REVIEW

on a dissertation submitted for the award of the educational and scientific degree "Doctor" in Professional Field 7.1 Medicine, Scientific Specialty "Hygiene"

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

Supervisor: Associated professor Vera Pavlova PhD

Reviewer: professor Irina Karadjova, Institute of General and Inorganic chemistry, Bulgarian Academy of Sciences

The review has been prepared on the basis of the submitted dissertation, abstract, publication activity, and participation in conferences. The submitted materials comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and its implementing regulations, as well as with the regulations of the National Center of Public Health and Analyses (NCPHA). The structure of the submitted dissertation meets the requirements of the NCPHA regulations.

Biographical data

Maria Mitreva obtained her Master's degree in the Master's program "Medical and Pharmacological Biophysical Chemistry" at the Faculty of Chemistry and Pharmacy, University St. Kliment Ohridski. In the period immediately after graduation (2014–2017), she was a full-time PhD student at the same faculty and successfully defended her PhD thesis in the professional field 4.2 Chemical Sciences, scientific specialty Analytical Chemistry.

She was appointed in 2017 as an assistant at the Analytical and Laboratory Activities Directorate of the National Center of Public Health and Analyses (NCPHA), and after successfully passing a конкурс in 2019, she became a Chief Assistant in the same directorate. She conducts research in the field of development and application of chromatographic methods for the analysis of pollutants in water, performing analyses of environmental, food, and beverage samples. Her scientific interests include methods for separation and preconcentration, as well as the determination of chemical element species in surface waters.

Evaluation of the submitted materials

The PhD thesis is presented in 139 pages (including one appendix) and contains 21 figures and 18 tables, with 153 literature sources cited. The main results of the presented research have been published in three peer-reviewed articles. The PhD candidate is the first author of all three publications. The contribution of the candidate to the conducted research, discussion, and presentation of the obtained results is undeniable. Results from the dissertation have been presented at nine international conferences and national forums.

Evaluation of the dissertation work

The dissertation of Maria Mitreva is undoubtedly relevant, aligned with the spirit of modern analytical science and with current requirements for drinking water quality. The new European framework for drinking water quality, introduced by Directive (EU) 2020/2184 and transposed into Bulgarian legislation through amendments to Ordinance No. 9, sets significantly higher requirements for the control and management of drinking water. Its main objective is to ensure a higher level of protection of human health by introducing a comprehensive, risk-based approach covering all stages of the water supply chain - from the water source to the consumer's tap.

A significant change in the new regulatory philosophy is the expansion of the scope of monitoring. While previous requirements were mainly focused on water quality within the distribution network, the new directive requires systematic monitoring and risk assessment already at the level of the water sources themselves. This includes the identification of potential contaminants, analysis of the factors influencing their presence, and the implementation of preventive measures to limit risks before they reach the end consumer.

The regulatory framework includes new indicators and contaminants reflecting current scientific knowledge regarding substances with potentially adverse effects on human health. In this context, cyanotoxins and in particular microcystins, toxic compounds produced by cyanobacteria (blue-green algae) gain special importance. One of the most significant representatives, microcystin-LR, is explicitly included as a mandatory parameter in the updated requirements of the directive.

The topic of the present dissertation is directly related to these new regulatory priorities, focusing on the study of microcystin-LR and the conditions under which it is formed. Of particular importance is the fact that, as of January 2026, the transitional period (derogation) granted to the Member States has expired, meaning that monitoring of this toxin is now a mandatory element of drinking water control.

According to the requirements of Directive (EU) 2020/2184, the analysis of microcystin-LR is not performed routinely in all samples but is applied when there are indications of

cyanobacterial blooms. This necessitates preliminary and in-depth studies, including determination of the species composition of microalgae, monitoring of their seasonal dynamics, and assessment of environmental factors (temperature, nutrients, light, etc.) that favor their development. It is precisely this integrated approach that enables timely identification of risk and effective management of drinking water quality.

In this respect, in the presented thesis Maria Mitreva demonstrates an in-depth understanding of the ecological, physiological, and toxicological aspects of cyanobacteria, as well as of the factors determining the risk of their proliferation and toxicity, which is essential for the effective management and monitoring of water resources. The PhD candidate consistently and convincingly presents the role of cyanobacteria as a key component of phytoplankton in eutrophic freshwater ecosystems, linking their ecological significance to current issues related to global climate change and anthropogenic eutrophication.

The candidate critically analyzes the complex impact of physical, chemical, and biological factors on the dynamics of cyanobacterial populations, while also highlighting species-specific adaptive mechanisms. A particularly strong impression is made by the systematic presentation of cyanotoxins, both in terms of their chemical classification and their toxic effects on the human body.

The section demonstrating the significance of the problem of cyanotoxins in drinking water sources is especially interesting, supported by diverse international examples. The reviewer highly appreciates the thorough and well-structured summary of the influence of environmental factors on cyanotoxin production. Physical, chemical, and biological parameters are considered simultaneously, with their interactions clearly demonstrated. Species-specific characteristics and the role of combined effects are emphasized, as well as the connection to current topics such as climate change.

The applicability to conditions in Bulgaria is also demonstrated, with consideration given to factors characteristic of local water bodies—eutrophication, seasonal stratification, and climatic influences. The review of analytical methods is very well structured and demonstrates in-depth knowledge of modern approaches for the determination of microcystins. Physicochemical, immunochemical, and biological methods are clearly distinguished, with their advantages and limitations presented in a balanced manner. Particularly positive is the emphasis on the need for a combined analytical approach, reflecting current trends in the field. Overall, the theoretical part under consideration is characterized by a logical structure, clarity of presentation, and skillful synthesis of a substantial volume of scientific information. It convincingly demonstrates that the PhD candidate is well acquainted with the subject matter,

possesses strong analytical thinking, and establishes a solid theoretical foundation for the subsequent research presented in the dissertation.

The presented results demonstrate a well-planned and consistently implemented study focused on key drinking water sources in the Sofia region. The stated aims and objectives are clearly formulated and fully aligned with the requirements of the current regulatory framework, and their implementation reflects a systematic and targeted approach.

Particularly worthy of positive evaluation is the development and validation of an analytical method for the determination of microcystins, which meets accreditation requirements and is applicable in routine laboratory practice. A comprehensive validation procedure is presented, including the evaluation of measurement uncertainty also at the sampling stage, which enhances the reliability of the obtained results. Participation in interlaboratory comparisons further confirms the quality of the developed method.

A significant contribution of the study lies in its practical application - a long-term monitoring of real water bodies has been conducted, providing valuable data on microcystin concentrations and their seasonal dynamics. The integrated approach is noteworthy, combining chemical analysis with monitoring of the species composition of cyanobacteria, allowing for a more comprehensive risk assessment.

It should also be positively noted that the study is not limited solely to microcystin-LR but also considers accompanying analogues, contributing to a deeper understanding of the processes involved. The presented comparison with ELISA analysis creates opportunities for optimizing future monitoring through the combination of methods.

The investigated drinking water reservoirs are characterized by a relatively stable ecological status, with low or trace concentrations of microcystins detected in most samples, indicating a low current risk to water quality. At the same time, a clear spatial and seasonal specificity in toxin occurrence has been identified, with the Studena Reservoir standing out as having a higher potential for episodic production. This does not contradict the overall positive assessment but rather demonstrates the sensitivity and analytical value of the study in detecting risk situations.

Possible correlations between elevated nutrient levels and the occurrence of microcystins as a result of bloom events are critically examined. A particularly significant contribution is the demonstration of the leading role of hydrophysical and climatic factors over nutrients in the formation of toxic events, which is consistent with current international scientific trends. The identified patterns in the seasonal dynamics of microcystins confirm the reliability and representativeness of the obtained data.

Overall, the conducted research is characterized by scientific soundness, practical relevance, and a meaningful contribution to the development of monitoring and risk assessment of cyanotoxins in Bulgaria.

Main scientific and applied contributions

The PhD candidate has carried out a substantial volume of experimental work and has demonstrated exceptional critical thinking in the interpretation of the obtained results; a very large number of samples has been analyzed. The methodological support of the study contributes significantly to its positive evaluation—the validated HPLC method and the application of ELISA analysis ensure both high sensitivity and analytical reliability, and their combined use ensure an effective approach for monitoring.

In summary, the results not only expand scientific knowledge on the distribution and dynamics of microcystins in Bulgarian drinking water reservoirs but also provide a reliable basis for optimizing monitoring programs and risk management. This defines the study as significant, relevant, and with clear practical value. The scientific contributions can be characterized as new scientific results with very good practical applicability and potential for implementation.

The thesis content is very well organized, with a concise description of the scientific problems and the scientific-applied aspects of the proposed approaches and the obtained quantitative results.

Critical remarks

I have no fundamental critical remarks regarding the research and the visualization of the obtained results. The PhD candidate has taken into account the critical comments in the preliminary submitted review and has presented a substantially new idea for assessing a possible correlation between increased nutrient levels and the occurrence of microcystins. The substantial volume of information and analytical results has been presented in a more appropriate and clearer manner.

Conclusion

In conclusion, I consider that the dissertation represents a complete scientific study on a topical subject determination of microcystins in which the research team has long term experience and is likely one of the few units possessing a long-term database on critical reservoirs. Significant scientific contributions of interest to the analytical community and laboratory practice have been achieved.

In terms of relevance, scope, and quality, the submitted dissertation and the published scientific papers fully meet the requirements of the Law on the Development of the Academic Staff in

Bulgaria and its implementing regulations at the National Center of Public Health and Analyses (NCPHA).

In addition, I believe that Maria Mitreva, whom I know personally, is an established researcher with a high professional level of scientific work, critical interpretation of results, and significant scientific achievements. I have sufficient grounds to confidently recommend that the Scientific Jury vote positively for awarding the educational and scientific degree "Doctor" to Maria Mitreva in the professional field 7.1 Medicine, scientific specialty "Hygiene."

Sofia, 7.05.2026 г. Reviewer: