REVIEW

by Dr. Atanas Krastev Bochukov – Associate Professor, Pensioner, former lecturer at the University of Plovdiv and the Thracian University – Stara Zagora

of a dissertation on the award of the educational and scientific degree "Doctor"

in the field of higher education: 4. Natural sciences, mathematics and informatics; Profesio-nal direction: 4.3. Biological sciences; Doctoral Program: Morphology

Author: Eleonora Tencheva Kovacheva

Topic: Assessment of aquatic ecosystem pollution with priority organic contaminants using morphofunctional biomarkers in Cyprinus carpio (Linnaeus 1785)

Scientific supervisor: Prof. Dr. Elenka Stoilova Georgieva – Faculty of Biology of Plovdiv University – Plovdiv

1. General description of the materials submitted

By Order No PD -21-519 of 28.02.2024 of the Rector of Plovdiv University "Paisii Hilendarski" (PU) I was appointed a member of the scientific jury to provide a procedure for the protection 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 acquiring the educational and scientific degree "Doctor" in the field of higher education: 4.1. Natural sciences, mathematics and informatics; Professional field: 4.3. Biolo-gic sciences; PhD program: Morphology. The author of the dissertation is Eleonora Tencheva Kovacheva – full-time PhD student at the Department of Developmental Biology under the supervisor Prof. Kovacheva. Dr. Elenka Stoilova Georgieva from Plovdiv University. At the first meeting of the scientific jury, I was selected as a reviewer.

The set of materials presented by Eleonora Tencheva Kovacheva is in accordance with Article 36 (1) of the Rules for the Development of the Academic Staff of Plovdiv University, includes the following documents: an application to the Rector of Plovdiv University for the opening of the dissertation procedure from 14.02.2024; CV in European format; Transcript – an extract from the minutes of the meeting of the Department Council, related to the reporting of the readiness for the opening of the procedure and preliminary discussion of the dissertation – 12.02.2024; dissertation and autore-ferati – 2 pcs.; List of scientific publications on the topic of the dissertation – the list includes 4 pcs. articles and their copies; a declaration of originality and authenticity of the documents affixed; Reference for compliance with the national minimum requirements for acquiring educational and scientific degree "Doctor"

The PhD student has attached 4 publications and additional information on participation in scientific forums (conferences – 8; participation in projects - 3). In the first submitted publication, however, the starting surname of the doctoral student is absent, and E. Petkova is recorded. I assume that this is related to a later change of the surname, but in this case a corresponding document should be attached.

The documentation in its entirety is available, complete and complies with university requirements.

2. Brief biographical data about the PhD student

Eleonora Tencheva Kovacheva was born on 16.12.1995. After graduating from high school in 2014, she was accepted as a student at the Faculty of Biology of Plovdiv University. He graduated in 2018 as a bachelor's degree, molecular biology. In 2019, he earned a master's degree in reproductive biology. From March 2020 starts training PhD student in the Master's program "Morphology" at the Department of Developmental Biology. In the period 2018-2022 he worked as a laboratory technician and organizer of sales and advertising in various companies. For approximately three years he worked in a histological laboratory, where he participated in diagnostic cytological and histological studies and mastered the histological technique. Since the beginning of 2023 the colleague is an assistant at the Department of Medical Biology at MU-Plovdiv, where she conducts practical classes with students in several disciplines.

3. Topicality of the topic and appropriateness of the objectives and tasks set

Modern industrial plant production widely uses as a recognized practice various chemical products (insecticides, herbicides and fungicides) for plant protection. Their use contributes to the easier cultivation of individual agricultural cultures, vineyards and fruit trees, as well as to their prevention and treatment of various diseases. Regardless of the envisaged durability periods of these chemical products, very often the same. Their influence has been studied by taking into account the tissue changes that occurred in carp organs (gills, liver and kidneys) exposed to these chemical agents that have fallen into the aquatic environment. Therefore, the study carried out is necessary and extremely up-to-date. It is both scientific and scientifically applied.

The use of chemical plant protection products in agriculture sometimes leads to non-reversible, negative changes in ecosystems and their inhabitants, loss of biodiversity and problems with human health. In order to reduce or avoid the toxicological effect of the said chemical means of plant protection, a variety of scientific studies shall be carried out to monitor soil and water systems. Such a study is also the presented dissertation. It includes

The main objective of the study is to assess the influence of short-term contamination of an aquatic ecosystem with 3 different chemical pollutants on the histological state of gills, liver and kidneys in carp. The histochemical examination of the liver complements the picture of the negative influence of the chemical agents used. The implementation of the set goal is achieved through the establishment of two main tasks. The first is related to conducting a short-term toxic test with three types of chemical agents and subsequent histological, histochemical and biochemical examination of the target organs. The second task is to assess the identified changes under the impact of applied pesticides and their application as biomarkers of pollution of aquatic ecosystems. In my opinion, the defined purpose of the study and the problems involved for its solution are quite appropriate for obtaining objective results.

4. Knowledge of the problem

The dissertation paper is written on 183 standard pages, including a literal tour report from 519 sources, of which 10 in Cyrillic. It is illustrated with 34 figures and 20 tables. The same has all the sections necessary for this kind of scientific development, which are divided as follows: thanks – 1 page, list of used abbreviations – 2 pages, introduction – 3 pages, literature review – 28 pages, purpose and tasks – 1 p., material and methods – 20p., results – 47 pages,

discussions., conclusion — 2 pp., conclusions — 1 p., contributions — 2 p., publications and participation in scientific fo-rums — 3 pp., literature cited — 45 pp.

The review of the scientific literature used in the conduct of scientific research and the writing of the dissertation is distinguished by a sufficient number (519) of literary sources, which are mainly from the last few years of our time. I accept as correct the author's approach to separate the literary review from her dissertations thematically 7 main sections (general characteristics and classification of pesticides, impact of pesticides on the environment, influence of them on animals and humans, influence of pesticides on aquatic ecosystems, influence on the body of fish, research in Bulgaria on the topic, changes in fish organs), some of which have several sub-points. The PhD student not only knows, but also possesses the huge volume and thematic variety of scientific information.

In conclusion, I would like to note that the colleague shows a thorough knowledge of the literatu related to the subject and tasks of the dissertation. In its entirety, the literature reference presents sources that are relevant to the research carried out.

5. Methodology of the study

The study included 70 carp, which were divided into 10 experimental groups. The three pesticides used were applied each in two concentrations. The experimental procedure and related studies were carried out in the scientific laboratories of Plovdiv University of Plovdiv. Three routine methods of obra-botka and staining of preparations for histological and histochemical examination were used in the histological examination – stained-not with hematoxylin/eosin of paraffin systes, ostaining of frozen lipid cuts with Sudan and staining with Schiff reagent for glyco-gene. The assessment of histological changes of gills, liver and kidneys was carried out on the scale of Bernet et al. The severity of the morphological changes was assessed according to the scale of Saraiva et al. The biochemical values of the different hepatic by-patients were determined spectrophotometrically. The methods and indicators used make it possible to make a reliable assessment of the biological mater studied.

This has made it possible to achieve the achievement of the set goal and reliable response to the tasks included in the dissertation

6. Characterization and evaluation of the dissertation

The dissertation work is made and presented professionally and with the necessary most scientific competence on the topic. The topic is well chosen and is up to date. The literary study carried out is purposeful and thorough. It has allowed to correctly determine the purpose of the study, the object of study, the use of current chemical agents for plant protection. Histological, histochemical and biochemical testing methods are reliable and reliable. The results obtained and their discussion were presented to 65 pages. Their presentation is made through the use of photographs, tables and figures. In my opinion, the pictures could have been presented in a larger format. The changes thus described would have been demonstrated more clearly. In addition, brief data on the presence or absence in behavioural responses of fish during the toxicological test had to be included. The picture for the evaluation of morphological changes could also be enriched with established macroscopic changes in the organs examined and especially in the two lethal cases with the first chemical agent used. The literature used in the "Results" section could be minimized and included either in the literature survey or in the discussion. The discussion of the results obtained has been carried out consistently and

thoroughly. This allowed the PhD student to make a logical conclusion about the reliability of the research methods used to assess the impact of the three chemical agents on aquatic organisms. The results obtained have allowed the PhD student to formulate 4 main conclusions related to pathomorphological changes in the target organs (gills, liver and kidneys), changes in glycogen and lipid content in the liver, as well as changes in its enzyme profile.

When I get acquainted with the dissertation, I have the impression that most of the most scientific research is the work of the PhD student herself.

In conclusion, despite the remarks, the dissertation thesis I believe meets the requirements for acquiring the educational and scientific degree "Doctor".

7. Contributions and relevance of development to science and practice

The dissertation work contains contributions of both a scientific and practical nature. Of an original scientific nature are the studies the impact of pirimophosme-thyl and promocarb hydrochloride on common carp. The morphophysiological study carried out in laboratory conditions is the first of its kind complex study by applying histopathological analysis of gills, liver and kidneys, as well as histochemical and biochemical analysis of liver to clarify the degree of three active ingredients of plant protection products on common carp. The dissertation contains 6 confirmatory contributions as well as three scientifically applied ones. All three types of contributions I accept.

8. Assessment of publications on the dissertation

From the section publications and participation in scientific forums it becomes clear that the doctoral student has 4 scientific publications related to the dissertation. In two of them, she is a leading author, and in the other two publications the PhD student is in second and eighth place. place. It has also attached a list of participation with 8 scientific products in conferences and seminars.

9. Autoreferat

The autoreferee consists of 34 pages and is properly structured. It contains all parts of the dissertation and includes illustrations from it.

10. Critical remarks and recommendations for future use of the results and presentations in the dissertation

I made my critical remarks in points 1 and 6 of the review. In my opinion, the dis-certification can benefit as a scientific work if a study on biological material was carried out some time after the normal aquatic environment is restored, without the presence of the chemical contaminants used. The contributions presented of an applied nature in the dissertation can serve as an inst-rument to assess the influence of various toxic substances on bioindicator species.

CONCLUSION:

In conclusion, I believe that the dissertation presented contains scientific and applied results that represent an original contribution to science and meet the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Great-vilnik for

the Implementation of the Law on the Implementation of the Law on the Development of the Academic Staff "Paisii Hilendarski".

The presented materials and dissertation results fully comply with the specific requirements of the Faculty of Biology, adopted in connection with the Rules of the University for the Application of the Law on Implementation of the Law on Combating Organized Crime.

The developed dissertation shows that the PhD student Eleonora Tencheva Kovacheva possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Morphology, with qualities and skills for independent scientific research.

Due to the above, I confidently give my positive assessment of the research presented by the dissertation thesis, autoabstract, achieved results and contributions, presented by the above-reviewed dissertation, and I propose to the honorable scientific jury to award the educational and scientific degree "Doctor" to Eleonora Tencheva Kovacheva in the field of higher education: 4. Natural sciences, mathematics and informatics; Professional field: 4.3. Biological sciences; scientific specialty / doctoral program: Morphology.

Apr 15, 2024

Prepared the review:

(Assoc. Prof. Dr. Atanas Bochukov)