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# REVIEW

**From:** Prof. Dr. Nadja Georgieva Ognjanova-Rumenova
Geological Institute at the BAS, member of the Scientific Jury, appointed by Order No. 26/05.03.2024. of the Director of the Institute for Biodiversity and Ecosystem Research at the BAS, Sofia

**Subject:** the submitted documents for the defense of a dissertation thesis for obtaining an educational and scientific degree "Doctor" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, Doctoral program "Ecology and protection of ecosystems", Research topic "Hydrobiology"

**Author:** Tsvetelina Yasenova Isheva

**Title:** Study on benthic diatom assemblages in intermittent rivers in Southern Bulgaria and their application for assessment of ecological status

**Scientific supervisor:** Prof. Yordan Ivanov Uzunov, PhD
**Scientific consultant:** Assistant professor Plamen Nikolaev Ivanov, PhD

## 1. General characteristics of the dissertation work

The set of materials presented by Tsvetelina Yasenova Isheva in electronic form is in accordance with the Regulations for the conditions and procedure for acquiring scientific degrees and for holding academic positions at the Institute of Biodiversity and Ecosystem Research at the BAS and includes the following documents: *Dissertation;*Abstract;*Reference on the contributions of the dissertation work;*Lists of dissertation publications; citations and participation in scientific conferences;*Curriculum vitae;*Diploma for obtaining a master's degree;* Minutes of the extended seminar of the section “Water Ecosystems Department”, directed the dissertation for defense;*Information on the study process and credits from the Scientific Secretary;*Summary of the dissertation in English;*Copies of dissertation publications.

The dissertation has a volume of 152 pages and is structured according to the rules for preparing a dissertation in 9 chapters, as follows: Introduction (2 pages), Research goals and objectives (1 page), Literature review (24 pages), Typology and characteristics of the studied rivers (17 pages), Material (2 pages), Methods (13 pages), Results and discussion (46 pages), Summarized results and conclusions (4 pages), Contributions (1 page), Bibliography (30 pages). The bibliography includes 310 titles, of which 15 in Cyrillic, 284 in Latin, 6 web pages, and 11 official documents. The dissertation contains 19 tables and 31 figures, as well as photographic material, united in the main text. The work is well balanced, with the chapter "Results and Discussion" being the most voluminous. In the dissertation there is also an additional Appendix, including 7 separate parts. The first of these contains 12 plates with photographs taken under a light microscope of certain species of diatoms. The good technical layout of the dissertation and above all the high-quality figures and tables that illustrate and support the interpretation of the results are impressive. Two lists of used abbreviations and terms have also been compiled. The goal is clearly and precisely set - "establishment of the taxonomic diversity, distribution, dominant structure, seasonal dynamics and ecology of diatom communities from Sub-Mediterranean intermittent rivers, distributed in southern Bulgaria (Ecoregion 07) and assessment of their ecological status". The tasks for its realization are concretely and correctly presented and justified. The main working hypothesis was formulated: "Whether the composition, structure and ecological spectra of the diatom communities during the different hydrological phases will differ significantly and the obtained results will confirm the need to update the national classification methodology and the ecological status assessment scale according to the BQE "phytobenthos". This hypothesis is a good basis for solving the tasks.

**Brief biographical data of the PhD student.** Tsvetelina Isheva has a master's degree in "Algology and Mycology" at the Faculty of Biology, Sofia University "St. Kliment Ohridski" (2011). She carried out two consecutive specializations at Ghent University, Belgium - in algae cultivation and at Durham University, Great Britain - in freshwater algae identification. She works as a biologist in the Department of Aquatic Ecosystems, Institute of Biodiversity and Ecosystem Research at the BAS. He has participated in 14 research and applied projects, financed by various Bulgarian and international organizations. Tsvetelina Isheva is a full-time doctoral student.

**2. Literary awareness and theoretical preparation of the candidate**

The author is well acquainted with research worldwide. This is confirmed by the attached bibliography, creatively used in the research and analysis carried out in the dissertation. The literature review is structured in five subsections. These several up-to-date directions are presented in depth and informatively *brief description of the studied organisms, *studies of rivers with temporary discharge, *diatom communities from rivers with a non-permanent water regime, *studies of diatom communities from rivers in Bulgaria, *application of diatom communities in the biomonitoring of rivers and * Water Framework Directive 2000/60/EU and intercalibration of state assessment methods.

The relevance of the dissertation topic is confirmed by the fact that in the near future the number of the intermittent rivers will rise dramatically in regions where pronounced climate changes are occurring. In addition to the natural cyclicity of drying, in areas with drought trends, water use and abstraction will increase, leading to an accelerated increase in the number, extent, frequency and duration of river drying. In this detailed literature review, there is a gap in the distribution, diversity and ecology of diatoms from drying rivers on a larger geographical scale, the role of recent studies is of utmost importance and provides a good basis for comparison with further studies.

The main focus is the creation of a classification system, as well as the development and updating of a methodology for assessing the ecological state and the most relevant period for collecting samples for BQE (biological quality element) "phytobenthos", in order to be adopted in Bulgarian and European legislation.

**3. Material and methodical approach**

I would also refer to the provided dissertation material the separate heading "Typology and characteristics of the studied rivers". The national river typology classifies small (with a catchment area of 10–100 $km^2$) and medium (100–1200 $km^2$) rivers and streams with a highly variable water regime, located in the south-eastern and south-western parts of Bulgaria (the basins of the Struma, Arda, Maritsa and Tundzha rivers), as Sub-Mediterranean small and medium-sized temporarily drying rivers (national river type R14), introduced into the Bulgarian legislation by Regulation No. H-4/14.09.2013. The distribution area of the type covers the Aegean

Water-Brine Basin (EBB), Ecoregion 07 (Eastern Balkans), based on the already published biogeographical zonation. The specific study is based on a wealth of factual material. 89 epilithic diatom samples were collected from 49 river points in the period 2012–2015. Field sampling was carried out during two hydrological periods: 1) during normal water levels (lotic/spring) and 2) low water levels (lentic/summer), which is a good basis for conducting scientific research.

The methodological approach in the development of the dissertation work is characterized by three strengths: (i) the focus on the well-formulated working hypothesis; (ii) the adequately selected study area; (iii) the modern methodology of analysis and verification of the hypothesis based on rich factual material;

The doctoral student has excellent theoretical and methodological training.

**4. Significance and conviction of the obtained results, interpretations and conclusions**

The obtained results of the field and ecological studies are bound in defense of the formulated working hypothesis. Diatom communities differ significantly during the different phases of water runoff and are composed of specific indicator species defining the individual classes of ecological status. The results are discussed in detail and at a high professional level in the text of the dissertation. They are organized in seven sections corresponding to the set goals and objectives. I believe that the doctoral student Tsvetelina Isheva is a well-established research scientist in the field of diatomology, hydrobiology and ecology.

The species richness of the diatom flora is very diverse (287 species and varieties), and the benthic diatom communities of the spring hydrological period are more diverse than those of the summer.

Physiological, morphological and ecological features of diatom communities are excellent indicators reflecting variability and heterogeneity in environmental conditions and can be successfully applied in primary studies of diverse habitats (aquatic, humid, terrestrial) in different biogeographical regions of the world, especially poorly studied such, as well as in the preparation of an indicative assessment of their ecological condition.

Of particular importance is the practical orientation of the research related to the creation of the classification system and assessment of the ecological status of the

national river type R14 according to the BQE "phytobenthos", in order to be adopted in the Bulgarian and European legislation.

**5. Critical notes to the dissertation work**

The presented dissertation work is developed precisely, the dissertation student has excellent methodical and theoretical training. My critical remarks may serve as a reference in her future work.

- In section 6. "Methods" - 6.2. Laboratory treatment - BDS 13946:2014 is cited as the research method, it is good to cite the original source of this method, namely: Hasle, G., Fryxell, G. 1970. Diatoms: Cleaning and mounting for light and electron microscopy. Transactions of the American Microscopic Society 89 (4), 469-474. This is also the scanning electron microscopy methodology used in the dissertation.
- The revision of the taxonomic list is well done, but the new taxonomic combinations used do not cite recent author studies. Most definitions are by authors who published before 2015. For example in the centric genera: *Cyclotella ocellata* Pantocsek already belongs to another genus (*Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & E. Ács 2016).
- Taxonomic descriptions should follow the terminology of Temniskova and Stoyneva (2011). Many foreigners words were used in the text.
- How are new species for the Bulgarian diatom flora defined? I am not aware of such a register being completed in at least the last 20 years.
- I have some doubts about the presented reason for the spread of *Sellaphora madida* (Kociolek) Wetzel, namely "global warming". On the occasion of the specific and rare distribution of diatom species, as well as on the question of the origin and colonization paths of the species, the most decisive, in my opinion, is "human activity" and the globalization of society.
- In the ecological analyses, I would not agree that *Cyclotella ocellata* Pantocsek is a characteristic species for marshes. It is very often and abundant found in artificial reservoirs, lakes and rivers. The species was first described in Lake Balaton (Pantocsek, 1902). Also *Aulacoseira granulata* (Ehr.)Simonsen has been referred to as a species abundant in large lake, but recently there have been many new published records of the species from river spills in China and India.

- In some of the figures used, the concepts are in English, for example: page 18 (fig. 4), page 33 (fig. 6), etc.

**6. Nature of scientific contributions**

As a result of the study, summaries and conclusions drawn, the doctoral student formulates five main contributions, presented in three groups: *Contributions of a floristic nature, **Contributions of a scientific applied nature and ***Contributions of a confirmatory nature.

I accept the reference of scientific contributions in the dissertation and consider them to be formulations of original and significant scientific achievements. I attach the greatest importance to one of the contributions of a scientific and applied nature, namely that the national river type R-14 has been aligned with the European types R-M1 and R-M2 of the Mediterranean Geographical Intercalibration Group, and that the classification system created and methodology has been adopted and promulgated - ORDER No. H-4/2013 (last amended and supplemented SG No. 85 of 02.10.2020 and No. 67 of 04.08.2023), as well as in European legislation.

**7. Abstract**

The submitted abstract fully and correctly reflects the essence of the dissertation and it can be stated that it fully meets the requirements of the Law. The abstract includes 38 pages with attached basic graphic material, the formulated contributions and publications on the topic correspond to those listed in the dissertation.

**8. Evaluation of the quality of scientific works reflecting dissertation research**

On the topic of the dissertation, two full-text publications written in English are attached, which fully cover the scientometric criteria for awarding the educational and scientific degree "Doctor". In both publications, Tsvetelina Isheva is the lead author. One is printed in Botanica Serbica (SJR Q3) and the second in Comptes Rendus de l'Academie Bulgare des Sciences (IF2020/0.329. SJR Q2). The presented articles are of a high scientific level, the conclusions are clear and well synthesized. Part of the results of the dissertation were included in poster reports at two international and two national scientific forums. A list of citations to the publications is also attached - 10 in number.

**9. Personal contribution of the doctoral student**

The significant personal involvement of Tsvetelina Isheva in the preparation of the dissertation work is undeniable. She is the first author on the papers on the dissertation topic. The presented dissertation work, as well as the formulation of the conclusions and contributions, are her personal merit. The scientific guidance of her scientific supervisor - Prof. Dr. Yordan Uzunov, as well as her scientific consultant - Assistant Professor Dr. Plamen Ivanov, which is a guarantee for the quality of the research.

My personal impressions of the doctoral student have been excellent since her student period, when she was finishing her Master's thesis related to the study of the diatom flora of the Vitosha mountain. We were also together at the 6th Central European Diatom Meeting (CE-DIATOM), 2012 in Innsbruck, Austria. I am happy that she took part in the training course organized by IBER, Reykjavík University, Eastern and Southern Network for Invasive Alien Species and Danube Delta for Invasive Alien Species on the topic: "Impact of invasive alien species on biodiversity and ecosystem services in environments with extreme conditions" in Sofia in 2017. I was a scientific consultant for her project DFNP No. 17-145/01.08.2017 "Investigation of the taxonomic diversity, ecology and distribution of benthic diatom communities in small and medium-sized Black Sea rivers (river type R11). Assessment of the ecological condition of rivers of this type" under the Program to support young scientists and doctoral students of the BAS (2017-2018). During our joint work, we have had the opportunity to communicate, exchange information and introduce her to colleagues working in the field of diatomology.

**Conclusion:**

The dissertation and the presented materials contain scientific and scientific-applied results which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of the ZRASRB and the specific requirements of the Terms and Conditions and the procedure for acquiring scientific degrees and holding academic positions at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, Sofia. The dissertation shows that the doctoral student Tsvetelina Yasenova Isheva possesses in-depth

theoretical knowledge and professional skills in the scientific specialty "Hydrobiology", demonstrating qualities and skills for independent scientific research.

Due to the above, I confidently give my positive assessment of the study presented by the above peer-reviewed dissertation, abstract, achieved results and contributions, **and I invite the esteemed scientific jury to award the educational and scientific degree "Doctor" to Tsvetelina Yasenova Isheva in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, Doctoral Program "Ecology and Ecosystem Protection ".**

**Reviewer:**

29.05.2024 г.
гр. София

(Prof. Dr. Nadja Ognjanova-Rumenova)