REVIEW

of a dissertation for the award of the educational and scientific degree "Doctor" (PhD) in Professional field 7.1. Medicine, Scientific specialty "Hygiene"

Title:
Hygienic and Analytical Aspects in the Study of the Seasonal Dynamics of Microcystins in Surface Waters Intended for Drinking Purposes

Author: Assist. professor Maria Angelova Mitreva, PhD
Department of chemical factors, Directorate of analytical and laboratory activities, National Center of Public Health and Analyses

Scientific Supervisor: Assoc. prof. Vera Pavlova, PhD

Reviewer: Assoc. prof. Rositsa Georgieva, PhD
Directorate of Analytical and laboratory activities, National Center of Public Health and Analyses

Brief biographical data

Maria Angelova Mitreva, PhD obtained a Master's degree in Medical and Pharmacological Biophysical Chemistry in 2013 from the Faculty of Chemistry and Pharmacy at Sofia University "St. Kliment Ohridski". In the period 2014–2017, she was a full-time PhD student in the same faculty in the doctoral program "Analytical chemistry", professional field 4.2. Chemical sciences.

Her professional career is associated with the National Center of Public Health and Analyses, where she has worked since 2017, initially as an assistant and, since 2019, as an assistant professor in the Department of chemical factors, Directorate of analytical and laboratory activities. Her research interests are focused on analytical methods for determining contaminants in environmental matrices.

In 2024, she was enrolled as an independent PhD candidate for the development of the present dissertation.

Relevance and significance of the topic

The topic of the dissertation is of high scientific and public importance. Cyanobacterial blooms and the associated cyanotoxins, particularly microcystins (MCs), pose a significant risk to the quality of surface waters used for drinking purposes.

Special attention should be given to microcystin–LR, the most widespread and toxic variant, characterized by hepatotoxic and potentially carcinogenic effects, as well as relative stability in aquatic environments.

The inclusion of microcystin–LR as a monitoring parameter in Directive (EU) 2020/2184 further emphasizes the importance of research in this field. In this context, the dissertation is timely, relevant, and fully aligned with current scientific and regulatory requirements. The study addresses a problem that remains insufficiently investigated under Bulgarian conditions.

General characteristics and evaluation of the dissertation

The dissertation is presented in a volume of 141 standard pages, illustrated with 31 figures and 24 tables, and includes 2 appendices. The bibliography comprises 153 references, including 12 in Cyrillic.

The work is logically structured and includes all required sections. There is a clear internal coherence and consistency in the presentation. The volume is fully adequate for a comprehensive examination of the research problem.

Literature Review

The literature review is extensive, well-structured, and demonstrates in-depth knowledge of the current scientific literature. It presents the main characteristics and classification of cyanotoxins, the processes of cyanobacterial blooms and their adverse effects on human health, as well as water treatment processes that may influence toxin formation.

The influence of environmental factors such as chlorophyll-A concentration, nutrient availability, temperature, pH, light regime, is discussed, including existing uncertainties and contradictory findings. A review of analytical methods for microcystin determination is also provided, covering both physicochemical and biological approaches, evaluated in terms of applicability, advantages, and limitations.

The author provides a critical analysis of the available information and clearly defines the scope of the present study. The review serves as a solid foundation for the formulation of the research objectives and tasks.

Aim and Objectives

The aim of the study is clearly defined and focused on investigating the seasonal dynamics of microcystins in surface waters intended for drinking purposes and assessing the associated hygienic aspects.

The objectives are clearly formulated, logically structured, and appropriate for achieving the stated aim.

Materials and Methods

The applied methods are modern and appropriate for addressing the research objectives. A complex approach has been adopted, including:

- Enzyme-linked immunosorbent assay (ELISA)
- High-performance liquid chromatography (HPLC)
- Nutrient analysis (nitrogen and phosphorus)
- Phytoplankton analysis
- Statistical data processing

The combination of these methods ensures a reliable and comprehensive evaluation of the studied problem.

Results and Discussion

The results are extensive and based on investigations of real water bodies, including dams of major importance for drinking water supply.

The method for the chromatographic determination of microcystins and its validation are described. The study includes characterization of the investigated dams – Iskar, Studena, and Krasava. A total of 99 samples collected during the period 2023–2025 were analyzed for microcystins, nutrients, and cyanobacterial composition.

The results demonstrate the presence of microcystins (MC–LR, –RR, and –YR) in samples from Studena dam, with total concentrations reaching 1.2 μg/L, MC–LR is 0.38 μg/L (~32% of the total content). Higher concentrations were detected in surface bloom accumulations near the dam wall.

A comprehensive comparative analysis of the studied dams is provided, including assessment of trophic and saprobic status, phytoplankton structure, and frequency of microcystin occurrence.

Correlation analysis between microcystins and nutrient concentrations (total nitrogen and phosphorus) did not reveal statistically significant relationships. The potential influence of other environmental factors, such as temperature and light, is also discussed.

The combined application of ELISA and HPLC methods is recommended, allowing both rapid screening and precise identification of microcistyns, which is particularly important for risk assessment in drinking water sources.

The results are compared with international studies and are consistent with global trends indicating localized and seasonally driven toxin production events.

Conclusions and Recommendations

- The conclusions are very well structured and logically follow from the completion of the assigned tasks.
- Recommendations have been formulated for monitoring vulnerable water bodies and for preventive monitoring of potentially endangered dams. They are practically stated and have real applicability.

Major scientific and scientific-applied contributions of the dissertation

Scientific contributions:

1. Original data on the occurrence and seasonal dynamics of microcystins in Bulgarian water bodies have been obtained.
2. Relationships between nutrient concentrations and toxin production have been investigated.
3. Knowledge about the behavior of cyanotoxins under local conditions has been expanded.

Scientific-applied contributions:

1. A high-performance liquid chromatography (HPLC) method for determining microcystins, suitable for use in official controls, has been developed and validated
2. The effectiveness of combined ELISA and HPLC application has been evaluated.
3. Practical approaches for monitoring of vulnerable water bodies have been proposed.

Remarks and Questions

No substantial critical remarks can be made regarding the dissertation.

The PhD candidate has addressed the recommendations made during the pre-defense review. The results from positive samples have been systematically summarized, and correlations between microcystins and nutrient concentrations have been analyzed.

The following questions may be posed:

1. What are the main sources of error in microcystin analysis?
2. How can the differences in concentrations between the studied dams be explained?
3. Can the nitrogen/phosphorus ratio be used as a reliable indicator for cyanobacterial bloom risk?

Conclusion

The dissertation of Maria Angelova Mitreva is a significant and original scientific study with clear relevance in both the scientific and applied fields. The objectives have been successfully achieved, and the results contribute to both scientific knowledge and practical applications.

The work complies with the requirements of the Law on the development of academic staff in the Republic of Bulgaria as well as the Regulations on the conditions and procedures for awarding academic degrees and holding academic positions at the National Center of Public Health and Analyses. The educational and scientific tasks of the PhD have been fulfilled successfully.

Based on the above, I confidently give a positive evaluation and recommend the scientific jury to award Maria Angelova Mitreva the educational and scientific degree "Doctor" (PhD) in the scientific specialty "Hygiene", Professional field 7.1. Medicine.

Sofia, 07 April 2026

Reviewer:
Assoc. Prof. Rossitsa Georgieva, PhD