PROJECT FINAL REPORT

IDENTIFICATION AND TOXIC POTENTIAL OF CYANOPROKARYOTA IN THE BULGARIAN WATER BODIES. ENVIRONMENTAL HEALTH RISKS.

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The **main aim** of the research project was to study the *Cyanoprokaryota* diversity and their toxic potential in selected Bulgarian freshwater dams used as sources of drinking water, to evaluate the health and ecological risks and to develop an effective screening test-system for presence of cyanotoxins.

The **objectives** of the project were:

- Collection of water samples at different time points from selected Bulgarian freshwater basins that are used as sources of drinking water and examination for presence of cyanotoxins.
- Collection and identification of the cyanoprokaryotic phytoplankton from the monitored Bulgarian freshwater basins.
- Study the ability of isolated blue-green algae to produce intracellular and/or extracellular cyanotoxins.
- Establishment of correlation between the environmental factors and toxin production.
- Development of an effective screening test-system for presence of cyanotoxins.

Our project was conducted during a key period for the country (2007-2010), when as a member of the European Union, Bulgaria had to meet the obligations for monitoring of the surface water as a part from the EU Water Framework Directive (2000/60/EC) for water protection and management. Bulgaria had to apply an integrated approach to assess the water quality and to harmonize the national legislation with the EU in this direction.

The implementation of our project resulted an accumulation and processing of information about the species *Cyanoprokaryota* that cause "algal blooms" and produce cyanotoxins during the summer months in some of the biggest dams in Bulgaria. As a whole, data related to phytoplankton in the Bulgarian dams are few and mainly concern the species composition and/or dominant species. Until 2007, for the existing 120 dams in the country, there were available data only for 28 of them. The typology of Bulgarian's lakes according to the system-B is significantly less developed than that of rivers, because of insufficient biological data and prevalence of modified water bodies. In practice, more than 90% of all water bodies are reservoirs (modified or artificial water bodies). Therefore, more essential for Bulgaria is determining the ecological potential for different types of "artificial lakes" than determining the ecological status of the small number of natural lakes (high-mountain glacial lakes, several landslide lakes and Black Sea or Danube lakes and swamps, which are usually strongly influenced by human intervention).

In the course of the project, normatively defined key indicators used in the monitoring and evaluation of the water quality, were determined: taxonomic composition, abundance and biomass of the phytoplankton with emphasis on representatives of *Cyanoprokaryota*. Concerning the taxonomic composition, an inventory list of cyanoprokaryotic species for each investigated period and dam was prepared. These data were used to evaluate the total species richness and for calculation of some taxonomic indices of diversity, and proportion of the major taxonomic groups. The presence of toxic species was analyzed for the selected dams. On the basis of the performed research is clear that all studied dams have a stable taxonomic composition and relatively low diversity of the phytoplankton. Green algae (*Chlorophyta*) have a dominant role for the summer phytoplankton, and the group of *Cyanoprokaryota* is relatively poor in terms of species composition. "Algal blooms" were determined by using the biomass and density, respectively.

Based on the average physico-chemical data for the water transparency (Secchi disk), the amount of total chlorophyll-*a* and total biomass as well as the amount of the total nitrogen and total phosphorus and their ratio (TN/TP), the trophic status of each dam was defined and the trophic state index (TSI) and Catalan index were calculated. The trophic potential and stress of each of the surveyed eleven dams were identified. For example, the values of the TSI index (50), the amount of chlorophyll-*a* (2.44 mg/l), total biomass (1.16 mg/l) and the ratio TN/TP (66) for the reservoir "Studen Kladenets", which is the second

largest dam in Bulgaria after the Iskar dam, showed a transition from a mesotrophic environment to a state of eutrophy and limitation of the phosphorus at community level.

Species composition, seasonal variation of the phytoplankton, including *Cyanoprokaryota* as well as the production of toxins is determined by the interaction between physical and chemical environmental factors such as temperature, pH, conductivity, dissolved oxygen, nitrogen compounds, total nitrogen, organophosphates, total phosphorus, transparency and turbidity of the water, which were also monitored within this project and whose values or relations gave us important information about the ecological status of the surveyed dams. Partial studies related to monitoring of these indicators have been performed for some Bulgarian dams, but they are also sparse taking into account the necessary information to meet the requirements of the Water Framework Directive. In this respect, we believe that our data concerning the physico-chemical parameters of the 11 dams subject of our research and found correlations, contributes to both fill the database and to clarify some important ecological relationships.

The relevance and innovation of the project is also determined from the launched in Bulgaria monitoring of surface water used for drinking, fishing or recreation regarding the cyanotoxins and their recognition in our country as a factor representing a danger to the flora, fauna and people. The presence of cyanotoxins (microcystins, nodularins, saxitoxins and anatoxin-a) in all dams subjects to this investigation was analyzed using biological (*in vivo* and *in vitro*), immunological (ELISA) and physico-chemical (HPLC) methods.

During the study period all four cyanotoxins were detected in the surveyed dams at concentrations slightly below the maximum permitted values. A large variety and quantity of cyanotoxins was detected in the «tail» of the dams. The «tail» part of the reservoirs are more eutrophic compared to the «wall» part. Obviously, there is an inflow of wastewaters, which cause loading with nutrients and organic matter. In order to limit further steps of organic and nutrient loading in the «tail» part of the studied dams, it is recommended to improve the control and quality requirements of the discharged water at these points.

In addition, an assessment of the water of the 11 monitored Bulgarian dams for the presence of heavy metals was conducted. Water samples, collected during the summer of 2008 and 2009 were tested for the levels of 5 heavy metals - zinc (Zn), lead (Pb), manganese (Mn), copper (Cu) and cadmium (Cd). Most often elevated values were determined for zinc (Zn), lead (Pb) and cadmium (Cd). Alarming data on the lead content was obtained for the water of the reservoirs Vacha and Studen Kladenets. Detected levels of lead in the reservoir Vacha (0.24 mg/L for 2008 and 0.29 mg/L for 2009) are 30 times more than the maximum permited concentration of 0.01 mg/L set by the World Health Organization. Reported elevated concentrations of lead in the water of the reservoir Vacha are dangerous to the aquatic ecosystem and the people chose this place for recreation or fishing. Moreover, this dam is used as a source of drinking water as well.

Detected levels of lead in the rezervoir Studen Kladenets (0.18-0.75 mg/L for 2008 and 0.26-0.83 mg/L for 2009) are at times more than the maximum permited concentration of 0.02 mg/L. It is assumed that this pollution comes mainly from the Lead-zinc factory "Kardzhali", unregulated landfills with municipal and medical waste in the beds of the rivers that flow into the reservoir.

All these data suggest that a continuous control and monitoring of water quality and timely awareness of potential risks for both aquatic ecosystems and human health, is required.

In **conclusion** we could say that:

- The purpose and objectives of our project were achieved and the project has its scientific value and contribution to the environmental research in Bulgaria.
- The relationship between the phytoplankton structure of a water basin, the presence of cyanotoxins and the water quality, makes it possible to create a good system for monitoring of the phytoplankton and its toxic potential.

The way to measure each research success of the academic staff at Plovdiv University is via career development and the amount of raised funds. Thus, in order to develop and maintain a reasonable research environment, funded projects such as the present one, have become essential. It laid the base for building up an active research group in the department. The international visibility of the group has clearly increased. Maintaining good contacts with researcher's collaborators, we could amplify already existing good international contacts and prepare the ground for new collaboration.

Dr. Ivanka Teneva has applied and won a position as an Assistant Professor in Botany at the Plovdiv University. This is a good example for a successful reintegration.