OPINION

from Ph.D. Dr. Pepa Koseva Atanassova-Hrischeva, professor,

Medical University - Plovdiv

of a dissertation for awarding the educational and scientific degree "doctor"

by: Field of higher education: 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)"

Scientific supervisor: Prof. d.b. Dr. Elenka Stoilova Georgieva, Paisiy Hilendarski University of Plovdiv

1. General presentation of the procedure and the doctoral student

By order No. P33-RD 21-509 of 28.02.2024. of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I have been appointed as a member of the scientific jury to ensure a procedure for the defense of a dissertation work on the topic "ASSESSMENT OF POLLUTION OF AQUATIC ECOSYSTEMS WITH PRIORITY ORGANIC POLLUTANTS THROUGH THE USE OF MORPHOFUNCTIONAL BIOMARKERS IN CYPRINUS CARPIO (LINNAEUS 1785)" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics and informatics; Professional direction: 4.3. Biological Sciences; Doctoral program: Morphology.

The author of the dissertation is ELEONORA TENCHEVA KOVACHEVA - PhD student in full-time studies at the Department of Developmental Biology, Faculty of Biology, PU with supervisor Prof. d.b. Dr. Elenka Stoilova Georgieva, Paisiy Hilendarski University of Plovdiv

The set of paper materials presented by Eleonora Kovacheva is in accordance with Article 36 (1) of the Rules for the Development of the Academic Staff of the PU, includes the following documents:

- a request to the Rector of the PU to disclose the procedure for the defense of a dissertation work;

– curriculum vitae in European format;

– minutes from the department council related to reporting the readiness to open the procedure and preliminary discussion of the dissertation work;

– dissertation work;

– abstract;

– a list of scientific publications on the subject of the dissertation;

– copies of scientific publications;

- declaration of originality and authenticity of the attached documents;

– certificate of compliance with national minimum requirements

The PhD student has attached 4 publications.

Brief biographical data of the PhD student

Eleonora Kovacheva obtained the BA "Bachelor", Molecular Biology at the Faculty of Biology of Plovdiv University "Paisiy Hilendarski" in 2018. She worked as a laboratory technician-biologist successively in the "Biochemistry" and "PKK" sectors, SMDL "KARIDAD"; SMDL "HISTO-2000", research and diagnostics in the field of histology and cytology; examination of biopsy samples, smear processing, gefrir examination. From 23.01.2023 - currently she is an assistant in the Department of "Medical Biology" of the Medical University - Plovdiv. Conducting practical classes in medical parasitology, molecular and cellular biology, immunology for first-year students majoring in "Medicine", "Dental Medicine" and "Pharmacy". Competence in Molecular Biology, Cell Biology, Genetics, Bio-

chemistry, Embryology and Histology, Microbiology, Human Anatomy, Plant Physiology, Botany, Plant In Vitro Cultures, Animal and Human Physiology, Molecular Virology, Regulation of Gene Expression, Immunology, Microbial Biotechnologies.

2. Relevance of the topic

The presented dissertation work is dedicated to an extremely topical question in the field of ecology, namely the study of the influence of priority organic pollutants in the contamination of water systems. The assessment was carried out through morpho-functional studies and the use of key biomarkers in CYPRINUS CARPIO (LINNAEUS 1785. The problem involved is important not only scientifically, but mainly in a scientific-applied sense.

3. Knowing the problem

The global use of pesticides, associated with an increasing risk of exposure to the environment and all organisms, has increased manifold in recent decades. The doctoral student is very well acquainted with the issues of water system pollution, which is evident from the extensive literature review. She has creatively evaluated the literary material, which gives her the opportunity to outline her own goals and objectives in solving an essential part of this problem.

In ecotoxicological studies, biomarkers are useful tools used in monitoring aquatic ecosystems. Eleonora Kovacheva's inclusion of complex biomarkers at different levels of biological organization is a suitable approach for the detection of pollutant-induced reactions. In aquatic ecosystems, one of the commonly used organs for monitoring complex biomarkers for ecotoxicological assessment is the gill, liver and kidney of fish. The dissertation student prepares permanent histological preparations, stained with HE and Sudan black, from the examined organs to report the pathological changes in them as a result of the impact of pesticides. The appropriate semi-quantitative scale described by Mishra & Mohanty (2008), but with own modification, was used to assess the extent of histopathological changes. In addition, the biochemical analysis of some indicators was used. Liver enzymes LDH, ASAT, ALAT, ChE and CAT were biochemically investigated as a diagnostic marker of the physiological and metabolic state of CYPRINUS CARPIO (LINNAEUS 1785). The amount of total protein was also measured to calculate specific enzyme activity.

The chosen lean methodology of the research, laid down in my presented dissertation, allows achieving the set goal and obtaining an adequate answer to the set tasks.

5. Characterization and evaluation of the dissertation work and contributions

The dissertation is written on 182 standard pages, observing all the requirements of the arrangement of the individual chapters. The literary overview includes 519 authors, of which 10 are in Cyrillic, the rest are in Latin.

Eleonora Kovacheva conducted a thorough histopathological examination of a number of organs - gills, liver and kidney of common carp (Cyprinus carpio L.). The obtained results show the presence of morphological disorders, including proliferative and degenerative changes, inflammation, as well as changes in the circulatory system. The index of histopathological changes in the gills, liver and kidney under the treatment with the different herbicides and fungicides was compared, showing that it was the highest in the gill, followed by the liver and the lowest in the kidney. The changes vary with the pesticide concentration applied. Biochemical changes under the influence of the experimental pesticides were also found, which confirm the observed histological and histochemical ones. All these data show a disturbance in the functions of the liver related to the processes of glycolysis, glyconeogenesis and lipogenesis.

There are three original contributions to the presented dissertation work. An in-depth and meaningful analysis of the obtained results and their comparison with already existing scientific and scientific-applied evidence in the literature is an achievement.

The present study on the effect of pirimiphos-methyl and propamocarb hydrochloride on common carp (Cyprinus carpio L.) is the first of its kind. The first complex morphophysiological study was carried out in laboratory conditions, including histopathological analysis of gills, liver and kidney, as well as histochemical and biochemical analysis of liver, to clarify the degree of toxicity of three active ingredients of PRZ on common carp (Cyprinus carpio L.). The performed histochemical analysis of the liver of common carp (Cyprinus carpio L.) for the influence of the toxicity of the herbicide 2,4-D is the first of its kind.

A methodical and applied contribution is the use of the scale to determine the degree of changes in the histological structure of the gills, liver and

kidney. Histopathological, histochemical and biochemical analyzes can-serve as tools to evaluate the effect of various toxic substances on bioindicator species. The established changes in gill, liver and kidney can be successfully applied as biomarkers and included in a model for the assessment of contamination of aquatic ecosystems with pesticides, in order to prepare an adequate regulatory framework concerning the presence of organic pollutants in aquatic ecosystems.

In general, the present work presents significant new aspects in the existing scientific problem of water pollution by using a new complex approach to evidence through the means of histopathological analysis. The main achievements of the dissertation are scientific and applied scientific contributions. The obtained results and approved research methods could be successfully applied in practice.

6. Evaluation of the publications and personal contribution of the doctoral student

4 published articles reflecting the results of the dissertation work are presented. Three of them were published in the journal Ecologia Balkanica with Q4 and one - in the journal Toxics with Q1 and IF=4.6.

In three of the articles, the doctoral student is in the first place, which emphasizes her personal contribution.

The obtained results have been presented at 7 scientific forums - 2 international and 5 national.

7. Abstract

The abstract is made according to the requirements of the regulations and reflects the main results achieved in the dissertation.

8. Recommendations for future use of dissertation contributions and results

In general, the present work presents significant new aspects in the existing scientific problem of water pollution by using a new complex approach to evidence through the means of histopathology and biomic analysis. The main achievements of the dissertation are scientific and applied scientific contributions. The obtained results and approved research methods could be successfully applied in practice.

CONCLUSION

The dissertation contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of PU "Paisiy Hilendarski ".

The dissertation shows that the doctoral student ELEONORA TENCHEVA KOVACHEVA possesses in-depth theoretical knowledge and professional skills, demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment for 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 Oblast of higher education: 4. Natural sciences, mathematics and informatics; Professional direction: 4.3. Biological Sciences; Doctoral program: Morphology.

04/7/2024 Prepared the opinion: .....

Prof. Ph.D.Dr. Pepa Atanassova-Hrischeva