# Mapping Areas under the Pressure of Cyanobacteria Blooms and Algal Toxins Emergence in the Danube Delta Biosphere Reserve (Romania)

*Cartarea ariilor aflate sub presiunea înfloririlor cu cianobacterii și dezvoltarea toxinelor algale din Rezervația Biosferei Delta Dunării (România)*

Liliana TÖRÖK, Marian MIERLĂ, Zsolt TÖRÖK

**Abstract**

*Cyanobacteria blooms can cause problems for ecosystems and human society. The present investigations, carried out in the frame of the project "Increasing the capacity of assessment the toxicity of cyanobacteria in order to improve the management and exploitation of water in the Danube Delta Biosphere Reserve (Creșterea capacității de evaluare a toxicității determinate de cianobacterii în vederea îmbunătățirii gestionării și exploatării apei în Rezervația Biosferei Delta Dunării)", financially supported by the National Authority for Research and Innovation (PN 47N/2016), aim the mapping and analysis of algal blooms using fluorescence emission techniques by quantification of different types of pigments produced by algae, the building a GIS-database and the providing of risk distribution maps for cyanobacteria in the Danube Delta Biosphere Reserve (DDBR). Some species of algae have reached the critical point defined as "limits of blooming" in the aquatic ecosystems of the delta and lagoon area. Due to the high density of pigmented cells, algal blooms modify the water colour. The discoloration of water in the aquatic ecosystems of the DDBR is often green, but can also be brownish (due to presence of mix population dominated by diatoms and Euglenophyta) or bright blue-green blooms (when cyanobacteria have high biomasses). Many species of cyanobacteria can produce harmful bloom events, involving releases of the toxic metabolites into the environment. The frequency, severity and distribution of the risk area (due to the occurrence of toxic events caused by cyanobacteria), is unknown, even if blooming is frequently observed in the Danube Delta's lakes. Scanning and mapping the area using Bbe Moldaenke GmbH environmental technology is the first step for the identification of the potential hazard areas.*

**Keywords:** *cyanobacteria, freshwater algal blooms, algal toxins, Danube Delta Biosphere Reserve, Romania*

## Introduction

The problems generated by the presence of cyanobacteria towards ecosystem services and human society on a global scale have led to increased attention for the management of the phenomenon by the administrators of the various protected areas, namely, the attention of managers of water resources destined for recreational use and human consumption. Assessing the risk of non-compliance with environmental objectives has become an extremely important issue for European Community countries. Romania has made remarkable progress over the last 20 years, but some issues addressed and discussed at the European level, such as those related to the management of the expansion of cyanobacterian blooming in surface waters, have remained little known at the level of environmental agencies, and responsible agencies for monitoring and management of surface water quality.

Previous studies have suggested that in the absence of major changes in water quality caused by accidental pollution, a minimum of six years of monitoring would be sufficient to determine the type and frequency of blooming in an open system, such as the delta.

As early as 2008, it was proposed that in the case of the Danube Delta a classification of lakes in risk classes should be made, taking into account both the values of the phosphorus and nitrogen concentrations, which are extremely important in the eutrophication dynamics, as well as the frequency and the type of blooming occurring in the delta aquatic ecosystems (TÖRÖK *et alii*, 2008). When drawing up the aforementioned classification, we took into account the recommendations and regulations related to the monitoring of algal blooming in surface waters in France (***, 2003) which use the numerical abundance of the species, rather than the biomass developed by them.

Inclusion of aquatic ecosystems in classes and/ or categories that will not comply with environmental objectives, in order to draw attention to the need to undertake ecological reconstruction work, namely to develop and implement risk management plans for algal blooming, or cyanobacteria blooming, should consider, in addition to the qualitative structure (species) of algal and/ or phytoplanktonic/ phytobentonic populations, the amount of biomass produced by them. This reporting system based on biomass quantification is more widespread in Europe than the one based on quantifying the numerical abundances of different species (****, 2001; KROKOWSKI *et alii*, 2002).

To have a general image of the phenomena for the entire studied water body there could be made maps that show the blooming image. These maps are made with the help of different interpolation methods. Many interpolation methods (GARNEO, GODONE, 2013; NAOUM, TSANIS, 2004) are used to develop the maps of the type presented in the paper, the most popular of which are IDW (Inverse Distance Weight), KRIGING and SPLINE. In the IDW method, it is assumed substantially that the correlation and neighbouring similarity ratio is proportional to the distance between them, which can be defined as an inverse function of spacing each point in the adjacent points. It is necessary to remember that the definition of the neighbouring radius and the power related to the reversal function are considered important problems in this method. This method will be used where there are sufficient sampling points with a local dispersion. One of the advantages of this method is to be appropriate to show barriers that show discontinuous lines such as fractures and rivers that break and discontinue on a surface (ZIARY, SAFARI, 2007). This interpolation works best with evenly distributed points. Similar to SPLINE functions, IDW is sensitive to extreme values. In addition, clusters of unequally distributed data result in errors (SHEPARD, 1968).

Similar to IDW, KRIGING uses a weighting that assigns more influence to the nearest data points in value interpolation for unknown locations. KRIGING, however, is not deterministic, but extends the weighting approach of IDW to include random components where the location of the exact point is not known by the function. KRIGING depends on spatial and statistical relationships to calculate the surface. The two-step process of KRIGING begins with the half-variability estimates and then performs interpolation. Some advantages of this method are the incorporation of variable interdependence and the amount of error area available (AZPURUA, RAMOS, 2010). A disadvantage is that it requires much more time to compute and model, and KRIGING requires more input from the user (LEGENDRE, LEGENDRE, 1998).

SPLINE estimates grid cell values by fitting a minimum-curvature surface to the sample data. It’s like a flexible sheet of plastic that passes through each data point but otherwise bends as little as possible. The difference between regularized and tension splines is one of flexibility: a regularized spline has more "give" in it and produces smooth surfaces with estimated values that may fall well outside the sample data range. Tension splines are less smooth but conform more closely to the sample range.
*https://www.nrem.iastate.edu/.../Surface_interp_tools.doc*

**Materials and Methods**

The analysis carried out in the present phase of the project aims at:

- establishing *in situ* biomass risk classes;
- testing the SPLINE data interpolation method;
- presenting the results of the first mapping of the risk areas to cyanobacterial exposure in the Danube Delta Biosphere Reserve.

By using algal biomass values expressed by ”*a*” chlorophyll values and phycoerythrin and phycocyanin values, the following three risk classes are proposed for the establishment of risk areas in the Danube Delta:

1. R (risk) if the cyanobacterial blooming frequency exceed 50% of the total number of blooms that occurs in one year, or if the concentration of phycoerythrin and phycocyanin produced by cyanobacteria is ≥ to 50% of the value of the expressed eutrophication threshold expressed in chlorophyll concentrations.

2. RP (possible risk) if the concentration of phycoerythrin and phycocyanin produced by cyanobacteria is below 12.5 µg/ liter.
3. FR (non-risk) if the concentration of phycoerythrin and phycocyanin is <10 µg/ liter, equivalent to less than 20% of total biomass and less than 20000 cells of cyanobacteria/ ml.

The identification of the risk areas for cyanobacterial blooming was carried out on the basis of the cyanobacterial distribution maps of two lacustrine complexes in the Danube Delta as follows:

- Matiţa Lake from Matiţa-Merhei lacustrine complex
- Puiu Lake from Roșu-Roșuleț lacustrine complex.

Risk maps were made in ArcGIS 10.0. in the Geographic Information System format using Stereo 1970 coordinate system, the SPLINE interpolation method being used in this paper. The SPLINE method estimates the values using a mathematical function that minimizes curvature of the total surface, resulting in a smooth surface that passes exactly through the sampling points. The advantages of SPLINE functions are that they can generate sufficiently accurate surfaces from just a few sampling points and keep small features. A disadvantage is that they may have different minimum and maximum values from the dataset and the functions are sensitive to extreme values due to the inclusion of the original data values at the sampling points (AZPURUA, RAMOS, 2010). In order to establish the distribution of cyanobacteria and total algal biomass on risk maps, the set of measurements taken in the morning, at noon and in the evening, in May and September 2016, was used.

## Results and Discussion

The analysis of the distribution of cyanobacteria on the surface of Matiţa Lake water and their concentration zones during one day in May revealed that there are no differentiated concentrations during one day. Maximum cyanobacterial biomass values (2.79 µg/ l) remain below the threshold alert. Analysis of the amount of algal toxins highlights the presence of microcystin (MC), in the traces.

With a distribution of rare aquatic vegetation, in Matiţa Lake it is observed that in September, cyanobacteria (Figures 1, 2, 3) exceed the alert threshold in almost all stations where measurements were made. This uneven distribution is also due to the wind that moves the water layer from surface of the water, allowing for more concentration in certain areas. The presence of cyanobacteria in the water mass can, in this case, endanger the life of aquatic organisms, due to the high recorded biomass 46,198 µg/ l of cyanobacteria. The gelatinous mass in which cyanobacterial cells are anchored, leads to blocking gills of fish and their inevitable mortality during algal blooms.

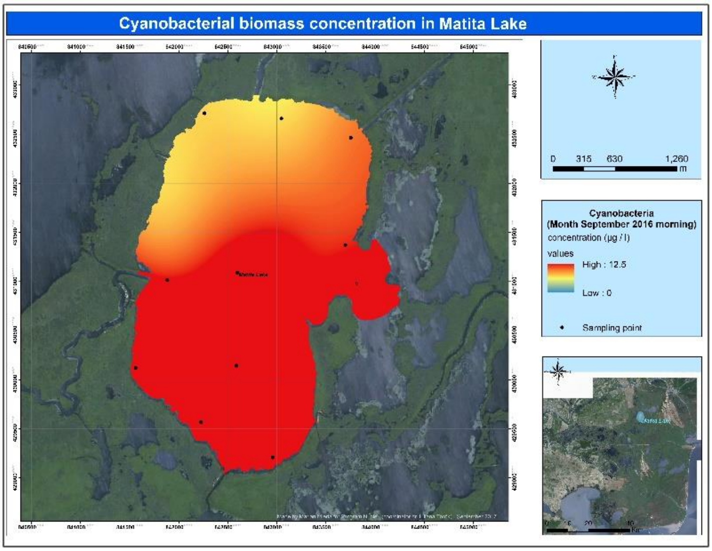


Figure 1. Circadian (September morning) highlighting of cyanobacterial biomass concentration zones in the Matita Lake

*Fig. 1. Evidențierea circadiană (septembrie dimineața) a zonelor de concentrație a biomasei cianobacteriene din Lacul Matița*

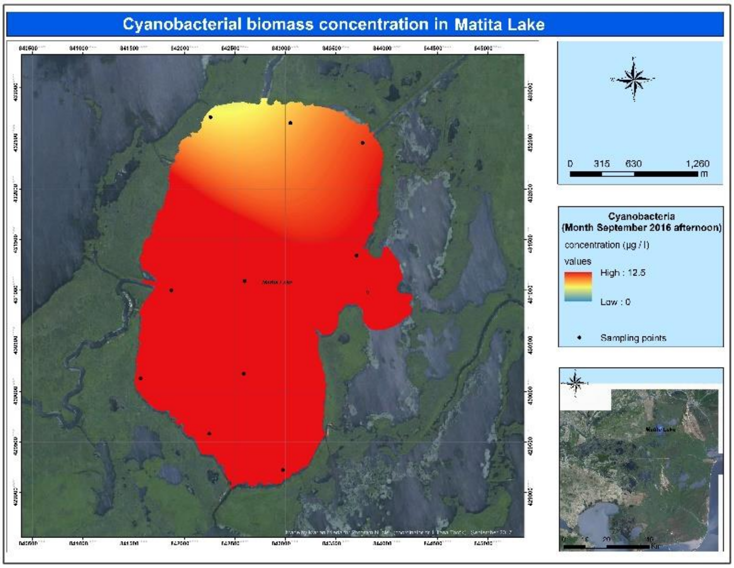


Figure 2. Circadian (September noon) highlighting of cyanobacterial biomass concentration zones in the Matiţa Lake

*Fig. 2. Evidențierea circadiană (septembrie amiază) a zonelor de concentrație a biomasei cianobacteriene din Lacul Matița*

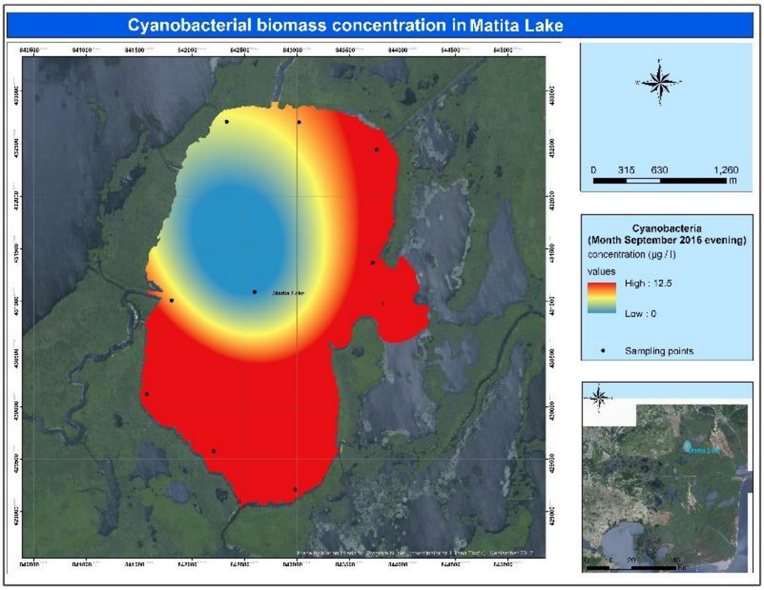


Figure 3. Circadian (September evening) highlighting of cyanobacterial biomass concentration zones in the Matiţa Lake

*Fig. 3. Evidențierea circadiană (septembrie seara) a zonelor de concentrație a biomasei cianobacteriene din Lacul Matița*

In the case of Matiţa Lake there is an increase in primary productivity during the summer season reaching maximum values of 101.7 µg/ l in September. Concentrations exceed the risk threshold.

The analysis of the distribution of cyanobacteria on the surface of Puiu Lake and their concentration zones over a day showed that in May, for all the spring, as in the case of the Matiţa Lake, an undifferentiated concentration occurs during a day. Maximum cyanobacterial biomass values are about 2.88 µg/ l, below the alert threshold. Analysing the amount of algal toxins also highlights the presence of traces of microcystin in this case. In September (Figures 4, 5, 6), the lack of submerged aquatic vegetation favoured the development of cyanobacteria, so that the minimum values recorded in Puiu Lake were 4.04 times higher, in 2016, than in the Matiţa Lake.

However, the Puiu Lake, strongly affected by the development of cyanobacteria, did not show significant changes in microcystin concentration in the water mass. This fact makes the working hypothesis (****, 2016 a; ****, 2016 b), whereby a large amount of algal toxins could signal in massive biomass accumulations of cyanobacteria, to not be confirmed. Moreover, it was found that PCR analysis (unpublished data) of gene coding sequences for the occurrence of toxic cyanobacterial population was inconclusive. In this case, taxonomy studies combined with genetic tests to identify which are the dominant cyanobacteria species and to certify that they have in their genome gene sequences coding for the production of microcystin or other algal toxins (cylindrospermopsin, nodularins) have not been analyzed in this study.

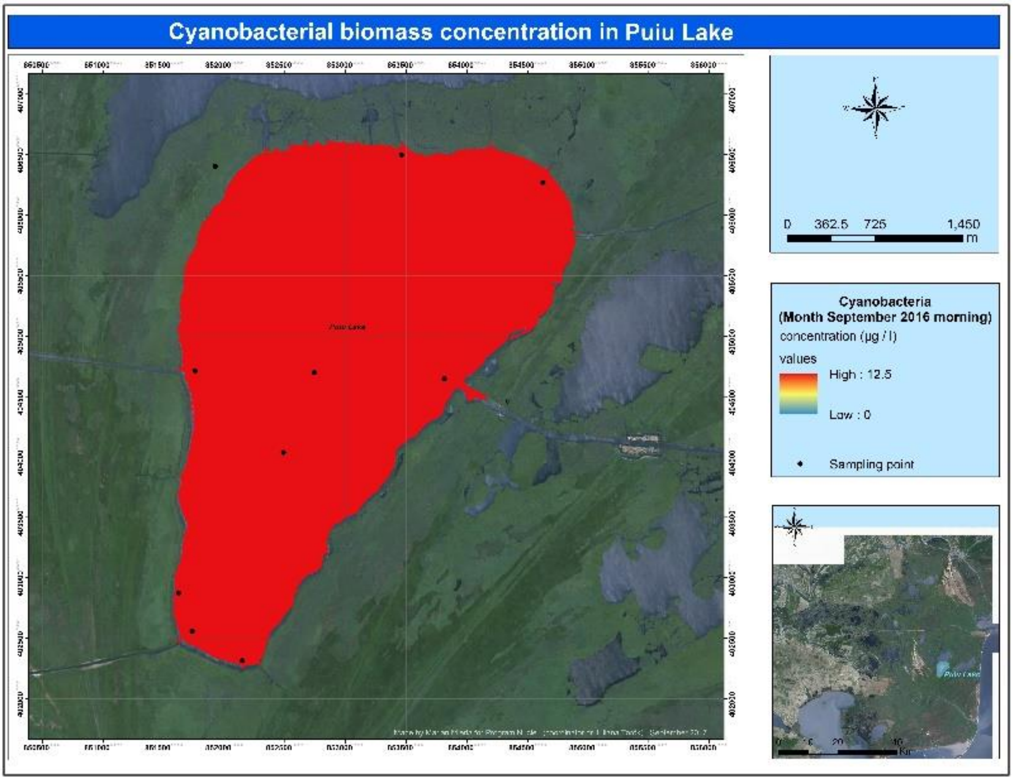


Figure 4. Circadian elevation (September morning) of cyanobacteria biomass concentration zones in the Puiu Lake

*Fig. 4. Creșterea circadiană (septembrie dimineața) a zonelor de concentrație a biomasei cianobacteriene din Lacul Puiu*

The ecological succession throughout the year and the analysis of the ecological requirements of cyanobacterial species and the feasibility of other phytoplankton systemic groups could provide clarification and additional information for such a classification. High values between 39.1 and 191.86 µg/l of biomass are recorded in all 10 stations where measurements were made.

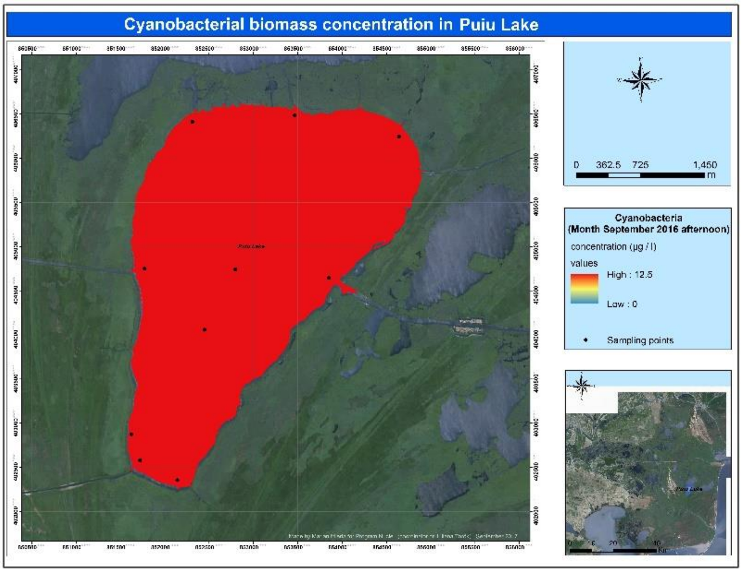


Figure 5. Circadian (September noon) highlighting of cyanobacteria biomass concentration zones in the Puiu Lake

*Fig. 5. Evidențierea circadiană (septembrie amiază) a zonelor de concentrație a biomasei cianobacteriene din Lacul Puiu*

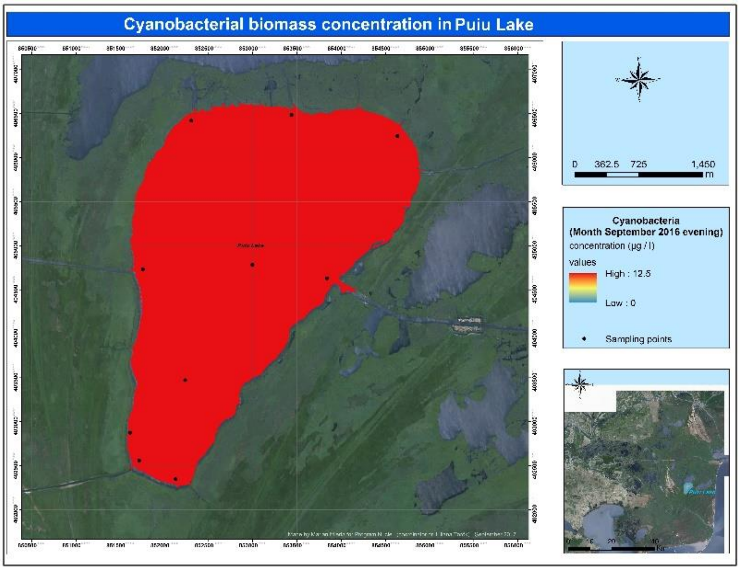


Figure 6. Circadian (September evening) highlighting of cyanobacteria biomass concentration zones in the Puiu Lake

*Fig. 6. Evidențierea circadiană (septembrie seara) a zonelor de concentrație a biomasei cianobacteriene din Lacul Puiu*

## Conclusions

By mapping the risk areas it was pointed out that:

- agglomerations of spring cyanobacteria occur mainly in the areas near the banks and the developed biomass does not pose a high risk for the aquatic organisms, the two lakes in the present case study can be classified in the no risk category;

- the concentration of cyanobacteria in risk areas was highlighted in September both for Matiţa and Puiu lakes; the lakes can be included in the risk category both for cyanobacteria presence in the water mass and for the total algal biomass;

- PCR analysis of coding gene sequences for the occurrence of cyanobacteria with toxic potential were inconclusive, although for both sampling periods were identified traces of microcystin in the water mass.

## Acknowledgements

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***Liliana Török***
*Institutul Național de Cercetare-Dezvoltare Delta Dunării, Tulcea*
*Str. Babadag nr. 165, Tulcea, România, tel./fax: +40240.531.520/533.547*
*e-mail: torokliliana@yahoo.com*

***Marian Mierlă***
*Institutul Național de Cercetare-Dezvoltare Delta Dunării, Tulcea*
*Str. Babadag nr. 165, Tulcea, România, tel./fax: +40240. 531.520/533.547*
*e-mail: marian.mierla@ddni.ro*

***Zsolt Török***
*Institutul Național de Cercetare-Dezvoltare Delta Dunării, Tulcea*
*Str. Babadag nr. 165, Tulcea, România, tel./fax: +40240. 531.520/533.547*
*e-mail: torok_zsolt2004@yahoo.co.uk*