

REVIEW

by Professor Dimcho Zahariev Ivanov, PhD

in connection with a procedure for the award of the scientific degree "Doctor of Science" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences (Ecology and Environmental Protection) for the needs of the Department of Biology at the Faculty of Natural Sciences of the Bishop Konstantin Preslavski University of Shumen

1. Information about the procedure

I participate in the scientific jury of the procedure according to Order No. RD-16-097/15.07.2024 of the Rector of the Bishop Konstantin Preslavski University of Shumen. I was selected as a reviewer by decision of the Scientific Jury with Protocol No.1/23.07.2024. I have been selected as a reviewer by decision of the Scientific Jury for the public defense of a dissertation on the topic: "Assessment of the ecological risk in the Bulgarian Black Sea water area through the study of microbial biodiversity in mussels and key fish species" with author Prof. Tsvetelava Veselinova Ignatova-Ivanova, PhD with Protocol No. 1/23.07.2024.

2. Information about the presented materials

The set of materials in connection with the procedure presented by Prof. Tsvetelava Veselinova Ignatova-Ivanova, PhD is in accordance with Art. 37, Para 1 of the Regulations for the Development of Academic Staff at Shumen University and contains: 1. Application to the Rector of the University of Shumen; 2. Autobiography; 3. Diploma for acquired educational and scientific degree "Doctor"; 4. Reference for the contributing moments; 5. List of publications on the topic of the dissertation work, together with the publications themselves; 6. Certificate of fulfillment of the national minimum requirements according to the Art. 26, Para 2 and Para 3 of the Law on the Development of the Academic Staff of the Republic of Bulgaria; 7. Declaration of authorship; 8. Protocol of the meeting of the council of the primary unit for preliminary discussion of the dissertation work; 9. Protocol of the check carried out by the anti-plagiarism system; 10. Abstract in Bulgarian and in English; 11. Dissertation work for the award of the scientific degree "Doctor of Sciences".

3. Fulfillment of the requirements for the award of the scientific degree "Doctor of Sciences"

The minimum national requirements for the award of the scientific degree "Doctor of Sciences", according to the Art. 26, Para 2 and Para 3 of the Law for

the Development of the Academic Staff in the Republic of Bulgaria, are fulfilled.

By group of indicators "A" Prof. Tsveteslava Ignatova-Ivanova, PhD has successfully defended a dissertation for the award of educational and scientific degree "Doctor", which is certified by a copy of the diploma issued by the Higher Attestation Commission in 2009. This ensures the required 50 points in this group.

By group of indicators "Б" a dissertation is presented for the award of the scientific degree "Doctor of Sciences", the successful defense of which ensures the obtaining of the required 100 points in this group.

By group of indicators "Г", 11 publications that are referenced and indexed in world-renowned databases of scientific information (Web of Science and Scopus), are presented. Of them, in the report on the implementation of the national minimum requirements in accordance with Art. 2b, Para 2 and Para 3 of the Law on the Development of the Academic Staff of the Republic of Bulgaria the following data are indicated: 4 publications are in scientific journals with quartile Q2, 4 publications are in scientific journals with quartile Q3 and 3 publications are in scientific journals with quartile Q4. Inspection of journal indexing shows the following results: 7 publications are in scientific journals with quartile Q3 and 4 publications are in scientific journals with quartile Q4. The reason for the difference found is the indexing of the BioRisk scientific journal in Scopus in quartile Q4 instead of Q2 in 2021 (1 article was published in the journal this year) and in quartile Q3 instead of Q2 in 2022 (3 articles were published in the journal in this year).

The distribution of the journals selected for publication is as follows:

Acta Zoologica Bulgarica – 2 articles

BioRisk – 6 articles

Ecologia Balkanica – 1 article

Journal of IMAB – 1 article

Pharmacia – 1 article

Various journals have been selected for the publication of the scientific results, which is appreciated as an advantage. Prof. Tsveteslava Ignatova-Ivanova, PhD is first author in 1 publication, second author in 2 publications, third author in 1 publication, fourth author in 1 publication, and last author in 6 publications. This distribution is very balanced and shows a built-in ability to work in a team.

With the required 100 points, Prof. Tsveteslava Ignatova-Ivanova, PhD has 153 points in this group.

By group of indicators "Д" a list with a total of 68 citations in journal articles, which are referenced and indexed in world-renowned scientific information databases (Web of Science and Scopus), is attached. This warrants 136 points, which exceeds the 100 point requirement in this group.

4. Relevance of the topic of the dissertation

The topic of the dissertation is integral and therefore relevant in several directions: seawater pollution, pollution control by using appropriate bioindicators, food safety, application of lactic acid bacteria or biologically active substances contained in them for the control of pathogenic microorganisms in seafood as a food source.

Seawater pollution is one of the global environmental problems. Many agents of contamination are known, including microorganisms. One of the main groups of pollutants are heavy metals. In recent decades, attention has also been paid to microplastic pollution. Reliable bioindicators are needed to track these three types of pollution. Given the small sizes of these pollutants, it is necessary to find suitable species in which they accumulate and which are suitable to be used as indicators. The black mussel (*Mytilus galloprovincialis* Lamarck, 1819), used as an object of study in this dissertation, is one of the few species that is accepted as a reliable indicator of the state of the marine environment. Other types of shells, as well as fish, have been used as objects.

The topic of the safety of the food that people use and the need to introduce stricter controls is particularly relevant: e.g. in the case of mussels, which must necessarily pass through microbiological and sanitary control. The use of lactic acid bacteria or their biologically active substances to control pathogenic microorganisms in seafood is a possible alternative to the use of antibiotics.

Proof of the topicality of the topic is the large number of citations (21 in number) of the results of research on the subject of the dissertation, published in scientific journals.

5. Characteristics of the dissertation

The dissertation contains 145 pages (not including the appendices) and is illustrated with 36 tables, 57 figures and 7 appendices. The text is structured in the following sections: List of used abbreviations (1 page), Introduction (2 pages), Literature Review (7 subchapters, 6 paragraphs and 23 pages), Aims and Tasks (1 page), Materials and Methods (10 subchapters and 5 pages), Results and Discussion (3 subchapters, 11 paragraphs and 77 pages), Summary (2 pages), Contributions (1 page), Conclusions (4 pages), Publications on the topic (2 pages), Participation in conferences (4 pages), References (19 pages), Appendices (44 pages).

The idea of active content is very good and makes accessing the sections and paragraphs in the dissertation much easier.

The list of abbreviations used in the text is also very useful, as it makes it easier for the author to present the information in the dissertation, and then helps to understand it.

In the introduction, current problems related to marine aquaculture, seawater pollution, the study of seafood and fish as a source of various biologically active substances and the lack of data on the diversity of

microorganisms in shellfish and fish in the Bulgarian Black Sea water area are highlighted. The three national projects of the Scientific Research Fund of the Ministry of Education and Science of the Republic of Bulgaria, on which the results in the dissertation were obtained, are presented.

The Literature Review presents the information known to date, which is necessary to justify the need to conduct the research in the dissertation work. More than half of the volume of this section is devoted to biologically active substances that are obtained from shellfish and fish, which is beyond the topic and purpose of the dissertation work.

In order to be precise about the purpose of the dissertation, a small correction is needed in the final part, where instead of "their biological status" it should be "their microbiological status". Since there is only one stated objective, it is correct to name the Aim and Tasks section. 8 tasks are marked. To achieve the aim, it is correct to include in tasks 3 and 5 not only the habitats of the black mussel (*Mytilus galloprovincialis*), but also at least the habitats of the white mussel, since they do not overlap. Including fish habitats would be really hard to do. Some of the tasks (e.g. tasks 2, 6, and 7) address problems highlighted in the Literature Review but are outside the scope of the stated aim. A possible solution is to formulate additional aims so that they are tied to the topic of the dissertation work.

Appropriate objects and methods for their research have been selected. A variety of methods have been applied that allow the realization of each of the set tasks.

The results obtained during the research are presented consistently and are well illustrated. The studied indicators are mainly presented graphically, and where necessary also in tabular form. Various graphic techniques have been applied to visualize the information, with the aim of avoiding uniformity, which I appreciate as a good approach. The results are compared with the data of other authors. In some of the paragraphs to this section (I.4, II.1, and III.1) a non-standard approach is applied: a brief literature review, a brief presentation of the material and methods used, presentation and analysis of the results. As a rule, each of these components should be included in the corresponding section, and here only the last part remains - presentation and analysis of the research results.

In the Summary section, it is stated that the results give a general assessment of the state of the Bulgarian water area of the Black Sea. This claim requires a much wider range of data than has been obtained. The statement that the microplastics isolated and determined in the course of the study "contain various poisonous impurities such as bisphenol A and DDT" is incorrect, because the content of these substances was not studied during the experimental work, but in the text of the Results and Discussion section on page 112 it only mentions the ability of microplastics to "absorb and release toxic chemicals". More care is needed in the use of the terms 'biodiversity' and 'ecosystem service' as they are used in a context other than their meaning.

As a result of the conducted research, 27 conclusions were formulated. A large part of them, however, present results rather than conclusions, which is also evident from the wording itself, which begins with the expressions: "It is shown", "It is established", "It is proven". Impressive is the conclusion for mussels that *Donax trunculus* does not have a permanent intrinsic microbiota, but only a transient one, and that a different type of Pseudomonas bacteria was found in each of the studied fish species.

A bibliographic description of the 13 published articles on the subject is presented in a separate section. A very good impression is made by the fact that all articles are in scientific journals indexed in Web of Science and Scopus.

The results of the research on the topic of the dissertation are presented with 33 participations in scientific conferences by the members of the team that carried out the research.

The list of cited literature includes 274 titles that are only in Latin. The number of literary sources is fully adequate for a serious and at the same time balanced study. The literature used is modern (the number of literary sources published before 2000 is only 30 in number), which is a mandatory condition given the topic of the dissertation work.

The dissertation includes five appendices. It is correct that they should also be indicated in the content of the dissertation.

No plagiarism has been found in the dissertation in the sense of the Law on the Development of the Academic Staff of the Republic of Bulgaria.

6. Contributions of the dissertation

The author indicates 6 scientific contributions. Four of them have an original character for the Bulgarian water area of the Black Sea, which proves the original approach in choosing a topic for conducting scientific research. The last two need reformulation to be able to play the role of contributions.

7. Evaluation of the abstract of the dissertation

The dissertation abstract is structured according to the accepted requirements and fully reflects the main points in the dissertation. The text has 92 pages and is illustrated with 6 tables and 25 colored figures.

8. Critical remarks and recommendations

I recommend Prof. Tsveteslava Ignatova-Ivanova, PhD to pay more attention to details: in the dissertation, in the abstract and in the materials for participation in the procedure. For each of these groups, I give one example: In the "Materials and Methods" section of the dissertation, only the methods used and the places of sample collection are included. It is proper to indicate the specific objects with their Latin names, which is done only in the text of the "Results and Discussion" section. At first mention, Latin names should be recorded with the author's name and the year. In the abstract of the dissertation, the numbering of the tables and figures is independent and does not repeat that

of the dissertation, which was considered by the author, but in several places in the text the numbers of figures and tables from the dissertation remain. The arrangement of publications in the Certificate of fulfillment of the national minimum requirements in accordance with Art. 2b, Para 2 and Para 3 of the Law on the Development of the Academic Staff of the Republic of Bulgaria and in the Appendix with the publications themselves is not in the same order and this makes it difficult to discover them: in the first case there is no specific dependence on inclusion in the list, and in the second case the publications are arranged in chronological order. On the other hand, 13 scientific publications are included in the Appendix, while only 11 scientific publications are included in the Certificate of fulfillment of the national minimum requirements.

9. Conclusion

The dissertation work of Prof. Tsvetoslava Ignatova-Ivanova, PhD, the submitted materials under the announced procedure and the author's abstract meet the criteria for awarding the scientific degree "Doctor of Sciences" of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation, and the Regulations for the development of the academic staff at Bishop Konstantin Preslavsky University of Shumen. Therefore, I give a positive assessment and recommend to the members of the respected Scientific Jury to award Prof. Tsvetoslava Veselinova Ignatova-Ivanova, PhD the scientific degree "Doctor of Sciences" in the field of higher education 4. Natural sciences, Mathematics and Informatics, professional direction 4.3. Biological Sciences (Ecology and Environmental Protection).

September 3, 2024
Shumen


Signature:
(Prof. Dimcho Zahariev, PhD)