НАЦИОНАЛЕН ЦЕНТЪР ПО ОБЩЕСТВЕНО ЗДРАВЕ И АНАЛИЗИ
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Opinion

on the PhD thesis for the acquisition of the ESD "Doctor"
on the topic: "Hygienic and analytical aspects in the study of the seasonal dynamics of microcystins in surface waters for drinking purposes"
of a doctoral student in independent training Maria Angelova Mitreva
with scientific supervisor Assoc. Prof. Vera Pavlova, MD

by Prof. Dr. Maya Petrova Stoyneva,
Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski"

This Opinion was prepared in my capacity as a member of the Scientific Jury in connection with the defense procedure in accordance with Order RD-146/18.03.2026 of the Director of the National Center for Public Health and Public Health, Prof. Dr. Plamen Dimitrov, MD.

The PhD thesis submitted for defense is dedicated to a systematic study of the seasonal dynamics of microcystins and basic biogenes in water bodies used for drinking water supply. Given that it is the first study for Bulgaria, the adopted Directive (EU) 2020/2184 of 16.12.2020 on the quality of water intended for human consumption and the amendment to Regulation No. 9/2001 on the quality of water intended for drinking and domestic purposes (promulgated in the State Gazette, issue 30 of 2001, last amended in the State Gazette, issue 9 of 23.01.2026), the relevance of the topic being developed is more than obvious.

The dissertation is 141 pages of text and contains 21 figures and 17 tables, as well as a bibliography containing 153 titles, of which 8 are in Cyrillic (two of them in English). It includes 9 chapters and an additional List of Publications and Participation in Scientific Forums. The abstract corresponds to the presented work and reflects all the results obtained.

This dissertation presents summarized results of studies conducted during the period 2023-2025 on the seasonal dynamics of microcystin–LR and its homologues –RR and –YR in water samples from main water sources for drinking water supply in the area of the city of Sofia and the region: Iskar Dam and Kokalyane Dam, Studena Dam and Krasava Dam. The experimental work was carried out in the laboratories of the National Center for the Protection of Biological and Biological Resources (where the doctoral candidate works) and modern equipment was used: High-performance liquid chromatograph with diode-array detector (DAD) Agilent 1200 with a Supelcosil ABZ+Plus column (150 mm × 4.6 mm, 5 µm), ELISA analysis kit consisting of: 96-well microplate reader HiPO MPP-96; thermo shaker PST-60HL; washing device for 96-well microplates Intelspeed IW-8; Thermoblock Digestor Unit DS6, type 2006, FOSS TECATOR and Specol 11 spectrophotometer from Carl Zeiss).

The original results obtained for microcystins, along with data on the composition of phytoplankton, temperature, biogenic nitrogen and phosphorus and from the correlation analysis, are presented in great detail and are grouped according to the tasks set. Due to the limited scope of the opinion, I will not list them all here, but I will emphasize that the results obtained from the study of Iskar Dam, Studena Dam and Krasava Dam show that the content of microcystins in most of the analyzed samples is low and often lies in the range of trace concentrations. Under such conditions, the choice of an appropriate analytical method is essential for the reliable detection of the presence of the toxin. Therefore, it is important that a chromatographic method for the determination of microcystins–RR, –YR and –LR in waters by HPLC has been developed and validated, with a limit of quantification of 0.2 μg/L and a limit of detection of 0.1 μg/L, a linear range from 0.2 to 1.5 μg/L, and an analytical recovery of 66.5 to 123.79%. The method meets the requirements of Directive (EU) 2020/2184 and Regulation No. 9/2001 on the quality of water intended for drinking and domestic purposes and is included in the scope of the accredited activity of the Health Center of the National Center for the Prevention and Control of Water Pollution. It has been shown that the results for microcystin content obtained by ELISA analysis are comparable to the concentrations obtained by HPLC analysis. The results obtained confirm the need for dynamic seasonal monitoring of drinking water reservoirs, especially during periods of increased temperatures and hydrophysical changes, in accordance with the requirements of European legislation. It is recommended that monitoring be carried out with increased frequency during the warm months and the beginning of the autumn season, when the risk of cyanobacterial events is highest.

The dissertation does not cite the only complete inventory of the wetlands of Bulgaria, including the drinking water dams, and there are individual errors in biological terms (e.g. "small fungi" instead of the scientific term "fungi", especially when it comes to symbioses with algae, "oxygenic photosynthesis" instead of "oxygen-emitting photosynthesis", "destruction of stratification", etc.), but they do not diminish the value of the presented dissertation.

According to the scientific articles presented and participation in scientific forums, Maria Mitreva is a promising young colleague, working with a desire to implement and apply new methods. This is evidenced by the scientometric data presented with three articles and her participation with five posters (one awarded with first prize) and four reports in nine scientific forums (two of which were international and one with international participation).

In conclusion, I would like to note that the presented dissertation work together with the credits received, the publications and the participations of the dissertation candidate fully meet the criteria of the Regulations on the terms and procedure for acquiring scientific degrees and occupying academic positions in the National Center for Health Protection and the Law for Obtaining of Scientific and Academic Degrees of the Republic of Bulgaria. Therefore, I believe that the PhD thesis presented to me for Opinion should be admitted to defense and I recommend that the members of the esteemed Scientific Jury evaluate it on its merits by awarding Maria Angelova Mitreva the educational and scientific degree of Doctor in the Scientific specialty "Hygiene".

4.05.2026 г.

Проф. дбн Майя П. Стойнева