cyanobacteria and may represent an important factor for their dominance. Cyanobacterial bloom principally affects nutrient-enriched water body, in particular, if they have extended low-flow periods during a hot season and persistent vertical

stratification that enable them to grow and fix atmospheric nitrogen under optimal conditions. Therefore, a dual, as opposed to single nutrient reduction strategies should be adopted to contribute to the resolution of eutrophication problem.

Cyanobacteria produce multiple types of toxins

Most of the cyanobacterial genera (i.e., *Anabaena, Aphanizomenon, Cylindorspermopsis, Lyngbya, Microcystis, Planktothrix*) generate various types cyanotoxins. Some of them are the most potent toxins, which pose a threat during the mass occurrence and release into the aquatic environment with the collapse of the bloom. Cyanotoxins are hepatotoxic, neurotoxic, dermatotoxic, genotoxic for higher organisms and cause general inhibition of protein synthesis. The greatest risk for human health is genotoxicity of microcystins and their effect on internal organs of higher organisms. Natural causes lead to a gradual collapse of the blooms. The cells are lysed and release all of their toxins into the water. Bloom collapses are therefore often associated with massive fish mortality, the death of freshwater and marine mammals, birds, and other animals, and alteration of water habitats or trophic structure through shading, overgrowth, or adverse effects on life history stages of fish and other water organisms (EU-US HAB Initiative, 2002). Humans, domestic and wild animals are exposed to cyanotoxins through ingestion of drinking water or food (e.g. shellfish, nutrient supplements), inhalation and during recreation (Funari and Testai, 2008; Backer et al., 2010). Acute health effects range from gastrointestinal and flulike illnesses to death, whereas repeated lower level chronic exposures are associated with chronic conditions such as cancers and neurodegeneration (Funari and Testai, 2008). Exposure to cyanotoxins can also occur through haemodialysis.

Multiple adverse effects of algal and cyanobacterial blooms

Besides production of toxins, algal blooms pose serious risks to human health, environmental sustainability and aquatic life, also due to the accumulated biomass. The algal biomass accumulation may cause a change in the water colour and may produce harmful effects and cause a reduction in biodiversity due to shading of the benthos and oxygen decline after the bloom dies offs. Low oxygen levels caused by the decay of the algae have in turn an impact on plant, animal and fish life and result in disruption of food webs (Sanseverino et al., 2016). Also, phosphorus released from sediments in anoxic zones can accelerate eutrophication.

Cyanobacterial blooms are increasing worldwide

There is a consensus among experts that the incidence of Cyanobacteria harmful blooms (CHBs) has been increasing worldwide in recent decades (Cheung et al., 2013), reflected in the increasing number of published reports and studies (250 reports during 1988-1992 to nearly 2500 during 2008-2012). Mass occurrence of cyanobacteria is today a major health risk related to surface waters in EU and worldwide. The occurrence of toxic cyanobacteria bloom is rapidly increasing worldwide because of increasing nutrient input into water bodies and rising temperatures due to the climate change. Although animal illness and deaths have been reported worldwide since the late 1800s, it has only been since around the end of the 1990s that the drinking water

industry and the public have begun to understand the negative impact of cyanotoxins on human and environmental health. Lately, many countries are faced with a bewildering array of toxic or harmful species and impacts, as well as disturbing trends of increasing bloom incidence, more impacted resources, larger areas affected, and higher economic losses (EU-US HAB Initiative, 2002).

Beside eutrophication, cyanobacterial growth is stimulated by climate changes

Even if exact causes of HABs are still unclear, climate changes are considered to contribute to the increased frequencies and magnitudes of HABs incidence besides human impacts (Sanseverino et al., 2016). The impact of climate changes on water resources includes warming of air and water, changes of precipitations, increased storm intensity, an increased carbon dioxide dissolution in waterbodies due to increased atmospheric carbon dioxide concentration and rising of salinity level (Sanseverino et al., 2016).

Increasing temperatures can directly favour bloom-forming cyanobacteria because the maximum growth rates are achieved by most cyanobacteria above 25°C. Meanwhile, the growth of other eukaryotic phytoplankton classes like *Chlorophytes, Dinoflagellates and Diatoms* decrease in response to warming. Therefore, in waters with high surface temperatures, cyanobacteria have better probabilities of out-competing other species (Sanseverino et al., 2016). Global warming will also favour the spread of tropical/subtropical species to temperate regions, like *Cylindrospermopsis raciborskii,* which was originally found in Australia and Central Africa (Sanseverino et al., 2016).

An increase in the average global temperature is strongly linked to changes in precipitation. Intense rainfall events increase pollution due to runoff of contaminants and nutrients into water bodies, which accumulate in waters and influence cyanobacteria growth. This is especially evident in water bodies with vertical stratification and long water retention time. Periods of dryness may affect cyanobacterial proliferation too. When a drought period occurs, water evaporation induces a higher concentration of nutrients and increases the area of still water where cyanobacteria can easily grow (Sanseverino et al., 2016).

The atmosphere is also an important source of trace metals such as iron, which is directly involved in promoting primary production and cyanobacteria growth, especially in industrialised regions impacted by iron-enriched rainfall (Sanseverino et al., 2016).

Different cyanobacterial genera can withstand a relatively high concentration of sodium chloride, and it has been observed that both atmospheric nitrogen-fixers (*Anabaena, Anabaenopsis, Nodularia, Lyngbya*) and non-atmospheric nitrogen-fixing cyanobacteria *(Microcystis aeruginosa*) can tolerate saline environments (Sanseverino et al., 2016).

The increase in atmospheric carbon dioxide concentration causes additional carbon dioxide dissolution in waterbodies and leads to lowering of pH and carbonate ion concentration in aquatic systems. This inhibits the biological production of corals and calcifying phytoplankton and zooplankton, while cyanobacteria grow is stimulated by high CO_2 concentration that may promote the intensification of blooms in eutrophic and hypertrophic waters (Sanseverino et al., 2016).

Cyanobacteria as principal group of organisms generating algal blooms

Besides cyanobacteria, diatoms and dinoflagellates are principal groups of organisms causing algal blooms (Sanseverino et al., 2016). Diatoms are unicellular, photosynthetic eukaryotes and include about 100 species spreading worldwide in both marine and freshwater. Dinoflagellates are microalgae forming a significant part of primary planktonic production in waterbodies. They can be heterotrophic, autotrophic or combine heterotrophy with photosynthesis. Their structure is characterised by the presence of two flagella providing them with the propulsive force to move in water and hold a position in the water column according to nutrients' availability. Both diatoms and dinoflagellates are mainly associated with the marine environment. Since **LIFE Stop CyanoBloom** project was dealing with the freshwater environment, the focus was put on cyanobacterial blooms.

The cyanobacteria or "blue-green algae" comprise a diverse group of photosynthetic bacteria with the ability to synthesise chlorophyll-a and other accessory pigments like phycobilin, phycocyanin and phycoerythrin proteins. The majority of cyanobacteria are aerobic photoautotrophs, and for their reproduction, they need only water, carbon dioxide, inorganic substances and light. Cyanobacteria are widely spread in many freshwater and marine ecosystems. Their long evolutionary history started 2,5-3 billion years ago, enabled them to adapt to climatic, geochemical and anthropogenic changes. Cyanobacteria can colonise water that is salty, brackish or fresh and can survive extremely low and high temperatures. Cyanobacteria are also inhabitants of infertile substrates like desert sand and rocks. Freshwater is the prominent habitat for cyanobacteria, and different species can spread along the water column dominating the epilimnion or deeper water layers (Sanseverino et al., 2016).

The photosynthetic apparatus of the cyanobacteria includes two kinds of reaction centres, Photosystem I (PSI) and Photosystem II (PSII), and the accessory pigments mentioned above allow to optimise the light absorption and most efficiently catch the light in differentially illuminated habitats. The vertical movement of many species of cyanobacteria along the water column is enabled by the presence of gas vesicles. These cellular structures are cytoplasmic inclusions impermeable to liquid water, but highly permeable to gases normally present in the air. Gas vesicles are necessary to optimise the cyanobacteria vertical position in the water column and thus to find a favourable habitat for survival and growth. Environmental stimuli such as photic, physical, chemical and thermal factors can also influence the buoyancy of cyanobacteria, enabling them to adjust their position in the water column (Sanseverino et al., 2016).

Cyanobacteria can perform the atmospheric nitrogen fixation, a remarkable metabolic process to convert atmospheric nitrogen directly into ammonium by using the enzyme nitrogenase.

Figure 1: Blooming of *Microcystis aeruginosa* on a fish pond in the north-eastern part of Slovenia.

4 Toxicity of cyanobacteria

Cyanobacterial species have the capacity to produce hazardous toxins that can find their way through levels of the food chain and are subsequently consumed by humans causing diseases or in the most serious cases, the death. Toxins are usually released when an algal bloom dies off. However, toxins can also be released into the water by live algal cells. When HABs happen, the presence of toxic algae is not always directly correlated to the effective production of harmful toxins. Moreover, a particular toxin can be produced by different algal species, and a single algal species can produce multiple types and variants of toxins. Low cell densities are often sufficient to reach dangerous toxicity levels of toxins. Also, water or seafood contaminated with toxins are odourless and tasteless, and toxins can not be destroyed by cooking or freezing (Sanseverino et al., 2016).

Toxic cyanobacteria are encountered all around the world, and problems related to safe drinking water production are common. The presence of cyanobacterial toxins in drinking and bathing waters has been recognised as a human health hazard by the World Health Organization. Chronic exposure to low levels of cyanotoxins in drinking water is a health risk which is still only partially understood (Meriluoto and Codd, 2005).

Cyanobacteria can produce toxins, which are classified in:

i) Hepatotoxins that act on liver
ii) Cytotoxins that produce both hepatotoxic and nephrotoxic effects
iii) Neurotoxins that cause injury on the nervous system and
iv) Dermatoxins that cause irritant responses on contact.

At present, the most severe episode in human associated with cyanobacteria occurred in Brazil, where kidney dialysis patients were exposed to toxins microcystins in the water used for dialysis. At least 50 deaths were caused by water used in dialysis contaminated by cyanobacteria toxins (Azewedo et al., 2002). In studying the health impact of cyanotoxins on human, it is necessary to consider that the exposure to cyanobacteria is possible through different routes like potable water, recreation water, dialysis and consumed fish and food supplements.

Table 1: Toxins produced by cyanobacteria: their effects and primary targets (Newcombe, 2009; Sedmak, 2012; Sanseverino et al., 2016):

Toxin classification	Toxins	Most common cyanobacteria genera producing toxins	The main organ affected	Effects	Main targets
Hepatotoxins	Microcystins	*Microcystis, Anabaena, Anabaenopsis, Aphanizomenon, Planktothrix, Oscillatoria, Phormidium*	Liver	Diarrhoea, vomiting, weakness liver inflammation, liver haemorrhage, pneumonia, dermatitis Possible carcinogen in liver and other tissues	Serine/ threonine protein phosphatases
	Nodularin	*Nodularia, Nostoc*	Liver	Diarrhoea, vomiting, weakness liver inflammation, liver haemorrhage, pneumonia, dermatitis, Possible carcinogen	Serine/ threonine Protein phosphatases
Alkaloids					
Cytotoxins	Cylindrospermopsin	*Cylindrospermopsis, Anabaena, Aphanizomenon, Raphidiopsis, Oscillatoria, Lyngbya, Umezakia*	Liver	Gastroenteritis, liver inflammation, liver haemorrhage, pneumonia, dermatitis	Protein synthesis
Neurotoxins	Anatoxins	*Anabaena, Aphanizomenon, Planktothrix, Cylindrospermopsis, Oscillatoria*	Nervous system	Muscle twitching, burning, numbness, drowsiness, salivation, respiratory paralysis leading to death	Nicotinic receptors or acetylcholinest erase

Toxin classification	Toxins	Most common cyanobacteria genera producing toxins	The main organ affected	Effects	Main targets
	Saxitoxins	*Anabaena, Aphanizomenon, Cylindrospermopsis Lyngbya, Planktothrix, Rhaphidiopsis*	Nervous system	Muscle twitching, burning, numbness, drowsiness, headache, vertigo, respiratory paralysis leading to death	Sodium channels
	BMAA*	*Nostoc, Microcystis, Anabaena, Aphanizomenon, Nodularia*	Nervous system	No specific clinical symptoms, ALS/PDC with consistent long-term exposure	NMDA* excitotoxicity, ROSproduction
Dermatoxins	Lipopolysaccharides	*All cyanobacterial genera (e.g. Synechococcus, Microcystis, Anacystis, OscillatoriaSchizothrix, Anabaena)*	Skin	Skin irritation, eye irritation, headache, allergy, asthma, fever	Toll-like receptors
	Lyngbyatoxins	*Lyngbya*	Skin	Skin and eye irritation, respiratory problems	Protein kinase C
	Aplysiatoxin	*Lyngbya, Schizothrix, Oscillatoria*	Skin	Skin irritation, asthma	Protein kinase C

* (**BMAA** stands for β-Methylamino-L-Alanine; **NMDA** stands for N-Methyl-D-Aspartate)

5 Algal bloom occurrence

Harmful algal blooms (HABs) events have been increasingly reported worldwide. For the marine harmful algal events worldwide, a Harmful Algal Events Dataset (HAEDAT, http://haedat.iode.org/) exists (Sanseverino et al., 2016). A less systematic evidence is available for freshwater bodies. For the frame of the **LIFE Stop CyanoBloom** project reporting, a List of water bodies affected by eutrophication and cyanobacterial blooms has been presented in Annex 20 of the Project Final Report.

Some exposed cases with adverse consequences of harmful cyanobacterial blooms are the following:

- Proven relation between CB in surface water accumulations and liver cancer in Serbia (Svirčev et al., 2010, 2013, 2014). Proven significant and persistent CB blooms in 9 out of 20 drinking water reservoirs in Central Serbia (Svirčev et al., 2014) - up to 650 µg/L microcystin-LR found in the reservoir Celije, 2.5 µg/L in the tap water of Kruševac.
- Proven presence of cyanotoxins in fish meat and pathohistological changes caused by the presence of cyanotoxins in Serbian fishponds (Svircev et al., 2015).
- Documented incidents involving CB in lakes used for drinking water abstraction in Finland (e.g. Lindholm et al., 1989). Surveys on 134 representative lakes in Finland showed that 15% of the investigated lakes suffered from microcystin production (Lindholm et al., 2003; Spoof et al., 2003). Since Finland has close to 190000 lakes, it can be estimated that at least 25000 lakes in Finland alone contain microcystins. Since most of the drinking water in Finland is surface water, there is a great need to minimise the presence of CB toxins in the raw water.
- In 155 freshwater lakes of Baden-Wurttemberg, Germany showed that 55% of the monitored lakes displayed a considerable amount of CB with the highest microcystin value up to 566 µg/L and a risk of development of toxic CB bloom during the bathing season (Wolf and Frank, 2002).
- The death of at least 50 haemodialysis treated patients in Brazil, 1996 due to microcystin and/or cylindrospermopsin (Azewedo et al., 2002).
- Closure of drinking water source for 2 million people from Lake Tai, China, 2007 due to *Microcystis* bloom (Qin et al., 2010).

Sustainable and low-cost approaches are therefore needed for CB control. The WHO guideline (2003) for recreational waters considers potential human health risk at 20,000 cells/ml; moderate risk at 20,000 to 100,000 cells/ml and high risk at over 100,000 cells/ml (visible CB scum formation). The WHO provisional guideline for microcystin-LR in drinking water is one µg/L (WHO, 2003). The concentrations of microcystin in drinking water are still too often above safe values.

6 Economic consequences of algal blooms

The societal impacts of HCBs are many and have led to a substantial financial commitments, in EU and other parts of the world, to reduce threats to local economies, living resources, and public health (The EU-US Scientific Initiative on HAB, 2002).

HCBs have an array of economic impacts, including:

- Increase of costs of enhanced water treatment,
- Conducting costly monitoring programs for aquaculture,
- Loss of revenue in the aquaculture business
- Losses on tourism and tourism-related businesses,
- Need of medical treatment of exposed populations, resulting in direct and indirect revenue losses.

To date, not many studies had specifically addressed the economic effects of HABs, in particular when HABs were caused by cyanobacteria (Sanseverino et al., 2016). The reason could be attributed to the spatially and temporally sporadic nature of these blooms. From the literature review, it has been found that freshwater HCBs are conservatively estimated to cost the U.S. economy between 2.2 and 4.6 billion $ annually (Dodds et al., 2009). Advanced treatment for cyanotoxin removal can be costly for public water systems and may even cause operational and maintenance issues (OEPA, 2011). For larger community water systems, costs for powdered activated carbon alone can exceed $200,000/month (Cheung et al., 2013). Routine monitoring of raw water and optimising the water treatment process if CB blooms are detected may be therefore a less costly solution. Preventing blooms in their early development stages by a sustainable and financially efficient method would also drastically reduce remediation costs.

In the following sections, the economic effects caused by HABs are grouped in four main impacts: (1) human health impacts; (2) fishery impacts; (3) tourism and recreation impacts; (4) monitoring and management costs (following the study of Sanseverino et al., 2016), with a focus on freshwater bodies. Some estimations have been already presented in the Report on socio-economic effects of the project, presenting the Annex 19 of the **LIFE Stop CyanoBloom Final report**.

6.1 Health impacts

When toxins are released into the water during harmful algal blooms (HABs), their toxic effects in humans are believed to occur through different routes: consumption of contaminated seafood, inhalation via aerosols or wind-dispersed particles of dried algal material, ingestion of water or scum and direct contact with skin or conjunctiva. These diseases may occur in humans with varying degrees of severity, and the

treatment of symptoms exhibited by affected people represent a cost for the healthcare sector. Hospitalisation and sickness due to intoxication incidents result in the costs of medical therapies, illness investigation, emergency transportation and are also responsible for the loss of individual productivities (Sanseverino et al., 2016). Severino et al. (2016) study report on four different estimated economic impact of HABs on human health about marine HABs with evaluated annual costs from 670,000 – 900 million US$. The costs represent the sum of medical expenses and personal expenses like lost wages, lost vacation time and transportation of patients to the hospital.

6.2 Commercial fishery impacts

Harmful algal blooms (HABs) episodes are strongly correlated to economic losses in the fish market. During HABs, toxins released by algae may be absorbed by fishes causing closure of fish trade, while algae proliferation may cause an oxygen depletion in water bodies leading to fish mortality (Sanseverino et al., 2016). The consequences are numerous: increase in fish production costs (e.g. production and harvest cost of fish, which die off or are later prevented from reaching the market, additional investment into maintenance and safeguards of fish quality, lower fish market price); decline in consumer demand due to increased costs or increased concern of fish safety. However, economic studies about the impact of HABs on the commercial fishing sector are mostly referred to seawater with respect to freshwater (Sanseverino et al., 2016). A study, in particular, recorded a monetary loss of about £29-118,000 per year in the United Kingdom due to a HABs in freshwater (Sanseverino et al., 2016 in Pretty et al., 2003).

6.2.1 Case study for Serbia fish ponds

During the **LIFE Stop CyanoBloom** project, we visited intensive carp breeding sites in Serbia. According to personal discussions, the production of carps reaches between 2 to 5 tonnes of fish per hectare per year, with more intensive production in small ponds and less intensive in larger freshwater ponds. With the fish market price of 2 EUR per kilogramme of fish and 13,500 and 14,000 hectares of fish ponds in Serbia (Čirić, 2013), this represents a potential income between 54,000,000 to 140,000,000 EUR per year. However, during the production, they confront with several problems. Besides higher costs for water abstraction and water release compared to neighbour countries, there are substantial fish losses due to a significant number of plundering cormorants and carp koi virus. The additional problem represents cyanobacteria, causing oxygen depletion and toxicity due to the release of cyanotoxins. The problems result in larger fish die-off, smaller fish growth and unpleasant fish odour, deterring people from buying them. The production costs increase due to a need of additional water aeration and other interventions. It should be mentioned, that presence of cyanotoxins in a fish meet is not being measured or regulated by legislation. Due to all mentioned problems, the production has declined for 50%.

6.3 Tourism and recreation impacts

The economic damage caused by HABs in freshwater tourism and leisure sector comprises losses due to fishing closures applied to recreational fishers, reduction in amusement and recreational experiences of visitors near the beaches and a drop in restaurants visits and lodging (Sanseverino et al., 2016). The tourism and recreation effects on the economy are influenced by the change in the coastal or freshwater environment triggered during bloom. These changes include changed water colour and turbidity, the accumulation of dead fish on beaches, unpleasant smell and algae biomass floating on the surface or deeper water levels. On a national level, the economic losses are not easy to measure, because the tourists travel to other locations. Local losses around affected water bodies should be taken into account.

6.4 Monitoring and management impacts

Monitoring programs for harmful algal blooms (HABs) refer to strategies adopted by different countries worldwide to control hazards from toxic algae to protect public health, fisheries and minimise ecosystem and economic losses caused by blooms (Sanseverino et al., 2016). The economic effects of HABs on monitoring and management programs include:

- The costs for a regular qualitative and quantitative sampling of phytoplankton cells in water.
- The costs for water sampling to assess the presence of toxins when the count of algal cells exceeds the limit values.
- The costs referred to expenses for water treatment required to remove toxins if water is used for a public water supply.
- The costs to eliminate odour compounds associated with blooms.
- The costs to identify the factors causing blooms.
- The expenses due to actions are taken to destroy HABs during the bloom process.

Looking in particular at the European data about annual monitoring plans against HABs, Denmark and Portugal spent about $500,000, France $800,000 and Spain (Galicia) $1,114,000 (Sanseverino et al., 2016 in Anderson et al., 2001).

About monitoring costs associated with Harmful Cyanobacteria Blooms (CyanoHABs), a study conducted in Australia fixed at $8.7 million the annual costs for monitoring and contingency planning for blooms caused by cyanobacteria (Sanseverino et al., 2016 in Atech, 2000).

In Germany, high microcystin concentrations reported in Lake Boehringer (Germany) was the cause of the closure of all water recreational activities in 2011, and 2012 and the country invested €10,000 per annum in monitoring the lakes for CyanoHABs (Sanseverino et al., 2016 in Hamilton et al., 2013).

7 Phytoplankton and bacterioplankton monitoring options

7.1 Importance of phytoplankton and bacterioplankton in water bodies

Phytoplankton is one of the most important groups of organisms on our planet. With the ability to obtain energy from sunlight, phytoplankton plays a key role in the formation of oxygen and the fixation of CO_2 and thus impact the maintenance of the atmosphere. With the formation of primary biomass, it represents the first link in the food chain and affects the quality of water resources.

Plankton consists of marine and freshwater organisms that float in the water column. Phytoplankton is photoautotrophic organisms, which are a more numerous part of the plankton. It is composed of tiny plant organisms and bacteria. To this group belongs the part of bacterioplankton, which can perform photosynthesis. These are numerous groups of cyanobacteria, which are also photoautotrophic since they use sunlight as the primary energy source.

The high concentration of phytoplankton in the water itself is not problematic because these organisms purify water by binding mineral nutrients and represent a rich source of food. Unfortunately, this is not true for all groups of organisms. Cyanobacteria are due to their particular physiology often more competitive than others, and usually in highly eutrophic water entirely prevail. With its high biomass, they prevent light penetration into deeper water levels and thus prevent the growth and existence of other phytoplankton organisms. In this way, they reduce biodiversity and slow down or interrupt the existing biogeochemical cycles.

7.2 Phytoplankton as an indicator of water quality

Phytoplankton is the only realistic indicator of the status of the water body. Phytoplankton are microscopic organisms, which through their presence respond to the state of the water. With phytoplankton analysis, we determine the quantity and species composition of autotrophs in water. This tells us about the current status, which is dynamic and varies depending on the geology, latitude, season, climate, insulation, temperature, weather conditions and the availability of nutrients. Generally, a great diversity of phytoplankton is an indicator of a good status, while the emergence of one group in a significant number, is an indication of the poor state of the water body.

Prevention of the eutrophication of water bodies (prevention of point and nonpoint pollution sources and plant nutrients input) is the first measure to achieve good water

status. The complete elimination of nutrient input is, however, impossible and impose high costs. It is, therefore, necessary to find a solution for early detection and prevention of bloom occurrence despite the conditions in a water body.

7.3 Traditional approaches to phytoplankton monitoring and its limitations

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 (Caspers, 1984). 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 (Walsby et al., 2004).

For example, it is recommended that a minimum of three sites be used when cyanobacterial counts exceed 2,000 cells mL^{-1}. The appropriate frequency of sampling will be dictated by a number of factors including the category of use, the current alert level status, the cost of monitoring, the season and the growth rate of the cyanobacteria. Apart from cost, the underlying consideration in operations monitoring is the possible health consequences of missing an early diagnosis of a problem. Cyanobacterial growth rates are generally related to seasonal conditions, and previous studies have shown that cyanobacteria in the field can exhibit growth rates from 0.1-0.4 d^{-1} (equivalent to population doubling times of nearly a week to less than two days respectively) (Newcombe (Ed.), 2009).

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.

7.4 Fluorometry as a rapid and straightforward tool for detection and quantification of cyanobacteria

Fast and simple detection and quantification techniques are therefore needed, giving real-time results on a detailed spatial and temporal scale. Monitoring should reflect the continuous dynamic of planktonic organisms in vertical and horizontal directions as

well as on a temporal scale. The method should offer the possibility to collect large quantities of data. One of such simple in-vivo technique is the use of in-vivo fluorometry. It is based on the direct measurement of the fluorescence of photosynthetic and other accessory pigments of autotrophic planktonic organisms (Richardson et al., 2010). While Chl *a* is a primary photosynthetic pigment present in all photosynthetic organisms, the accessory pigments are unique to particular groups of algae and CB and have a variety of roles in the organisms.

Cyanobacteria contain besides Chl *a,* accessory pigments from the phycobiliprotein family, among which phycocyanin (Phy) is predominant in freshwater CB. Chl *a* and Phy have a high fluorescence response at different wavelengths of the visible part of the light spectrum, which enables differentiation of both plankton groups in water and detection of their concentration (Gregor and Maršalek 2004; Gregor et al., 2007). The method allows processing of a large quantity of data on a real-time scale and therefore a faster reaction of water managers in case of detected exponential growth trend of CB. Of all the phytoplankton groups that massively appear in the freshwater systems, only cyanobacteria contain phycocyanin as an auxiliary photosynthesis pigment.

The instruments that measure this fluorescence are called fluorimeters. In the last ten years, many new fluorimeters have been introduced on the market: bbe Moldaenke, YSI, TriOS Optical Sensors, JFE Advantech Co., Ott, Photon Systems Instruments, Wetlabs/Satlantic, Tuner Designs, Chelsea Technology Groups (Pires, 2010). Different fluorimeters have different tools, options and prices. Consequently, it is difficult to find the right fluorimeter for a particular need or to combine optimal combination. There is a large variation in price between the different fluorimeters on the market. The range is from € 1.400, for the UniLux from Chelsea Technology Groups to € 75.000,- for the LOBO, Satlantic (Pires, 2010). This significant difference mainly has to do with the purpose of the instruments. The cheapest fluorimeters are suitable for quick scans and do not monitor other parameters, while the more expensive ones are more suitable for more intense measurements. The prices of the instruments increase with the number of possibilities on the instruments. The difference in weight between the instruments is even larger than the differences in price. The weight and the price depend on of the purpose of the instrument. The fluorimeters meant for long-term monitoring are the heaviest, but in this case, the weight is usually not a relevant category.

To detect the harmful algal and CB bloom potential in due course, measurements should be not only easy, efficient, and economical but also enable real–time field estimates of phytoplankton biomass that can be directly correlated to standard laboratory quantitative measurements (e.g. Chl *a* concentration, cell number or biovolume). For this concern, advanced measuring environment and calibration protocols should be established, where variables that affect in vivo phytoplankton measurements could be controlled. Such variables represent water temperature, water turbidity, light history, phytoplankton health (growth stage), cell aggregation

dynamics and sensor response quenching at high cell concentrations. From the technical point of view, the preferred sensor should have the flowing characteristics:

- Resistance to biological and particulate fouling in long-term deployments,
- Low detection limit,
- Light in weight,
- Gathering data for an extended period for a wide parameter set,
- Available accessories (data reading and analysing, connection to data reader and depth profile, battery pack), and
- Reasonable cost.

For our purpose in the **LIFE Stop CyanoBloom** project, submersible fluorescence sensors were used. Among eukaryotic algae and cyanobacteria, there are some differences in chlorophyll fluorescence. Eukaryotes, which include green algae, can be followed very precisely by fluorescence, which is detected when excited by blue light, while the prokaryotes - cyanobacteria absorb the light of this wavelength more weakly and the concentration of their chlorophyll can be underestimated (Gregor et al., 2007). For the purpose of **LIFE Stop CyanoBloom** project, we, therefore, decided to use the excitation with blue light (chlorophyll sensor) to conclude on the joint presence of phytoplankton (chlorophyll fluorescence measurements), while for the determination of cyanobacteria, we used orange light (fluorescence measurements of phycocyanin with phycocyanin sensor).

Phycocyanin is one of the key indicators by which can be defined particular blooming of cyanobacteria as harmful or dangerous. Measurement of fluorescence of phycocyanin *in situ*, which is typical for the presence of cyanobacteria, is an extremely sensitive indicator of their presence in a water body (Izydorczyk et al., 2005). The signal response can be already detected at low biomass of cyanobacteria, which are in accordance with the warning levels set by WHO (WHO Alert Levels Framework) (Bartram et al., 1999). Correlation between the concentration of cyanobacteria and phycocyanin is much higher than the correlation among cyanobacteria and their chlorophyll (Ahn et al., 2002). In vivo and *in situ* determination of the presence of cyanobacteria using phycocyanin fluorescence is a sufficiently reliable method and is suitable for monitoring potential producers of microcystins in drinking water sources and for early warning in case of mass occurrence (McQuaid e soil., 2011; Bastien e soil., 2011).

8 In-lake cyanobacteria control options

8.1 Catchment management as primary measure for cyanobacteria growth control

The most important step to prevent excessive algal blooms is limiting the external nutrient loading into the lake or prevention of eutrophication from non-point and point sources of pollution. The non-point sources of nutrients are runoffs from agriculture, erosion from urban and deforested areas. The runoff of water and nutrients from these sources can be prevented by revitalisation of regulated rivers and streams, rehabilitation of riparian zones and especially wetlands restoration, sustainable catchment management, etc. (Drabkova, Marsalek, 2007). The point sources of the nutrient load from municipal and industrial wastewater outflows can be reduced by improved sewage treatment plants operations, with tertiary treatment and removal of nitrogen and phosphorous from water.

The possibility to sufficiently decrease nutrient runoff from the watershed is often limited, or this measure may be insufficient due to the internal recycling of nutrients in the lake or reservoir. Therefore, it is necessary to control the internal phosphorus turnover (preventing releasing of P from sediments), decrease nutrient bioavailability and use a combination of other direct in-lake treatment methods. The effectiveness of different in-lake methods depends on a number of circumstances and good knowledge of the specific water quality situation. Significant differences exist particularly between the possibilities for shallow versus deep lakes (Drabkova, Marsalek, 2007).

8.2 Comparing LIFE Stop CyanoBloom solution with existing in-lake methods of cyanobacterial growth control

The **LIFE Stop CyanoBloom** project was dealing with in-lake cyanobacteria control applying electrochemical stimulation of cyanobacteria. A review of different in-lake methods is presented in Table 2 with information about methods` effectiveness, advantages and limitations to provide cost and efficiency comparison.

There are several measures, which can be used to reduce the occurrence of CB and cyanotoxins in surface waters. First, the total capacity of the waterbody that supports biomass production needs to be reduced, as presented in the paragraph above. One of the comprehensive reviews of in-lake methods and measures with watershed methods has been provided by CyanoData, Centre for CB and their Toxins (CCT), as the national representative for UNEP and the project CYANONET (Drabkova and Marsalek, 2007). The review of the methods in presented in Table 2.

Existing conventional water treatments such as precipitation, coagulation, flocculation, chlorination, or addition of $CuSO_4$ have several limitations. For example, added chemicals and herbicides are not CB specific, *Microcystis aeruginosa* cells are Cu resistant, coagulants, flocculants cause additional sediment depositions, and renewed release of nutrients is possible, cellular death is caused by the bloom peak development resulting in the release of toxins, etc. (Villada et al., 2004). The latter is dangerous for humans and has detrimental impacts on aquatic ecosystems. Additionally, reports can be found on illness development following the use of copper sulphate as hepato-enteritis occurred in the community of Palm Island (Australia) (Sanseverion et al., 2013). An addition of any chemical into the water leads to an increase of the total capacity of the water body to support biomass development rather than decrease. The effect of treatments using compounds like permanganate, aluminium sulphate, activated carbon and zeolite on the environment has not been clearly understood, and many management authorities prefer to adopt physical methods for removing CyanoHABs. The costs are also not a negligible fact. A study conducted in Australia estimated the value of copper sulphate treatments within the reservoir and in the water treatment intakes at $1 million per annum (Sanseverion et al., 2013 in Stefensen 2008).

Another approach is nutrient reduction without chemical treatment (removal by flotation, hypolimnetic oxygenation, dredging), which is often ineffective and cost prohibitive over the long term. Alternatively, the calm water requirement for HCBs can be targeted through hydrologic manipulations. Increasing flow rates, for example by artificial waterfalls or fountains, can effectively eliminate HCBs formation even in nutrient-rich freshwaters. However, vertical transport of nutrients through destratification and mixing of the entire water column can stimulate HCBs. The approach of hypolimnetic syphoning is used to remove nutrient-enriched water from water bodies and this method, used in Lake Varese (Italy) in combination with oxygenation, is reported to cost $150,000 per annum (Sanseverion et al., 2013 in Premazzi et al., 2005). Also, mechanical mixers, used in a small reservoir in Perth (Australia) was estimated to cost $30,000 per month (Sanseverion et al., 2013 in Kolman, 2001).

Devices producing ultrasound that disrupt gas vesicles represent an advanced approach without chemical additions or resistance development but may have only a short-term effect and need further research and proof of their efficiency. Methods such are ultrafiltration and nanofiltration were found effective but are not appropriate for the large water bodies or as an in-lake treatment option (Gijbertsen-Abrahamse et al., 2006). Novel methods for removal of CB exploit the CB adaptations. Air flotation, for example, exploits the ability of CB to adjust their buoyancy to their need for sunlight (Teixeira et al., 2010). Novel methods are the use of photocatalytic coatings for degradation of cyanotoxins (Antoniou et al., 2009) and the use of allelopathic substances - natural compounds, which are used by aqueous organisms to fight other competing organisms including CB (Vardi et al., 2002), or the simple method of using

barley straw. Considering their ecological speciality the harmful bloom of CB can be prevented by changing hydrological conditions: destratification of water masses, using different mixers and by aeration (Huisman et al., 2004). This approach does not lead to cellular death and release of toxins into the water, yet it is energy-demanding and costly. The efficiency of the approach is expected to be lower at higher water temperatures (Joehnk et al., 2008).

Promising results have been lately obtained by introducing H_2O_2 to water (Matthijs et al., 2012), which, however, also has its drawbacks due to manipulation with high quantities of reactive chemical, issues to be solved in how to construct dispersion devices for large lakes and metalimnetic/deep water CB populations and need of synchronisation of CB monitoring and H_2O_2 dispersion with regard to population density. Besides, it treats the whole waterbody.

Understanding the mechanisms of occurrence of CB blooms is a necessary precondition to intervene in their development effectively. Efficient, low cost and sustainable methods for CB growth control are needed, which do not cause negative effects to other organisms in the lake environment, development of CB resistance or accumulation of deposit on the lake bottom and resuspension of nutrients. The methods must also not increase the total capacity of the waterbody for biomass development.

8.3 New trends in controlling cyanobacterial populations: phage induced cell lysis and stimulation with reactive oxygen species

In the marine waters, the main natural factor controlling the density of CB populations are viruses – lytic phages (Wommack and Colwell, 2000, Stopar et al., 2004). The first studies of freshwater cyanophages were carried out in the 1960s and 1970s, with viruses (tempered phages) noted as potential “algaecides” (Safferman and Morris, 1963; Safferman et al., 1969; Whitton and Potts, 2000). Phage induced lysis in freshwater filamentous CB (which are major primary producers) is well known since the early 1970s, (Suttle, 2000 and references therein). Several studies provide evidence that viruses establish lysogenic associations and were induced to the lytic mode (Hilker et al., 2006 and references therein). The potential of viruses to control bacteria growth has only just started to be seriously explored in freshwater ecosystems (Middelboe et al., 2008) with recent studies of the phenotype, abundance and diversity of viruses in freshwater (Dorigo et al., 2004; Tucker and Pollard, 2005; Baker et al., 2006 ; Wiedner et al.,2007). There are two main replication cycles of viruses that infect CB plankton (Hilker et al., 2006 and references therein): (i) lytic (or virulent) infections with destruction and without reproduction of the host and (ii) lysogenic (or temperate) infections in which viruses integrate their genome into the host’s genome and multiply along with the host until the lytic cycle is induced. The shift to the lytic mode can be triggered by a variety of environmental stresses such as radiation, pollution, temperature changes and nutrient depletion or may occur

spontaneously (Hilker, 2006 and references therein). It can also be triggered by an artificial factor (Sedmak et al., 2008, 2009) like exposure to oxidants in the water. Such artificial triggers could serve as efficient tools to control CB blooms by simultaneous lysis of virus-infected cells.

Today the role of viruses as natural controlling agents of CB growth is emerging as an important potential factor in bloom collapse by "Killing the Winner" (Pollard and Young 2010). Beltrami and Carroll (1994) showed that a lytic viral infection could destabilise an otherwise stable phytoplankton–zooplankton food chain. Chattopadhyay and Pal (2002) demonstrated that zooplankton feeds ten times more on infected CBplankton and infected CBplankton is more vulnerable to predation by zooplankton (Singh et al., 2004.). New models propose a lysogenic infection before the switch to the lytic cycle (thus actually resembling a chronic infection). Hilker and Malchow (2006) have provided a detailed mathematical and numerical analysis of the local model for lysogenic/chronic and lytic infections.

The main innovation of the proposed technological solution **in LIFE Stop CyanoBloom project** for the prevention of CB bloom formation was based on above presented scientific findings. With the research performed so far, Arhel Ltd has proven in cooperation with National Institute of Biology (Leštan, Sedmak, Laković: Preventing of mass occurrence of harmful CB, patent No *23987 (A), 2013*) the potential to trigger CB lysogeny and further lysis with oxidant molecules, like hydroxyl radicals, produced directly from water in the electrolytic cell**.** The photosynthetic apparatus of CB is very sensitive to hydroxyl radicals, which affect CB easier than most other organisms.

In comparison to unselective methods of CB growth suppression or degradation, our method is focused on detected "emerging clouds (mass occurrences)" of CB in waterbodies and acts localised and selectively on CB. In such clouds of CB, they can be effectively reduced with the proposed approach, while other phytoplankton species remain unaffected. Their reduction is based on CB phages induced lysis increased liability to bacterial infection and degradation and zooplankton grazing. In a waterbody CB reduction results in increased biodiversity, reduced turbidity of water and positive impact on inter-specific relations between different ecological groups (an increase of green algae positively affect zooplankton development, fish, amphibians, crustacean and birds, because it presents a better nutrient source than CB).

The operation of the electrolytic cell also causes (besides production of reactive oxygen species, which are hindering cyanobacteria proliferation) mixing of the water in the local area of operation. This activity provides secondary positive 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.

Table 2: Review of existing in-lake cyanobacterial growth control options (adopted by Drabkova, Marsalek, 2007).

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
1 In-lake sediment treatment and phosphorus inactivation - Limitation of main nutrients				
1.1 Removal of sediment	The release of water and removal of sediment, or direct (suction) dredging of the sediment.	With the removal of phosphorous, the cyanobacteria inoculum is also removed.	Problems with deposition of the sediment. Destruction of the benthic fauna (organisms from the bottom)	High costs of the method (excavation, deposition, tax for deposited material) 150 – 300 €/m^3 [1] $ 17,984/ ha [3] 0.77 km^2, 29m depth (max depth 6.2m): 1.8 Mio €; Average 30 – 50 €/1m^3 of sediment
1.2 Sediment capping	Capping with clean sediment Active barriers: calcite materials ($CaCO_3$), aluminium salts, zeolites, modified clay, caolines, other ballast substances, modified humic substances	Prevents remobilization of nutrients (P, N) and cyanobacteria.	Difficult to achieve uniform coverage of the bottom (30-40 cm thickness). Reduces the volume of the accumulation. Possible side effects (Phoslock – contains rare earth lanthanum – toxic for fish).	High costs of the method. Commercial product Phoslock[i]: 2800$/ton; needed 2.5 ton/ha 5.13 km^2; 4.9m depth (max depth 29m): 300,000€ (coverage

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
				with lime; without accompanying measures).
1.3 Hypolimnetic Withdrawal	Establishment of syphon for withdrawal of hypolimnetic anoxic water rich with nutrients. Pumping with the pump.	Great removal of nutrients possible.	Suitable only for deep stratified lakes. Destratification should be avoided. Suitable of larger lakes >2.5 x 10^6 m^3 Possible negative effect on recipient.	Relatively high costs For the lake of 41 ha with 3.4 m^3/min flow, $304.000; for 287 ha lake with 6.3 m^3/min flow- $45.000.[3] 0.25 km^2, 9m depth, 17m max depth: 10.000-150.000 € investment costs
1.4 Hypolimnetic aeration and oxygenation	Oxidising the bottom layers of the lake sediment, which binds phosphorous very strong and prevents its release back into the water column. Application of hypolimnetic aerators, injection of air into the lake bottom layers	Prevents release of P from the bottom layers. The concentration of Fe and Mg is decreased, the taste of water improves, less unpleasant odours, zooplankton penetrates in deeper layers. Active degradation of organic compounds in bottom layers, which prevents the survival of the cyanobacterial inoculum.	If it is not restricted to the lower hypolimnion layer may even accelerate the growth of cyanobacteria in the upper layer. Appropriate for deeper water bodies >15m.	Electricity connection needed; potentially high operation costs. 2000 – 10.000€ for the aerator. $6500/ha for 6 month operation ($3.40/kgO2) [2] 14.4 km^2, 44m depth (max depth 87m): (RISTELOX system with O_2 input, LIMNO, TIBEAN) - 1.5 Mio € Investment costs; annual operation costs

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
				250.000€
1.5 Phosphorous precipitation and inactivation: several options (A – E)	Input of coagulants and flocculants from late autumn to the beginning of the summer, when the phosphorous concentrations are the highest	Besides phosphorous, cyanobacteria and algae can bind on floccules.	Less effective in shallow lakes	
A/ Aluminium salts	Aluminium sulphate (alum, $Al_2(SO_4)_3.14H_2O$) Formation of aluminium hydroxide precipitates, which binds inorganic phosphate in pH range 6 – 8. Reports on inputs of 5 – 100 g Al m^{-2} or 5 – 25 g Al m^{-3} to achieve efficiency.	Phosphorus binds on sediment, the water transparency increases, the phytoplankton biomass decreases. With the addition of poly aluminium chloride $PAX_{18,}$ in 5 mg Al/L they have achieved efficient decrease of cyanobacteria and retained hygienic minimum for the bathing waters also six weeks after the treatment	At pH <4 produces Al^{3+}, which toxic. At pH >8 phosphorous releases back into water. In waters with low hardness (<30 to 50 mg of $CaCO_3$ L-1) the pH can be greatly reduced after the introduction of aluminium salts and leads to the formation of toxic $^{Al3+}$, so it is necessary to add puffer (NaOH, calcium hydroxide, ..). At lower temperatures, lower efficiency of phosphorous binding.	Subject of allowances
B/ Ferrous salts	Input of $FeCl_3$, $FeCl_2$, $Fe(SO_4)_3$ → formation of ferrous hydroxides → phosphate binds on Fe or floccule of Fe hydroxide.	The stability of floccule is not pH dependant. Fe^{3+} is not as toxic as $Al^{3+.}$	The highest sorption at pH 5 - 7, which is not usual for eutrophic lakes. After introduction, hydrogen ions can release from water, which lowers pH and is toxic for fish. The stability of P and Fe binding	Subject of allowances

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
			depends on redox state: if the concentration of O_2 drops below 1mg/L P start to release; the process is fast – sufficient is already small anoxia at the sediment; Additional aeration is needed during treatment. Fe binds only inorganic forms of phosphorous and not P bound on particles. If Fe is limiting factor in the lake, the treatment can even increase the growth of cyanobacteria. The water may become brown in colour during the treatment.	
c/ Calcium carbonate or calcium hydroxide (lime)	**$CaCO_3$**, $Ca(OH)_2$ Phosphorous precipitates at pH>9 Lime input: 25 – 300 mg Ca/L	No toxic effect. After application, cyanobacteria can precipitate besides phosphorous.	If the pH drops, the P release quickly back into the lake (this is frequent in the active zone of bacterial oxidation). Possible side effects on water organisms, since lime increases the pH. Not appropriate for bigger natural lakes; performed only in fishponds (carps breading waters).	Relatively low cost method Dispersion of lime needed.

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
D/ Clay materials	Zeolites, modificated clay materials (**Phoslock™**), bentonite, kaolin	Cyanobacteria are also precipitated and removed.		
1.6 Chemical methods for sediment oxidation	Method **RIPLOX ($(Ca(NO_3)_2)$, $(FeCl_3)$ and $(CaCO_3)$)** – several substances, which enable binding of P, regulation of pH and redox potential **Depox®**: preparation containing Fe(III) and NO^{3-} Initiating in upper lake layers Dose: 16 to 140 g N m^{-2}	Successful binding of phosphorous late in the spring.	Appropriate for shallow lakes with flat bottom. RIPLOX needs dispersal with harrow. Where inputs of P large, the efficiency lower (suitable for less polluted waters). Long-term data on its efficiency not available.	Relatively high costs Commercial product RipLox[3]: $5200/ha
1.7 Biological treatment and sediment mineralization	Addition of microorganisms, biocatalisers, and enzymes for enhancing the mineralisation, Several commercial preparations available	Organic matter degrades → less organic matter present in the sediment → less oxygen depletion in the bottom → oxyc conditions → phosphorous is not releasing; as there is no organic matter, the conditions do not support cyanobacteria overwintering.	No scientific evidence on the efficiency of commercial products on cyanobacteria.	
2. Technical and physical in-lake measures				
2.1 Artificial destratification (mixing)	Water circulation → increases the aerations with input of air bubbles, pumping of water. Application during summer period	Oxygenated conditions → phosphorous is not releasing Because of mixing, cyanobacteria buoyancy	Water can become supersaturated with dissolved nitrogen → fish kill Negative effect on zooplankton	2.22 km^2, 23.9 m depth (max depth 40.5 m): Investment costs 165,000€, 20,000€ annual

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
		capacity is no more an advantage → algae grow with the same efficiency The system more appropriate for deeper waters	Negative effect on fish adopted to lower temperatures by warming the hypolimnion Possible even more intensive blooming of cyanobacteria due to input of nutrient rich water into the surface layers. Enables control of cyanobacteria in limited conditions only (when presence of cyanobacteria is not excessive and the amount of P is relatively low)	operation costs
2.2. Ultrasound	Influencing air vacuoles of cyanobacteria	Reduces buoyancy of cyanobacteria; the cells are not destroyed and the cyanotoxins are not released into water. Can effect on photosynthetic apparatus.	The vacuoles can quickly re-establish (within 20 hours) → cyanobacteria active again. Possible negative effect on fish when direct application into the water body (better are closed continuous flow systems). Scientific evidences on lakes not available.	The cos of the equipment 1000 – 5000 €[2]
2.3. Mechanical removal of cyanobacterial biomass	Removal of cyanobacterial slime from the surface (the same equipment as at removal of oil from the surface)		Enables removal of smaller part of the cyanobacterial population in the lake.	The same equipment as for the removal of oil spills.

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
	Filtration		Applicable, when cyanobacteria in their full blooming stage and negative effect visible (release of toxins). Applicable in combination with flocculation (bentonite, polyaluminium chloride).	
2.4 Dilution and flushing	Dilution of nutrients Shortening of the retention time of water in the lake.		Rarely used because of high quantity of water needed.	
2.5. Sediment drying	Release of water, drying and removal of upper layer of sediment	Removed are cyanobacteria, which overwinter in the lake sediment.	Suitable for shallow lakes, which are possible to be dried out.	
2.6 Production of hydroxyl radicals with electrochemical oxidation of water	Use of electrolytic (electrochemical) cell with high capacity electrodes (e.g. boron doped diamond electrodes) for production of hydeoxyl radicals directly from water media. Dose depending action: direct lysis of cyanobacteria cells or trigger cyanobacterial lysogeny and further cyanobacterial-phages induced lysis.	Selective effect on cyanobacteria; no effect on fish, macrophytes or green algae Inactivation of free cyanotocins	To reduce costs, retain method selectivity and prevent sudden cyanotoxins release from lysed cyanobacterial cell, the application is provided for before the onset of full cyanobacterial bloom.	Evaluated in this report
3. Biological control				
3.1 Biomanipulation	Intervention into the food chain (catchment of non-predator fishes →		Not applicable in very eutrophic lakes.	

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
	zooplankton prevails → regulates the concentration of phytoplankton) Removal of bentivorous fish, such as carp, which resuspend phosphorous and cyanobacterial cells from the sediment with their movements.		Suitable for smaller lakes Ineffective if predominates the cyanobacteria *Microcystins*; zooplankton is not feeding on it. Just certain fish (pumpkinseed *Lepomis gibbosus*) may weaken cyanobacteria in its digestive tract. The fish that feed on aquatic plants can even increase the growth of cyanobacteria due to release of plant nutrients (N, P) with the release of faeces.	
Herbivorous fishes				
3.2 Macrophytes and periphyton	The plants limits the resuspension of sediment, shade the water body, offer dwelling to water organisms feeding with algae; uptake the nutrients (N, P), release allelopathic substances, which inhibit growth of cyanobacteria		Macrophytes prevail up to the concentration 50-100 µg P L^{-1}; if the P concentration is higher, phytoplankton and cyanobacteria prevail. Full plant growth possible only in shallow lakes Requires continuous monitoring and manipulation.	
Combined biological treatment				
3.3 Other organisms	Known effect of groups of organisms on	Known positive effects from	Experiments from the natural	

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
(viruses, bacteria, algae, fungi, protozoa	prevention of growth (allelopathic effect) and lysis (cyanophages) of cyanobacterial cells	laboratory experiments.	environment missing.	
4 Algaecides	Application of substances with cyanocide and cyanostatic effect. Treatment should start at the beginning of the season, before full development of the bloom.		Degradation of cells from the mature cyanobacterial bloom can release substantial amount of cyanotoxins. Due to the onset of decomposition of large amounts of dead cyanobacterial biomass, anoxia may occur leading to fish deaths. Resistance to chemicals may establish. Effects are usually temporary.	
4.1 Copper	Copper sulphate $CuSO_4.5H_2O$ Inhibition of photosynthesis, binding of P in fixation of N Efficient dose already 5-10 µg Cu L^{-1} Usually needed dose: 1 mg L^{-1} For extensive blooms need of 30-300 mg Cu L^{-1}	More efficient activity on cyanobacteria than on green algae.	Possible occurrence of cyanobacteria resistance possible. Cu toxic for animals, accumulates in the sediment. A general usage in lakes is usually not permitted – usual ban on use.	Low cost of chemicals Banned application for natural lakes. 0.67 km^2, 6.5 m depth (max depth 10.8m): 1.1 Mio € for flocculent manipulation, 0.6 Mio € investment costs for deep aeration; 50.000€ annual operational costs

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
4.2 Other inorganic chemicals	Ag nitrate (Ag NO_3, dose 0.04 mg L^{-1}), potassium permanganate ($KMnO_4$, dose 1-3 mg L^{-1}), sodium hypochlorite NaOCl, dose 0.5 – 1.5 mg L-1		Nonselective toxicity on a number of water organisms. Limited application / ban on application	
4.3 Hydrogen peroxide (H_2O_2)	Dose: 0.3 to 5 mg L^{-1} Dose depends on species, growth and light conditions Application in powder form as $2Na_2CO_3.3H_2O_2$ Solid form as $2Na_2CO_3.3H_2O_2$	Selective effect on cyanobacteria; no effect on fish, macrophytes or green algae Because of local and short-term activity not toxic for other water organisms.	Short-term effect Local effect – localisation of cyanobacterial »clouds« needed Method still in testing	Compound relatively low cost
4.4 Photocatalysis	Input of photosensitive compounds (free chlorophyll, humic acids, porphyrins, phthalocyanines, titanium dioxide (TiO_2)) , which form under light (UV) reactive oxidative compounds (radicals: $^1O_2$, superoxide $O_2^{\bullet -}$ hydroxyl peroxide H_2O_2, perhydryl HOO^-, hydroxyl $HO^\bullet$ radical	Similar principle of activity as peroxide.	Radicals short-lived Micrometer working distances TiO_2 has low solubility; the contact times are usually too short to be effective.	Input of TiO_2 subject of environmental permits
4.5. Organic chemicals - herbicides	Reglone A (diquat,1,1-ethylene-2,2-dipyridium dibromide, dose 2-4 mg L^{-1} simazin (2-chloro-4,6-bis(ethylamine)-s-thriazine, dose 0.5 mg L^{-1})		The use is not safe from a toxicological and environmental point of view - banned	

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	COSTS
	didecyldimethylamonium chloride or n-alkyl-dimethyl-benzyl-ammonium chloride Diuron (DCMU; 3,4-Dichlorphenyl 1–1 dimethyl urea; dose 0.1 mg L^{-1} paraquat (N,N'-Dimethyl-4,4'-bipyridinium dichloride, dose 0.026 mg L^{-1} Roundup (glyphosate)			
4.6. Natural compounds and materials	Release of allelopathic compounds from mactophytes Algistatic reaction of barley straw (distribution of straw bale on the water surface).		It is difficult to place bales of straw in the larger lakes, especially if intended for recreation. The exact mode of effect is not known.	Barley straw bales – cost efficient solution

[1] http://aqua.wisc.edu/publications/pdfs/sedimentremediation.pdf
[2] http://www.rwmwd.org/vertical/sites/%7BAB493DE7-F6CB-4A58-AFE0-56D80D38CD24%7D/uploads/New_Tech_March_2012-Phoslock.pdf
[3] http://www.waterboards.ca.gov/lahontan/water_issues/programs/tmdl/indian_creek/docs/icrtable.pdf
[4] http://www.solitudelakemanagement.com/sonicsolutions-ultrasonic-algae-control-facts-and-specifications

9 In-lake cyanotoxin control options

When potentially toxic cyanobacteria have been identified in a water source, toxin analysis is required to determine if the cyanobacteria are, in fact, a toxic strain. The presence of cyanotoxins in fresh-water bodies can be a reason for fish die-off in aquacultures, poisoning of swimmers and pets during swimming or because of consuming fish and other water organisms. Most undesirable is the presence of cyanobacterial toxins in drinking water and the cyanotoxin removal techniques are mostly dedicated to drinking water treatment.

Even if the treatment is aimed at removing cells intact with their intracellular toxins, there is the possibility that dissolved toxins may be present. Conventional treatments such as coagulation etc, are not effective for the removal of dissolved cyanotoxins. The usual water treatment processes used for the removal of microcontaminants like cyanotoxins are (Newcombe (ed.), 2009):

- physical processes (such as activated carbon and membranes),
- chemical processes (oxidation with chlorine, ozone and potassium permanganate)
- biological processes (such as filtration through sand and granulated activated carbon supported by health biofilm)

All these processes are, however, not applicable for direct in-lake cyanotoxins removal. Different cyanobacteria also have different susceptibility to algaecides. For example, chlorine dose, to be effective in inactivation of microcystins (100% destruction), saxitoxins (70% destruction) and cylindrospermopsin 100% destruction), should be > 3 mg/L (Newcombe (ed.), 2009).

Hydroxyl radicals, as very strong oxidants, have, however, a high potential for cyanotoxins inactivation.

10 LIFE Stop CyanoBloom cyanobacteria detection and in-lake growth control

10.1 LIFE Stop CyanoBloom Robotic vessels

Several models of robotic vessels or unmanned surface vessels can be found in the market, which were designed to perform specific chemical and biological monitoring, measurements of the hydraulic characteristics of different water bodies, or cyanobacterial control: Siralab Robotics, ETH Limnobotics, ARC-Boat HR Wallingford, SeaBED-class AUV Hanumant Singh, Oceanscience Q-Boat 1800D, Riverwatch, Clearpath Robotics, LG Sonic MCP-Buoy, SolarBee, Hans C.P. Matthijs water harrow for H2O2 dispersion. The following prices for the robotic vessels were found: Clearpath Robotics ($50,000), Yonzhou tech (€60,000) – both for monitoring purposes; SolarBee $150,000/ 25 ha lake (monitoring and control).

The **LIFE Stop CyanoBloom** robotic vessel for detection and control of cyanobacteria is powered by solar energy. During the navigation, the vessel can automatically follow the points on the water specified by the user by a computer program. Three-dimensional localisation of cyanobacteria is obtained by measuring the fluorescence of cyanobacterial pigments. For this purpose, the chlorophyll and phycocyanin fluorescence sensors are used. By the application of the external electric potential on the electrolytic cell, short-lived hydroxyl radicals are produced directly from the water, which prevents further cyanobacteria proliferation. With the selected intensity and duration of the electrochemical stimulation, a degradation of cyanotoxins is achieved, cellular decomposition of cyanobacterial cells or only an inhibition of their further proliferation. A specially developed mechanical system allows the collection of water samples from variable depths for accurate laboratory processing. The vessel reports real-time data to the user via GSM communication. The user, therefore, obtains an accurate picture of the current state of the water body, which can also be followed via a web application. In addition to fluorescence sensors, the vessel reports the information on water temperature, electrical conductivity, pH, dissolved oxygen concentration, the position in the water body, the strength of the wind and the photographic image. User-friendly software allows processing of measured data and their graphical display. The vessel is equipped with an advanced system for automatic docking and charging of the spare battery during inactivity.

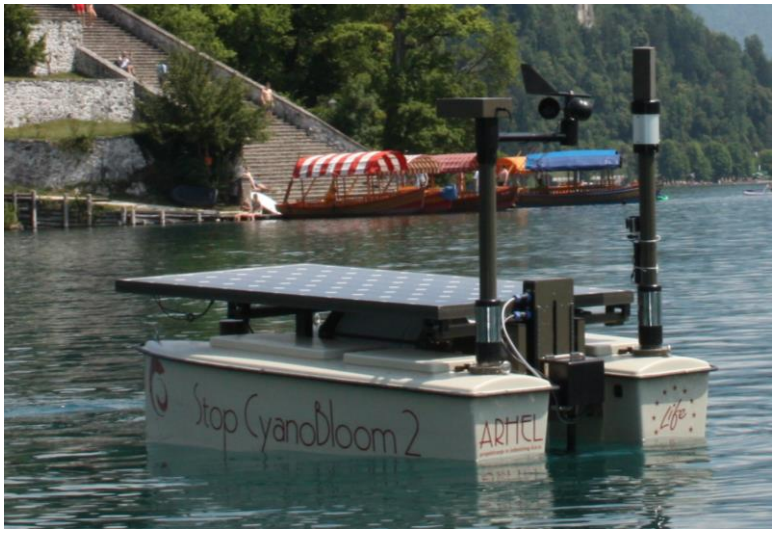

Photo 1: LIFE Stop CyanoBloom robotic vessel in operation on Lake Bled.

10.2 In-vivo cyanobacteria sensing (detection and quantification) by LIFE Stop CyanoBloom vessel

The main innovation in our early detection of CB proliferation is in the simultaneous use of Phy and Chl a sensors in a specially designed continuous flow measuring chamber placed on-board the vessel for selective determination and quantification of the CB plankton and phytoplankton at different depths and in different spatial locations. Pumping of the water into the chamber enables its storage in the on-board samplers for later advanced laboratory analysis when needed. Additional on-line sensors installed in the chamber and on the vessel enable simple and effective determination of other important parameters. Their selection and placement depend on the monitoring requirements or calibration needs. Simultaneous transmission of measured data and their presentation on the website platform is possible. Databases with long-term monitored data of an individual water body and developed calibration algorithms enable interpretation of changes in phytoplankton and CB plankton composition and represent a basis for the early warning system.

In the project, commercially available submersive Turner Design Cyclops Chl a and Phy sensors have been used. They have been selected as the best choice among accuracy, compact size, weight and price. Arhel`s engineering group has solved other needed features for the effective sensing, storage, transition and interpretation of the results. By this innovative solution, the water is continuously pumped into the measuring chamber equipped with two fluorescence sensors. The depth of the measurement is checked by the use of a double hose, placed on a winch, enabling accurate readings of the depth of the analysed water. The sensor heads, an expensive part of the equipment, are protected by placement in the chamber. The pumping of the water can be detained and the abstracted sample measured in different modes (after light adaptation, with and without mixing, etc.). The elimination of bubbles and cleanliness of the sensor surface is solved by water detention and automatic sweeper. In the case

of detected excessive CB concentration, the water runs through the electrolytic cell, which is activated automatically. The measured and treated water is returned to the abstracted location in the water. The continuous-flow measuring chamber with additional electronic and mechanical equipment for water treatment, therefore, offers a new monitoring approach on the market. To solve the problem of precise water flow and connectivity of several elements (chamber, electrolytic cell, and sampler unit), an additional innovation has been added in the form of series of "smart" electronic valves.

10.2.1 Advantages of the continuous flow monitoring chamber of the LIFE Stop CyanoBloom robotic vessel

The determination of the presence of phytoplankton using sensors in an especially designed **LIFE Stop CyanoBloom** monitoring chamber is an effective complementary method to conventional monitoring and laboratory analysis because it enables:

a) Obtaining information on the state of water in the natural environment in real time. The data detected by the sensors are transmitted by the **LIFE Stop CyanoBloom** platform in a real-time via the mobile network to the server and for the security reasons also simultaneously stored in monitoring system own memory. Thus, ensuring their safety and accessibility at the same time.
b) Better accessibility to all parts of the water body and collection of samples at a selected location. The device excludes arbitrariness in determining the sampling points with its mobility.
c) Higher spatial and temporal resolution - a three-dimensional snapshot of the water body. The device enables horizontal and vertical measurements in a relatively short time.
d) Optimizing sampling strategies. Basic information on the presence and quantity of phytoplankton is obtained from the sensors and sent to the control centre in real time. This allows us to give a reasoned decision on the further time and location of sampling.
e) Quantitative and qualitative detection of phytoplankton. The combined use of chlorophyll and phycocyanin sensors allow us to gain information on phytoplankton biomass and cyanobacteria share. With retrospective analysis of the signals, we obtain additional information on the nature of the phytoplankton.
f) Assessment of the physiological state of the phytoplankton. To change the ratio between the fluorescence CHL and PC indicates changes in the physiology of autotrophs.
g) Determining the presence of stress and stressors in the aquatic environment. Depending on the nature of the stress changes the size and the ratio of single signals.
h) The establishment of early warning. Detection of the phycocyanin signal above the warning value gives the opportunity for immediate action.
i) Detecting and tracking the position and movement of cyanobacterial bloom. Due to specific ecophysiological specificities of cyanobacteria, which are reflected in

their tendency to combine into various forms of clusters, colonies and migrations in larger communities as well as the formation of cyanobacterial clouds, we can locate them in the space and follow their integration and migration. It also allows us to assess their abundance and actual threat, which they may constitute in certain places such as recreational areas and sampling sites.

j) Local effect (treatment) only in selected areas with an established presence of dangerous cyanobacteria. In the event of a decision, to start with the in-lake cyanobacteria control a **LIFE Stop CyanoBloom** platform allows to determine specific and suitable sites for intervention. The scope of intervention into a water body in this way localised, which brings energy and cost savings. In addition, the release of a biological material from the affected cyanobacteria into the environment is controlled and limited in time and space.

10.3 Principles of electrochemical oxidation in electrolytic cell

The principle of electrochemical oxidation used in the **LIFE Stop CyanoBloom project** and the results obtained during the project demonstration period have been presented in detail in the Annex 15 of LIFE Stop CyanoBloom Final Report.

Electrochemical oxidation also called anodic oxidation belongs to the group of Advanced Oxidation Processes (AOP). Implementation of advanced oxidation processes can take place in various combinations (Rozina et al, 2016), as chemical AOPs (application of combination of O3/H202 and Fenton reagent (Fe2+/H202), ultrasound) or photochemical AOPs (application of O3/UV, H202/UV, photo-Fenton reaction (Fe2+/ H202/UV) and UV/TiO2 catalysis). The processes are mainly used for the treatment of wastewater and drinking water, alone or in combination with physico-chemical and biological methods.

Electrolytic cells, which were used in the project consists of two BDD electrodes, positioned in an aluminium housing (Zupančič Justin et al., 2016). Supporting part of the electrodes made from niobium is covered with a thin diamond layer doped with boron, which makes the electrodes conductive, therefore the name boron-doped diamond electrode, or BDD electrodes. The BDD electrodes are advanced, inert electrodes (high stability in strong acid media) with wide potential window and high over potential for oxygen evolution, producing the highest amount of •OH (Liao et al., 2014). Degradation and inhibition of toxicity of microcystins, cyanobacterial toxins, with the use of electrochemical oxidation in laboratory electrolytic cell equipped with BDD electrode has also been proven (Zhang et al., 2009; Liao et al., 2014; Meglič et al., 2016). Hydroxyl radicals have the capacity to destroy the toxicity of cyanotoxins, such as microcystins, by inducing oxidative cleavage (Antoniou et al., 2008). The •OH reactivity is namely second only to fluorine (2.8 V and 3.0 V oxidation potential, respectively). Hydroxyl radicals as well as other produced reactive oxygen species in electrolytic cell (e.g. O_3, H_2O_2, and $\bullet O_2^-$) promote cell lysis through inhibition of photosynthetic activity in cyanobacteria by impairing electron transfer and oxygen

evolution, which can lead to the inactivation of photosystem II and eventual cellular death (Barrington et al., 2013; Samuilov et al., 2004). Damages after the exposure to electrochemical oxidation influence cell proliferation, buoyancy mechanism, thinning of extracellular mucilaginous sheaths, cell shrinkage with the loss of nucleoid homogeneity and loss of auto fluorescence (Meglič et al., 2016).

10.4 Description of the main LIFE Stop CyanoBloom robotic vessel functional components

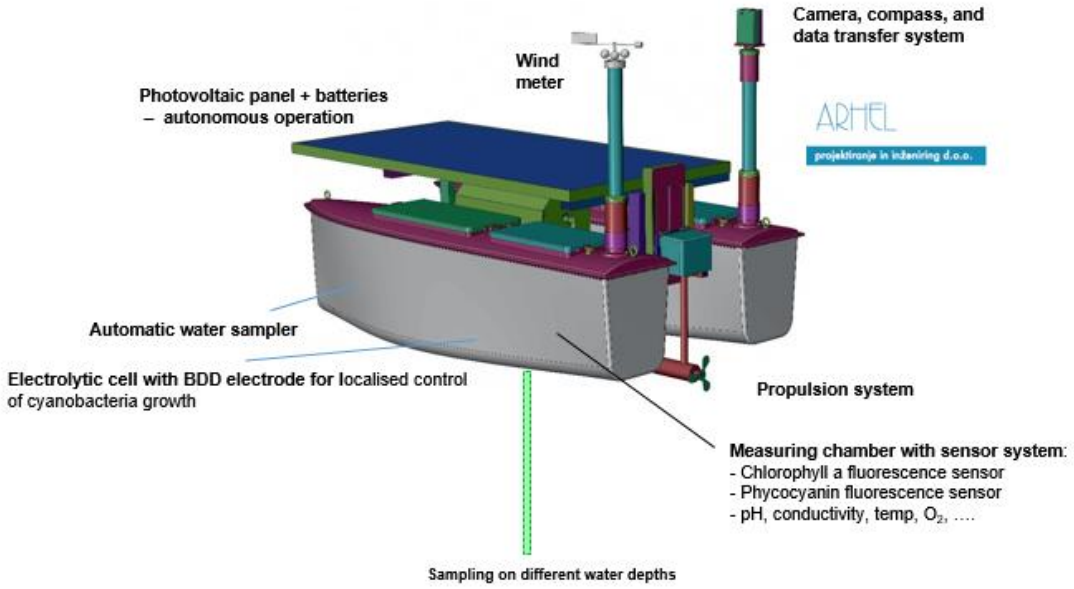

Figure 2: LIFE Stop CyanoBloom robotic vessel main components

10.4.1 Combined smart water measurements, treatment and storage

A specially developed hardware within the project allows the robotic vessel the abstraction of water from different depths for measurements of water parameters, the decision on turning on the electrolytic cell, or to abstract water samples for further laboratory analysis. All the systems on board are interconnected and communicate via an RS485 network.

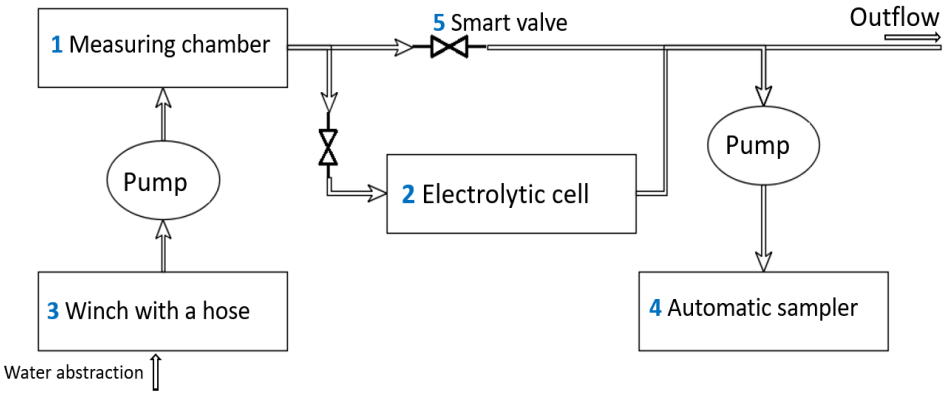

Figure 3: Combined smart water measurements, treatment and storage

10.4.2 Water quality continuous flow measuring chamber

Newly designed water measuring chamber allows online measuring of water quality parameters on board. It enables simultaneous reading from five sensors (e.g. phycocyanin, chlorophyll, temperature, oxygen, conductivity, pH). Submersible fluorescence sensors with the automatic cleansing system are used for cyanobacteria and algae quantification. A magnetic stirrer is incorporated into the bottom of the chamber to prevent settling of the phytoplankton. Signals are transferred to a printed circuit inside of an electronics enclosure, where they are converted to a digital form and transferred to a PC for further data analysis.

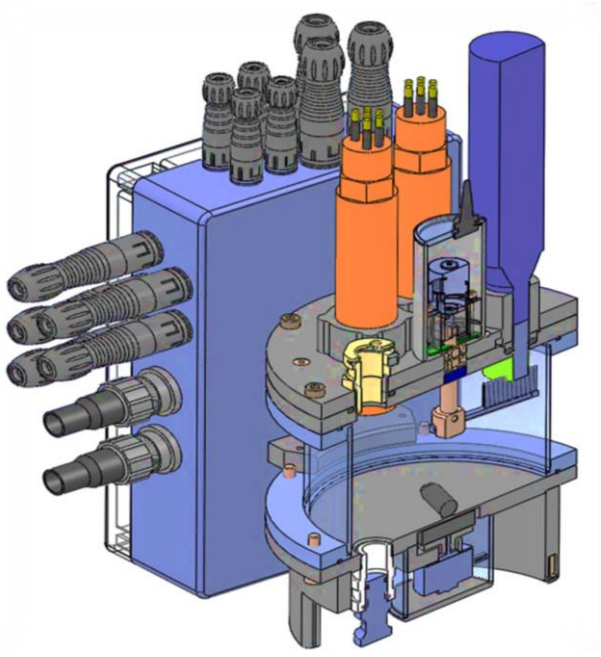

Figure 4: Water quality continuous flow measuring chamber with attached electronics

10.4.3 Electrolytic cell for prevention of cyanobacteria proliferation and degradation of cyanotoxins

A single-compartment electrolytic cell equipped with boron-doped diamond (BDD) electrodes is used for electrochemical stimulation of detected cyanobacterial directly in water. Water is pumped through the cell equipped inside with small flow deflectors to achieve laminar flow. Within the cell, short-lived hydroxyl radicals (•OH) are produced directly from water with the use of electric power. The BDD electrodes are advanced inert electrodes with the highest potential of •OH production achieving inhibition and degradation of cyanotoxins, suppression of further cyanobacterial proliferation or their lysis, depending on the electrolysis length and current density applied.

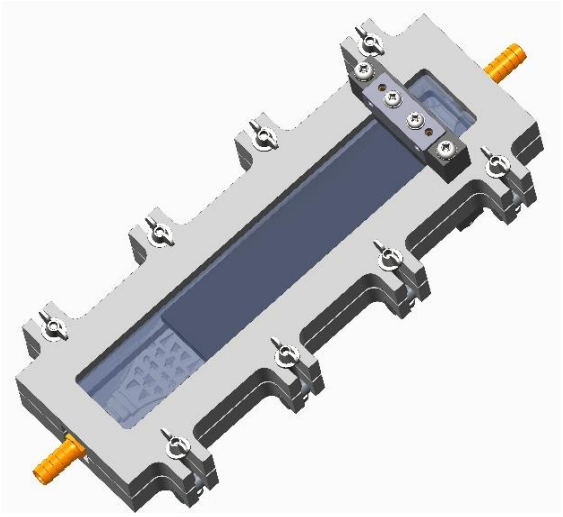

Figure 5: Electrolytic cell for prevention of cyanobacteria proliferation and degradation of cyanotoxins

10.4.4 Automatic water sampler with a winch

The sampler enables abstraction of six 1-litre water samples using a winch with a hose. The command for the sample abstraction is transmitted via RS485 network (in the vessel), via PC through USB connection, or by pressing the button on the control console (when used as an autonomous device). Innovative smart valves allow abstraction of samples without contamination with the water already taken. Sampler`s electronics allows automatic collection of samples at the programmed time and depth, detection of possible failures (leaking containers, pump failure, clogged pipe) and avoidance of spills with automatic shut-off.

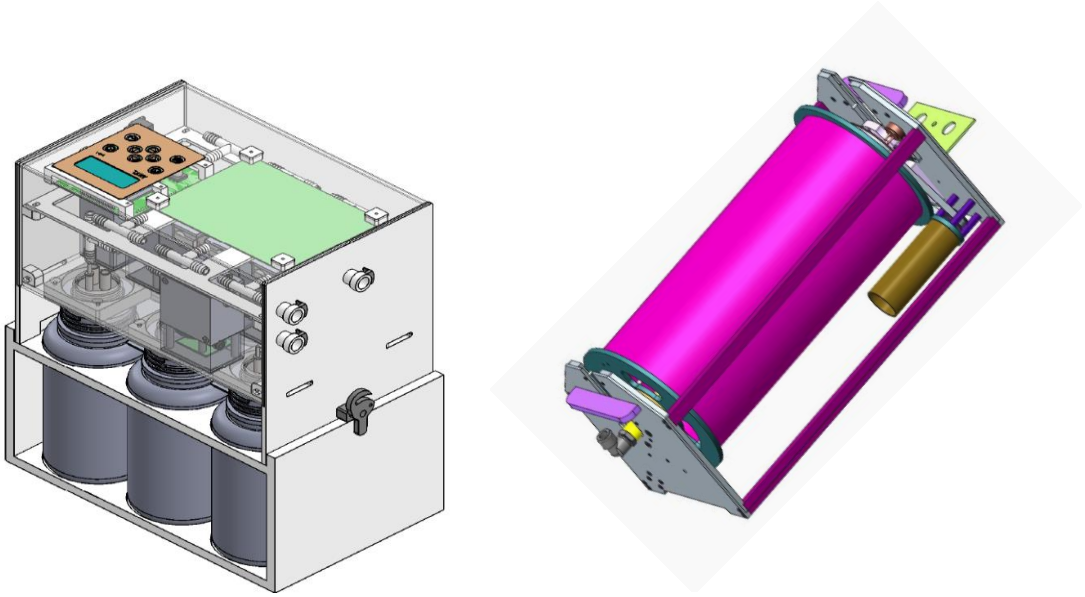

Figure 6: Automatic water sampler with a winch

10.4.5 Water flow regulation with smart valves

The water flow system in the vessel and the automatic sampler are equipped with smart valves. Valve`s drive motor and integrated electronics allow for automatic control and integration of the valves into a larger system with the synchronised operation. Flow regulation is carried out with a stepwise compression of the hose, which is inserted into the valve. The design of the valve allows for easy replacement of the hose (e.g. to prevent contamination of the sample), the use of hoses of different diameters, and mechanical properties. The valve controls the flow directly by measuring the motor power consumption. This function provides reliable operation of the valve with no need for additional sensors or switches.

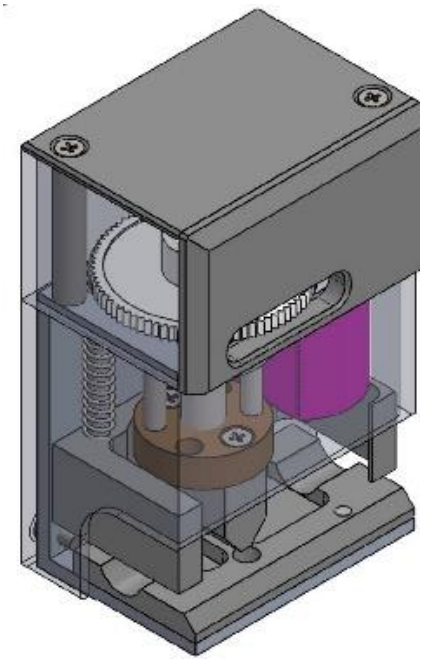

Figure 7: Water flow regulation with smart valves

10.4.6 Information system for transfer and presentation of measured data

The integrated information system allows three-dimensional inventory of the status of a water body. With all its sensors, the vessel reports to the operator its GPS location, depth, the presence of algae and cyanobacteria, water temperature, electrical conductivity, dissolved oxygen and pH. The vessel reports real-time data via GSM communication to the user, providing an accurate picture of the current state of the water body, which can be displayed by a web browser. GSM module sends the data also to the TCP / IP server, which can be located at a remote location of the operator, where the data are stored in MySQL database. The user-friendly data processing software enables analysis and graphical representation of the stored data adapted to the geographical picture of the water body.

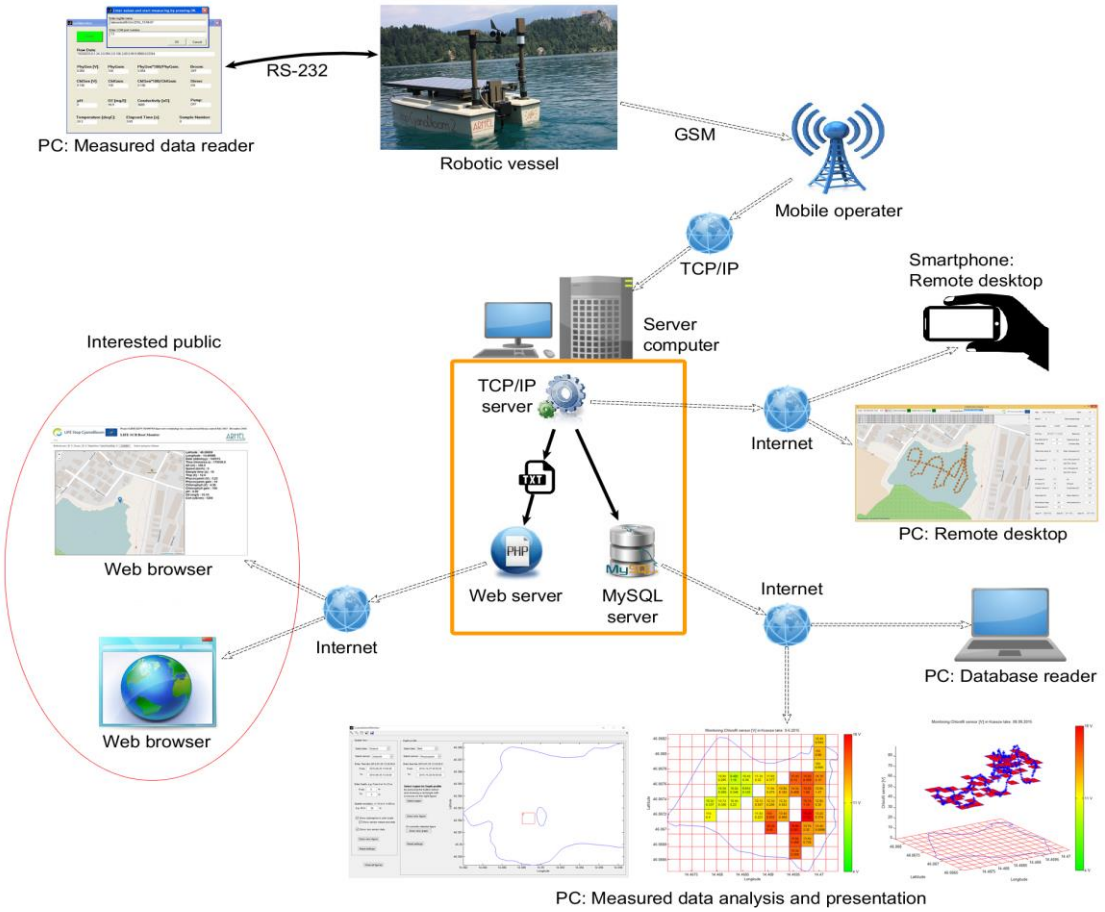

Figure 8: Information system for transfer and presentation of measured data

10.4.7 Automatic docking

The vessel is navigated manually, or a route plan is set by a PC. After a completed mission, the vessel returns to its home position (1) and the automatic docking starts. With the use of a compass (2) and a robot vision (3), the vessel determines its precise position relative to the docking station. When the position is known, robot vision (4) and measured values of obstacle detection sensors (5) are used as input variables of a fuzzy logic controller, which adjusts the vessel's heading and speed accordingly to enter the dock safely (6).

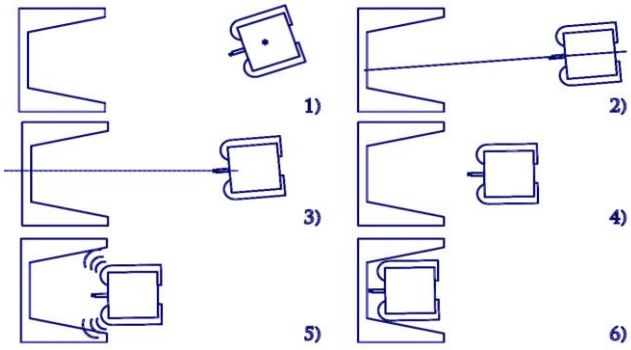

Figure 9: Automatic docking

11 Cost evaluation of LIFE Stop CyanoBloom monitoring and in-lake cyanobacterial control approach

Within the LIFE Stop CyanoBloom, we accomplished successfully on-line monitoring with the robotic vessel at two water bodies, fishpond Koseze (2 hectares) and Lake Bled with 145 hectares of lake surface. The performed parallel measurements with the use of fluorescence sensors showed high correlations with laboratory determination of phytoplankton biovolume and extracted pigments (presented in detail in Annexes 10A, 10, 13, 14 of the Final Report). In further monitoring activities, the sampling of water for laboratory determination of phytoplankton would be therefore needed only once per year and during the events of detected increased concentrations of cyanobacteria or other significant changes in fluorescence signals response. It can be, therefore, concluded that with the on-line monitoring, we could substantially reduce the need for laboratory determination of phytoplankton and related costs (to approximately one tenth of existing costs related to laboratory measurements), while substantially 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.

For the purpose of the LIFE Stop CyanoBloom project, the robotic vessel was equipped with the 60cm^2 electrolytic cell with 12ml volume. With the obtained results from the laboratory and pilot scale experiments and observations (presented in details in the Annexes 12 and 15 of the Final Report), we evaluated the needed size of the system for real scale environment presented below.

The size of **one electrolytic cell** used in the project (60cm^2 electrode surface, 12 mL working volume) would serve for **3 m^3 and 0.92 m^3 of water** if we would like to treat the detected affected water volume in **one week** and **two days**, respectively and achieve **80% reduction** of cells in the treated cloud of cyanobacteria. This would be sufficient for controlling **0.32 hectares and 0.09 hectares** of water surface, if we presumed that the treatment of 10 cm water layer (with the highest cyanobacterial concentration) would be needed and that cyanobacterial cloud covers 1 % of the water surface. The applied current density of 50mA/cm^2 was taken into account. Since the goal is to keep the cyanobacterial population below the critical level (and not to completely destroy the cells), a lower reduction efficiency could be needed, which then **allows the control of a larger surface area**. We evaluate, that the treatment would need to be repeated several times during the cyanobacterial blooming season.

The existing boat size and the capacity of the photovoltaic panels would allow installation of **3 electrolytic** cells of the same size (60cm^2 electrode surface), which would increase the capacity of the system to **9 and 2,8 m^3**, with the goal of treating the complete affected water volume in **one week or two days**, respectively. This would allow controlling **1 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.

Permanent monitoring and treating during cyanobacterial blooming season would increase area capacity of the vessel by keeping cyanobacterial concentration below the critical level.

In the following table, we have calculated the needed estimated size of the electrolytic cells with investment and operational costs for the monitoring and treatment of water from fishponds of three size classes. The problems with cyanobacterial overgrowth usually occur during the two summer months, and they aggregate in the upper 10 cm water layer. To start with the control of cyanobacteria before their critical outbreak, nine months of the electrolytic cell operation was envisaged. Besides, due to high eutrophic conditions, 2% of the affected water surface has been taken into account.

Table 3: Robotic vessel with monitoring and cyanobacterial control equipment investment and operation costs

	Fish pond surface (ha)		
	1	**10**	**100**
Investment			
Monitoring system with monitoring chamber, water sampling system, data transfer and data analysis software	17,400 €	17,400 €	139,200 €
Electrolytic cells (electrodes, pumps, housing, power supply adapter)	4,440 €	22,200 €	177,600 €
Vessel with photovoltaic panels for internal charging, batteries and navigation system	16,440 €	42,000 €	336,000 €
Docking station (boat house)	27,000 €	43,200 €	86,400 €
Total investment costs	**65,280 €**	**124,800 €**	**739,200 €**
Operation costs			
Annual electricity costs from outer network supply	300 €	3000 €	30,000 €
Annual maintenance and operation costs	11,700 €	25,000 €	150,000 €
Total annual operation and maintenance costs	12,000 €	28,000 €	180,000 €
Annual costs with amortisation (10 years for the equipment)	**18,528 €**	**40,480 €**	**253,920 €**

With the purchase of the robotic vessel, with the indicative market price 124,800€ (the distributor costs not included), the return of the investment for the 10 ha fishpond can be expected already in 1.5 - 6 years. Here we took into account that 2-5 tonnes of fish

are produced by 1 ha of fishpond with market price 2 €/kg, and considering that fishpond owner would avoid the annual loss of 50% to 100 % of produced fish, which usually occurs due to harmful cyanobacterial blooms.

12 Replicability and transferability potential of LIFE Stop CyanoBloom robotic vessel and its components

The aim of the development work on the project was to contribute to the improvement of the state of water resources and therefore contribute to economic benefits on other sectors dependent on clean and cyanotoxin-free freshwater resources.

As presented in the review above, 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,
- 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,

12.1 LIFE Stop CyanoBloom competitive advantages

The advantages of application of **LIFE Stop CyanoBloom** approach with on-line fluorescence based algal, cyanobacteria monitoring, and in-lake cyanobacteria control by direct production of hydroxyl radicals from water can be combined in the following:

- Preventive action based on synchronised cyanobacteria monitoring and growth control, preventing cyanotoxins release;
- Curative actions with simultaneous cyanobacterial and cyanotoxins destruction
- Obtaining detailed spatiotemporal data on water quality with lower costs and less labour-demanding way;
- Cost competitive and technologically advanced robotic vessel (advanced control operation, navigation and docking, autonomous position maintenance, and real-time data generation/interpretation);
- Providing solution for localised control of metalimnetic/deep-water cyanobacterial populations (existing measures work on the whole water body);
- Sustainable energy use with solar powered operation without artificial chemicals input.

- Standalone use of the individual components of the vessel (e.g. electrolytic cell or monitoring chamber as stationary unit)

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

- Lake water management for tourism,
- fishing and aquaculture,
- wastewater stabilisation ponds,
- irrigation reservoirs,
- drinking water abstraction and
- Protection of endangered freshwater habitats.

12.2 Replication potential in aquacultures

The very important market represents ponds and accumulations for aquaculture. While water availability has traditionally governed the maximum production capacity for a location, environmental legislation on waste loading has largely replaced water availability as the criterion.
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 problems confronted in intensive fish production.

According to FAO 2016 report, world fish harvested from aquaculture amounted to 73.8 million tonnes in 2014, with an estimated first-sale value of US$160.2 billion, consisting of 49.8 million tonnes of finfish (US$99.2 billion), 16.1 million tonnes of molluscs (US$19 billion), 6.9 million tonnes of crustaceans (US$36.2 billion), and 7.3 million tonnes of other aquatic animals including frogs (US$3.7 billion). Measured at the national level, 35 countries produced more farmed than wild-caught fish in 2014. This group of countries has a combined population of 3.3 billion, or 45 percent of the world's population. Countries in this group include five major producers, namely, China, India, Viet Nam, Bangladesh, and Egypt (FAO 2016). The other 30 countries in this group have relatively well-developed aquaculture sectors, e.g. Greece, the Czech Republic and Hungary y in Europe, and the Lao People's Democratic Republic and Nepal in Asia. All mentioned indicates also on a large market potential for the equipment for providing water quality monitoring and cyanobacteria control.

There are four clear market areas of freshwater fish: the food market, restocking, production of ornamental fish and sport fisheries. There is also a degree of interest in the production of organically certified freshwater fish products. Higher water quality can enable the production of agricultural goods with higher added value.

12.3 Replication potential in natural and artificial lakes for recreation

A very important market represents also natural and artificial freshwater bodies with a high recreational potential. There are for example 1939 registered (big) lakes across

Europe (EEA European Union, 2014) with bathing waters. In OECD countries, tourism is a big business (4.7% of GDP, and 6% of employment; 12 % of total EU members employment). Good recreational water quality is of a high importance and lake resorts would therefore be an important market. In 2013, there were 22,076 bathing waters identified in Europe, out of which 21,836 are in the 28 EU Member States and 29.6 % (nearly 6000) of them were inland waters (EEA Water bathing quality report, 2014). Globally the bathing water number is even higher. Here, high costs are spent for intensive water quality monitoring programmes as well as for rehabilitation and remediation measures to, for example, prolong the bathing season with removal or other cyanobacterial control measure.

12.4 Replication potential in drinking water and irrigation reservoirs

In the EU, water supply is mainly fed by groundwater and by surface water, including artificial reservoirs. Water sources vary considerably between Member States. Differences among countries are apparent when looking at the breakdown of water abstraction between groundwater and surface water resources. In Belgium (2009), Bulgaria (2011), Hungary (2008), Romania (2011) and the Netherlands (2010), surface water abstraction accounted for around ten times the volume of water abstracted from groundwater resources (Eurostat water statistics). The surface water abstraction in Germany, France, and Spain reached 26 000 million m^3 in 2004 and 2010, respectively. Some Member States recorded an increase in surface water abstraction, such as Estonia (with a 31 % increase), the Netherlands (22 %), Czech Republic (15 %), Sweden (14 %), and Bulgaria (14 %).

High water quality is an urgent precondition. The total abstraction of freshwater across Europe is around 288 km^3/year and represents, on average 500 m^3 per capita/year. Overall, 24 % of the total abstracted is for agriculture and 21 % for the public water supply (Water resources across EU, EEA, 2009). The countries with the highest percentage of stored water in relation to their total freshwater resources (over 20 %) are Turkey, Spain and Cyprus. These countries also use the highest percentage of their resources for irrigation. With increasing water need and regional long-term droughts, the needs for efficient water quality control solutions, as it is offered by LIFE Stop CyanoBloom approach, are huge.

The advantage of the technology is in the combined automated on-line cyanobacterial monitoring with the early warning system for CB blooms and in-lake CB proliferation suppression at the detected hot spots. This is done without the use of chemicals or sediment-developing inputs, thus not changing the total capacity of the water body. The method supports and retains healthy water biodiversity enabling full ecosystem services in the fields of aquaculture, drinking water abstraction and bathing water use.

12.5 Identified end-users of LIFE Stop CyanoBloom robotic vessel and its components

The following end-users of the technology and its components can be identified:

- Concessionaries of water bodies responsible for maintaining their quality (managers/operators of tourist bathing waters and multipurpose reservoirs (fish ponds used also for irrigation and firefighting, hydropower plant accumulations, etc.)
- State environmental agencies responsible for state monitoring of waters of national importance
- Certified laboratories performing water analysis on water bodies of national and local importance
- National institutes for public health monitoring drinking water sources
- Higher education institutions with environmental orientation
- Research institutes (with water and environmental departments)
- High schools with environmental and electro-technical (mechanical engineering, robotics, mechatronics) orientation

The following needs can be expected at identified end-users:

- Complete robotic vessel with monitoring, cyanobacteria control and data transfer module
- A need ro robotic vessel without automatic docking and boathouse lifting mechanism
- Stationary platform (measuring systems and electrolytic cell)
- Floating platform with navigation
- Floating platform with navigation and docking electronics
- Continuous measuring chamber
- Electrolytic cell for cyanobacteria control with charging system
- Deep water sampling winch
- Automatic sampler
- Smart valve system
- Information system for data transfer and graphical presentation
- Service on data interpretation and database management
- Service on supporting water analysis
- Repair and maintenance of robotic vessel, education on upgrades

The identified institutions could fulfil the following goals with the purchase of robotic vessel or services:

a) reduction of monitoring costs;
b) getting detailed spatiotemporal insight into water quality characteristics and providing early warning control;
c) self-maintenance of water quality under concession;
d) reduction of CB control curative costs;
e) performing research (water ecology / CB control / robotics / mechanics / electronics);
f) educational purposes;
g) becoming service provider for CB control in waters

12.6 Life Stop CyanoBloom transferability potential

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 on 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 has 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 a bigger geographical scale).

13 Open issues and proposals for further work

With the implementation of the project activities, we managed to fulfill all set project objectives:

1. A pilot scale demonstration of the innovative technology for prevention of cyanobacterial bloom occurrence has been performed in natural conditions.
2. With the set experiment in the natural environment we demonstrated, that the new technology didn`t affect the green algae population and that improvement of the ecological status of the chosen water bodies can be expected.
3. Introduction of a simple and effective method for determining certain physical and chemical parameters and concentration of cyanobacteria in the entire water body using innovative devices constructed for the proposed project.
4. Introduction of the project and innovative technology in international events and gathering interest from potential end users.

The limited project duration period, dependence on the seasonal occurrence of the cyanobacterial blooming, the absence of cyanobacterial blooms in the selected water bodies and complexity of the activities, however, ask for further tests and research. There are vast differences in cyanobacteria occurrence comparing oligotrophic natural lakes, artificial drinking water accumulations or eutrophic aquacultures. All three areas ask for their attention to meet, for example, the criteria of bathing and drinking water quality and prevent mortality and toxicity of aquaculture products for human consumption. The cyanobacterial species occurrence is different in mentioned three environmental as well as intensity and mode of occurrence (dispersed, hypolymnic, superficial, etc.) in the water column. Geographic locations of water bodies also bring differences in bedrock, temperature regimes, the amount of precipitation and length of sunlight. All mentioned requires different cyanobacteria control approaches.

The development of techniques in cyanobacterial control during the project also implies on the possibilities and needs of the application of the combination of methods, due to a high diversity of cyanobacteria growth intensity and occurrence in different geographical areas and water bodies.

With the tests performed during the project, it has been proven that hydroxyl radicals produced during the anode oxidation, have the potential to degrade cyanotoxins or reduce their toxicity. Further observations are proposed, performed during the longer period in the natural environment to study also the triggering/preventive mechanisms of cyanobacterial toxin production.

New possibilities show up in the area of reduction of energy costs with the use of new (nano) materials in anode oxidation, applicable for low water conductivity environments.

The possibility of triggering the lytic cycle with the induction of proliferation of cyanophages after electrochemical stimulation remained untested part of the project. To follow the cyanophages in the cyanobacterial population, a special laboratory equipment would be required.

14 Literature

A.K. Rai, and L.J. Stal. 2013: John Wiley & Sons.

Anderson, D.M., et al., Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters 2001: APEC Report # 201-MR-01.1, Asia Pacific Economic Programme and Intergovernmental Oceanographic Commission of UNESCO, Technical Series No. 59, Paris, France.

Antoniou, et al. 2009. Impact of the morphological properties of thin TiO

Atech, Cost of algal blooms. 2000, Canberra, ACT, Australia, : LWRRDC Occasional Paper 26/99, Land and Water Resources Research and Development Corporation,.

Baker, et al., 2006. Identification of a diagnostic marker to detect freshwater cyanophages of filamentous cyanobacteria. Appl. Environ. Microbiol.

Barrington, D.J., Reichwaldt, E.S., Ghadouani, A., 2013. The use of hydrogen peroxide to remove cyanobacteria and microcystins from waste stabilization ponds and hypereutrophic systems. Ecol. Eng. 50, 86–94. doi:10.1016/j.ecoleng.2012.04.024

Bricelj, Sedmak. 2001. Transport of biologically active substances through gravel strata. Netherlands, Swets & Zeitlinger Lisse

Caspers, H. 1984: OECD Eutrophication of waters, Monitoring, Assessment and Control. Int. Rev. Ges. Hydrobiol. Hydrograph.

Chattopadhyay, Pal 2002. Viral infection on phytoplankton–zooplankton system—a mathematical model. Ecol. Model. 1

Chen, G., 2004. Electrochemical technologies in wastewater treatment. Sep. Purif. Technol. 38, 11–41. doi:10.1016/j.seppur.2003.10.006

Cheung et al. 2013. Toxin-producing Cyanobacteria in Freshwater: A Review of the Problems, Impact on Drinking Water Safety, and Efforts for Protecting Public Health. Journal of Microbiology

Ćirić, M.D., 2013. Uticaj različitih tipova dodatne hrane u poluintenzivnoj proizvodnji mlađi šarana (Cyprinus carpio) na strukturu i dinamiku ribnjačkog ekosistema. Doctor disertation. Beograd, 2013.

Demmig-Adams , Adams. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci.

Department of Environmental Engineering. 2001, University of Western Australia.

Dodds, et al. 2009. Eutrophication of U.S. fresh-waters: analysis of potential economic damages. Environ. Sci. Technol43, 12–19

Dorigo, et al., 2004. Cyanophage diversity, inferred from 20 gene analyses, in the largest natural lake in France, Lake Bourget. Appl. Environ. Microbiol.

Drabkova M., Marsalek B.: A review of in-lake methods of cyanobacterial blooms control and management. CyanoData – The Glogal Database of Methods for Cyanobacterial Blooms Management, Centre for Cyanobacteria and their Toxins.www.cyanodata.net; April 2007

Drabkova M., Marsalek B.2007. A review of in-lake methods of cyanobacterial blooms control and management. CyanoData – The Glogal Database of Methods for Cyanobacterial Blooms Management, Centre for Cyanobacteria and their Toxins. www.cyanodata.net

effective cell population density control mechanism in cyanobacterial blooms. Microb. Ecol.

GijsbertsenAbrahamse, et al. 2006. Removal of cyanotoxins by ultrafiltration and nanofiltration. J. Mem. Sci.

Gregor, et al. 2007. Detection and estimation of potentially toxic cyanobacteria in raw water at the drinking water treatment plant by in vivo fluorescence method. Water Res., 41: 228–234.

Gregor, Maršalek 2004. Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water Research.

FAO, 2016. The state of world fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome 220 pp.

Hamilton, D.P., et al., Costs of harmful blooms of freshwater cyanobacteria. In book: Cyanobacteria: An Economic Perspective, Chapter: 15, ed. N.K. Sharma,

Hilker , et al. 2006. Oscillations and waves in a virally infected plankton system Part II: Transition from lysogeny to lysis. Petrovskii ecological complexity 3, 200–208.

Hilker, et al. 2006. Strange periodic attractors in a prey–predator system with infected prey. Math. Popul. Stud.

Huisman, et al. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology

Isabella Sanseverino, Diana Conduto, Luca Pozzoli, Srdan Dobricic and Teresa Lettieri; Algal bloom and its economic impact; JRC101253, EUR 27905 EN; doi:10.2788/660478, European Union, 2016.

Joehnk, et al. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global Chan. Biol.

Kolman, P., Mixing in a small, artificially destratified Perth reservoir, in

Liao, W., Murugananthan, M., Zhang, Y., 2014. Electrochemical degradation and mechanistic analysis of microcystin-LR at boron-doped diamond electrode. Chem. Eng. J. 243, 117–126. doi:10.1016/j.cej.2013.12.091

Meglič A., Pecman A., Rozina T., Leštan D., Sedmak B. 2016. Electrochemical inactivation of cyanobacteria and microcystin degradation using boron doped diamond anode (BDDA) - A potential tool in cyanobacterial bloom control. Unpublished.

Middelboe, et al., 2008. Viruses in freshwater ecosystems: an introduction to the exploration of viruses in new aquatic habitats. Freshw. Biol.

Miller, et al. 2005. The adsorption of cyanobacterial hepatoxins as a function of soil properties. J Water Health

Newcombe G. (Ed.), 2009. International Guidance Manual for the Management of Toxic Cyanobacteria. Global Watger research Coalition. United Kingdom. edepot.wur.nl/210694

Pires, 2010. Evaluation of fluorometers for the in situ monitoring of chlorophyll and/or cyanobacteria. Deltares. 1203593-000-ZWS-0004

Pollard & Young, 2010. Lake viruses lyse cyanobacteria, Cylindrospermopsis raciborskii, enhances filamentous-host dispersal in Australia. A cta Oecologica.

Premazzi, G., et al., Hypolimnetic withdrawal coupled with oxygenation as lake restoration measures: the successful case of Lake Varese (Italy). Limnetica, 2005. 24: p. 123-132.

Pretty, J.N., et al., 2003. Environmental costs of freshwater eutrophication in England and Wales. Environ Sci Technol, 2003. 37(2): p. 201-8.

Richardson, et al.2010. Spectral fluorometric characterization of phytoplankton community composition using Algae Online Analyser®. Water Res

Rozina, Tinkara, Klančar, Anita, Trontelj, Jurij, Roškar, Robert, Kristl, Albin, Meglič, Andrej, Čater, Maša, Finžgar, Neža, Zupančič Justin, Maja. Tehnologija elektrokemijske oksidacije za zmanjševanje odpadnih onesnažil v sledovih iz vode. 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, 2016, str. 361-368, ilustr. http://www.bc-naklo.si/index.php?id=2948. [COBISS.SI-ID 4072817]

Safferman, et al., 1969. Phycovirus SM-1: a virus infecting unicellular blue-green algae. Virology

Safferman, Morris, 1963. Algal virus: isolation. Science 140,

Samuilov, V.D., Timofeev, K.N., Sinitsyn, S. V, Bezryadnov, D. V, 2004. H 2 O 2 Induced Inhibition of Photosynthetic O 2 Evolution by Anabaena variabilis Cells. Biochem. Moscow 69, 926–933.

SEDMAK, B. Cyanobacteria and their toxins : what are they, where can we find them, why are they able to prevail and how do they behave?, (Vse živo, 03). Ljubljana: National Institute of Biology, 2012. 166 str., ilustr. ISBN 978-961-92543-8-7. [COBISS.SI-ID 260887040].

Sedmak, et al. 2009. Cyanobacterial cytoskeleton immunostaining: the detection of cell lysis induced by planktopeptin BL1125. J. Plankton Res.

Sedmak, et al. 2008. "Nontoxic" cyclic peptides induce lysis of cyanobacteria

Singh, et al. 2004. The role of virus infection in a simple phytoplankton zooplankton system. J. Theor. Biol.

Steffensen, D.A., Economic cost of cyanobacterial blooms. Adv Exp Med Biol, 2008. 619: p. 855-65.

Stopar, et al. 2004. Viral abundance and a high proportion of lysogens suggest that viruses are important members of the microbial community in the Gulf of Triest. Microb. Ecol.

Suttle, C.A., 2000. Cyanophages and their role in the ecology of cyanobacteria. In: Witton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer, Dordrecht, pp. 563–589.

Teixeira, et al. 2010. Investigating dissolved air flotation performance with cyanobacterial cells and filaments.Water Res.

The EU-US Scientific Initiative on Harmful Algal Blooms, European Commission, 2002 Workshop report.

TOXIC : cyanobacterial monitoring and cyanotoxin analysis / Jussi Meriluoto and Geoffrey A. Codd (editors). – Ĺbo : Ĺbo Akademi University Press, 2005. – (Acta Academiae Aboensis. Ser. B, Mathematica et physica, ISSN 0001-5105; vol. 65, no. 1) ISBN 951-765-259-3

Tucker Pollard, 2005. Identification of cyanophage ma-lbp and infection of the cyanobacterium Microcystis aeruginosa from an Australian subtropical lake by the virus. Appl. Environ. Microbiol.

Vardi, et al. 2002. Dinoflagellatecyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr. Biol.

Villada, et al. 2004. Occurrence of copper resistant mutants in the toxic cyanobacteria M. aeruginosa characterisation and future implications in the use of copper sulphate as algaecide. Water Res.

Walsby, et al. 2004. Comparison of the depth where Planktothrix rubescens stratifies and the depth where the daily insolation supports its neutral buoyancy. New Phytol.

WH, O., Guidelines for safe recreational water environments. Coastal and Fresh Waters. 2003: Geneva: World Health Organization.

Whitton, Potts, (Eds.), 2000. The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic, Boston, USA, p. 669

Wiedner, et al., 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia

Wommack, Colwell R. R. 2000. Virioplankton: viruses in aquatic ecosystems. Microb. Mol. Biol. Rev.

Zhang, C., Fu, D., Gu, Z., 2009. Degradation of microcystin-RR using boron-doped diamond electrode. J. Hazard. Mater. 172, 847–853. doi:10.1016/j.jhazmat.2009.07.071

Zupančič Justin Maja, Tinkara Rozina, Gorazd Lakovič, Lovro Pokorn, Anita Klančar, Luka Teslič, Mario Marinović, Jošt Grum, Jurij Trontelj, Maša Čater, Robert Roškar, Andrey Yakuntsov, Neža Finžgar, Branko Hamiti, Albin Kristl, Marko Gerl. (2016) Projekt LIFE PharmDegrade – demonstracija elektrokemijske oksidacije z elektrolitsko celico kot obetavnega pristopa odstranjevanja obstojnih mikroonesnaževal v procesu čiščenja odpadne vode. LIFE PharmDegrade project – demonstration of electrochemical oxidation with electrolytic cell as a promising approach to removal of persistent micropollutants in the wastewater treatment process. Ekolist13 Revija o okolju. http://www.ekolist.si/datoteke/ekolist_13/S121.pdf