

RECENSION

By Prof. Mima Ivanova Nikolova, PhD, DBSc

On a dissertation submitted for the acquisition of the educational and scientific degree "PhD"

Field 4. Natural sciences, mathematics and informatics

Professional direction 4.3. Biological Sciences

Doctoral program Morphology

Author Eleonora Tencheva Kovacheva

Topic „Assessment of pollution of aquatic ecosystems with priority organic pollutants using morphofunctional biomarkers in *Cyprinus carpio* (Linnaeus 1785)”

Supervisor Prof. Elenka Stoilova Georgieva, PhD

1. General description of the presented materials

By order No. RD-21-519 dated 28.02.2024 of the Rector of Plovdiv University "Paisiy Hilendarski", I have been appointed as a member of the scientific jury for ensuring a procedure for the defense of a dissertation on the topic „Assessment of pollution of aquatic ecosystems with priority organic pollutants using morphofunctional biomarkers in *Cyprinus carpio* (Linnaeus 1785)” for the acquisition of the educational and scientific degree "PhD" in

Field 4. Natural sciences, mathematics and informatics

Professional direction 4.3. Biological Sciences

Doctoral program Morphology

Author Eleonora Tencheva Kovacheva, full-time PhD student at the Department of Developmental Biology with supervisor Prof. Elenka Stoilova Georgieva, PhD, from PU “P. Hilendarski”

The set of paper materials presented by the PhD student is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of Plovdiv University. All references are signed and stamped and contain reliable

information. Four scientific publications on the topic of the dissertation are attached.

2. Brief biographical data about the PhD student

It is evident from the professional resume that in 2018 the PhD student received a bachelor's degree at the Faculty of Biology of the University of Plovdiv, and in 2019 she received a master's degree in "Reproductive Biology" at the Faculty of Biology of the University of Plovdiv.

From 2020 to 2023, she is a PhD student at the Department of Developmental Biology, Faculty of Biology. In 2018 and 2021 she was a biologist in specialized medical laboratories, and in 2022 she was a manager at the Vela Clinic medical center. From January 2023 until now, she is an assistant professor at the Department of Medical Biology of the Medical University- Plovdiv, where she conducts practical classes in medical parasitology, molecular and cellular biology and immunology for first-year students of the Medicine, Dentistry and Pharmacy specialties. She conducted exercises in embryology and histology with students from the "Medical Biology" specialty at the "Developmental Biology" department, Faculty of Biology, University of Plovdiv.

Her interests are in the field of medical parasitology, molecular and cell biology. She participated in three scientific research projects financed by the Scientific Research Institute at PU "P. Hilendarski"

3. Relevance of the topic and appropriateness of the set goals and tasks

Along with the undeniable benefit of pesticides in agriculture, there is clear evidence that their widespread use causes irreversible damage to ecosystems, their inhabitants, including humans.

The impact of pesticides on organisms and the environment is an important medicobiological issue. Pesticides cause biodiversity loss and ecosystem damage. There are very few studies that are aimed at establishing the morphological and functional state of individual organs, systems and the organism as a whole as a

result of their living in aquatic ecosystems burdened with pesticides and their deposits in the relevant tissues.

I believe that the PhD student has very precisely chosen the direction of the proposed study, seeking an answer to the questions: What are the actual patterns of action of pesticides? What is their fate in the aquatic environment? What morphological and biochemical changes occur at the cellular and tissue level both in individual organs and in the whole organism under the influence of a certain pollutant?

These are the questions that guide the PhD student's research efforts and determine the relevance and significance of the proposed research in both scientific and scientific-applied terms.

4. Knowledge of the subject

An in-depth analysis of the research on the questions was made. The literature review references 519 sources – 10 in Cyrillic and 509 in Latin, mostly from the last decade. It is structured in 7 sections, considering in sequence: characterization and classification of pesticides; their impact on the environment, humans, aquatic ecosystems and fish organisms, as well as research in Bulgaria on these issues.

My overall assessment is that the PhD student shows very good knowledge of the subject and competence in analyzing the issues under consideration. The large number of literature sources gives her the opportunity to bring out the main questions for which she did not find an unequivocal answer in the specialized literature - sufficiently detailed information on clarifying the cause-and-effect relationship between the risk to the health of aquatic organisms and the concentration of the toxicant, as well as reliable biomarkers in determining the degree and type of pollution of aquatic ecosystems.

Thus, consistently and purposefully, she substantiates the need and significance of a complex study and shapes the purpose of her dissertation development. The 7 specific tasks set for its achievement convincingly

demonstrate the creative approach of the dissertation student to solving the scientific problem.

5. Research methodology

The methodological part of the study is described in detail and precisely. The experimental individuals of common carp, the tissues and organs subjected to analysis, as well as the experimental three types of active ingredients of PPPs - the insecticide pirimifos-methyl, the fungicide propamocarb hydrochloride and the herbicide 2,4-D - were chosen for reasons. For the experiment, two concentrations were used for each pesticide and 10 individuals for each experimental concentration and for the control group.

The complex study includes the following methodical approaches:

1. Histopathological analysis of common carp gill, liver and kidney to determine pathological cellular changes in the organ and their comparison with normal morphology. The changes are classified into three groups for the gills - circulatory, degenerative, and proliferative, and for the liver and kidney into four groups, adding the inflammatory changes. The 5-point scale of Saraiva et al was used to determine the severity of each alteration. Histopathological change indices for each group and organ pathological alteration index were calculated.
2. Histochemical analysis for the detection of lipids and polysaccharides in the liver of common carp by modifying the used semi-quantitative scale for reporting changes.
3. Biochemical analysis to determine the activity of 5 liver enzymes.

The data were processed statistically and the average values for all subjects of the experimental concentrations were obtained, and the differences between the concentrations and the control were tested for significance by Student's t test.

The entire methodical part convinces that the author aptly complements and motivatedly offers appropriate methodical and statistical approaches for solving the set tasks.

6. Characterization and evaluation of the dissertation work and the contributions of the development to science and practice.

The presented work is the product of extensive research work. The first thing that impresses is the neat logic of the dissertation structure, arranged in the generally accepted pattern. In 9 comprehensive parts - introduction, literature review, aim and tasks, material and methods, results and discussion, conclusion and conclusions, contributions, publications and literature, the dissertation presents the path and results of her scientific research. The data are presented in 20 tables and illustrated in 34 figures.

The "Results" chapter represents the most essential and largest part of the dissertation.

A neat system of analysis was built, which includes a complete and detailed characterization of the normal histological structure of the organs, the histological structure of the organs of the experimental individuals and the degree of manifestation of the histopathological, histological and biochemical changes in the organs of common carp after treatment with the pesticides used. I find the results of the comparative analysis of the toxic effects of the used pesticides on the histological structure of the organs to be valuable both theoretically and practically. This gives the doctoral student the opportunity to discuss the relationship between the appearance of the individual categories of changes in the organs and the degree of toxicity of the applied pesticide concentrations.

Thus, the following important conclusions are proven: 1. All three applied pesticides negatively affect the histological structure of the gills, liver and kidney of common carp. Along with this, the compensatory-adaptive mechanisms in the body are activated, which affects the functionality of the carp's organs and leads to a deterioration of the health of the entire organism. 2. The degree of appearance of the morphological changes is directly proportional to the increasing concentration of the applied pesticides. 3. All tested concentrations of the pesticides caused a change in the carbohydrate and

lithide profile of the bioindicator species and inhibition of the specific enzyme activity in the liver.

The whole study has scientific, applied and methodical contributions. The following points can be highlighted as particularly significant:

1. For the first time, a complex morphophysiological study was conducted to clarify the degree of toxicity of three active ingredients of plant protection preparations on the organs of common carp.
2. Additional new data on changes in the macro and microstructure of metabolically active organs of common carp under the influence of pesticides are presented. Such scientific search is important not only in a purely scientific aspect for fundamental studies, but it is also important for the medico-biological health status of organisms and especially relevant for humans.
3. The scale used to determine the degree of changes in the histological structure of the gill, liver and kidney of common carp has a methodical nature.
4. The combination of the proposed biomarkers are reliable tools for evaluating the effect of various toxic substances on bioindicator species and can be useful in the preparation of an adequate regulatory framework regarding the presence of organic pollutants in aquatic ecosystems.

In the "Conclusions" chapter, 4 main conclusions are presented in a convincing manner and with categorical evidence. They are an illustration of the scientific and scientific-applied contributions of the dissertation, which do not arouse doubt.

It completely coincides with my opinion and the competently made self-assessment of the contributions of Eleonora Kovacheva's dissertation work.

7. Assessment of dissertation publications

In connection with the topic of the dissertation, the author has presented a list of 4 scientific publications and 8 reports at national conferences and international seminars. One of the articles was published in a journal with an impact factor (Q1) and three were published in refereed journals (Q4), indicating fulfillment of the

specific requirements of the law. In two of the publications, the doctoral student is the first author.

The dissertation research was carried out independently by the author, appropriate and modern methodology was used in its development, creativity was shown in the analysis and solving of the tasks.

8. The abstract corresponds to the structure and content of the dissertation.

9. Critical remarks and recommendations

Based on the fact that the present study was conducted in laboratory conditions, I believe that the results for the chronic impact of toxicants on aquatic ecosystems in real conditions, where there are complex processes of synergism and antagonism, will be interesting.

I recommend the doctoral student to direct her future efforts in this direction.

CONCLUSION

The dissertation work of Eleonora Kovacheva contains **scientific and scientific-applied results**, which have extremely great medical-biological significance. The work meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations of PU "Paisiy Hilendarski". The presented materials and dissertation results fully correspond to the specific requirements of the Faculty of Biology, adopted in connection with the Regulations of the PU for the application of the LDASRB.

The dissertation shows that the doctoral student Eleonora Tencheva Kovacheva possesses **in-depth theoretical knowledge and professional skills** in the scientific specialty "Morphology", demonstrating qualities and skills for independent conduct of scientific research.

Due to the abovementioned, I confidently give my **positive assessment** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and **I propose to the honorable**

scientific jury to award the educational and scientific degree "doctor" to Eleonora Tencheva Kovacheva in Morphology doctoral program.

22.03.2024

Plovdiv

Reviewer:

Prof. dbs M. Nikolova