STATEMENT

on dissertation work for obtaining the educational and scientific degree "doctor" in: field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.4. Earth Sciences; the scientific specialty: "Ecology and Protection of Ecosystems"

Author of the dissertation: Petya Georgieva Zaharieva, Full-time doctoral student at the Department of "Agroecology and Environmental Protection" at the Agricultural University, Plovdiv

Topic of the dissertation: "Content of heavy metals in fish and their parasites from the Danube River - ecology and bioindication"

Reviewer: Prof. Dr. Diyan Mihailov Georgiev, Trakia University, Faculty of Agriculture, Department of Ecology and Animal Hygiene, 4.3. Biological Sciences, "Ecology and Protection of Ecosystems", designated as a member of the Scientific Jury by Order No. RD-16-1117/31.10. 2022 by the AU Rector.

1. Relevance of the problem.

In a global aspect, the problems related to the pollution of the environment with heavy metals have gained particular importance. This is caused by the biogenic nature of the elements on one hand, and on the other, that at certain concentrations and factors they become toxic and change the environment by passing through the trophic levels.

Studies on natural objects, in particular water ecosystems located in urbanized areas, are relevant in order to preserve their purity and biological diversity, as well as to develop regional and national strategies for environmental protection.

Often in these areas, activities leading to pollution are combined with inadequate measures of neutralization, the effect of which is poisoning of the environment, costly pollution control and human health. The national and strategic importance of the Danube River, the exceptional diversity of the ichthyofauna, intensive fishing and the sale of fish products, define the present study as timely and justified. The analyzed amount of information allows Petya Zaharieva to convincingly present the relevance of the problem and to provide a targeted literature review of studies in this area, in a global aspect.

2. Purpose, tasks, hypotheses and research methods.

Doctoral student Petya Zaharieva develops the literature on the subject based on 193 scientific publications that provide data on the concentrations of heavy metals and metalloids in water and sediments, freshwater fish and their parasites from the Danube River and the Danube Basin.

The goal is clearly formulated, 5 tasks and subtasks are set for implementation. On the basis of a good literature awareness, the need to include all components of the water body - water, sediments and biota - is convincingly indicated in this kind of research. The research methodology includes a complex of field sampling and laboratory analyses. In "Materials and methods" the selected methods, the equipment used and accredited laboratories with the help of which the analyzes were performed are described in detail. In three tables, values for heavy metals of certified reference standards for water, sediments and biological elements are

presented, which enables the obtained results to find an adequate answer in the dissertation work.

3. Transparency and presentation of the obtained results.

The dissertation meets the requirements of ZRASRB. It is written in 250 pages. It contains scientific and scientific-applied results that represent an original contribution to science. On the face are all sections in sufficient volume and ratio between them.

The information is illustrated by 106 figures and summarized in 42 tables. The dissertation shows that the candidate has in-depth theoretical knowledge and abilities for independent scientific research.

Extensive field and laboratory work was carried out. During the period 2019-2021, a total of 810 specimens (270 specimens of each species) of three types of fish - *Alb. alburnus*, *Abr. brama* and *Ch. nasus* from the upper reaches of the Danube in Bulgaria (Kudelin). For the presence of copper, cadmium and arsenic, the liver, skin and muscles of the three freshwater fish species were examined.

4. Discussion of the results and used literature.

The obtained results for the physico-chemical indicators of the waters of the Danube River, Kudelin section and the analysis of the content of heavy metals in the sediments and organs of three types of fish are scientifically sound. In each studied indicator, established regularity and expressed hypothesis, the author looks for interdependencies and connections, which he often supports with data from the literature review, with data from norms in national and international documents (Ordinance No. 31 of July 29, 2004 on the maximum permissible amounts of pollutants in Food, the World Food Organization (FAO) and the World Health Organization (WHO)).

The obtained results are subjected to statistical processing, correlation dependences and degrees of credibility of the discussed problems are highlighted. In the dissertation, acquired new knowledge and proposed new approaches to solving the scientific problem stand out.

In order to present the relationship between the content of heavy metals in the examined organs and their content in the waters of the Danube River, a bioaccumulation factor was used. In order to answer the question to what extent fish tissues/organs can accumulate chemical substances directly from waters and sediments, the bioconcentration factor was used.

12 conclusions were formulated, which logically and concisely summarize the conducted studies and achieved results.

Real recommendations for science and feasible activities for the practice are made.

The list of literature contains 349 sources, of which 43 are in Cyrillic and 306 are in Latin (including websites of ministries, municipalities, etc.).

5. Contributions of the dissertation work.

20 scientific and scientific-applied contributions of the dissertation are presented.

Scientific contributions

The more important scientific contributions are related to the presentation of new data on the content of Cu, Cd and As in *Ch. nasus, Alb. alburnus, Abr. brama* and their parasites *P. laevis* and *Contracaecum sp*.

The data on the seasonal changes in the content of As in the studied fish species from the Bulgarian section of the Danube River have been updated with the results from the Kudelin biotope.

The scientific literature on the content of Cu, Cd and As in tissues and organs of *Alb alburnus, Abr. brama and Ch. nasus*, parasites (*P. laevis*, Contracaecum sp.), as well as in waters and sediments from the freshwater ecosystem of the Danube River, has been enriched.

Scientific and applied contributions

Scientific contributions of applied nature are the established excesses of Cu, Cd and As in liver, skin and muscles of *Alb. alburnus, Abr. brama and Ch. nasus* as well as their parasites. In this connection, the circulation of heavy metals and metalloids in the ichthyocenosis, water and sediments of the Bulgarian section of the Danube River was studied.

As a result of the study, data on the values of the bioconcentration factor are reported for the first time and its values are updated for the river and the study area Kudelin.

The bioindicative role of the liver of carp and uklei for the Cd content was established; bream liver for As content; *Contracaecum sp.* about the contents of the CD; *P. laevis* for the content of As.

6. Critical Notes and Questions.

I have no critical notes and recommendations

7. Published articles and citations.

Two articles have been published on the dissertation, which are in a global database, and through them the doctoral student covers the MNI for the acquisition of the ONS Doctor":

1. Zaharieva, P., Kirin, D., 2020. A contribution to the studies on the content of Cu, Cd and As in Alburnus alburnus (Linnaeus, 1758) from the Danube River. Scientific Papers. Series D. Animal Science, LXIII (2), 405-412, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-226.

2. Zaharieva, P., Kirin, D., 2020. Content of copper, cadmium and arsenic in Chondrostoma nasus (Linnaeus, 1758) from the Danube River. Scientific Papers. Series D. Animal Science, LXIII (1), 481-488, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260

8. Abstract

The presented abstract is structured in accordance with the requirements of the ZRASRB, PPZRASRB and PRAS of the AU. It objectively reflects the structure and content of the dissertation. It is written on 34 pages and has an English version. This makes the scientific achievements of PhD student Petya Zaharieva noticed in the world's databases and accessible to a large circle of scientists.

CONCLUSION:

Based on the various research methods learned and applied by the doctoral student, the correctly performed experiments, the generalizations and conclusions made, I believe that the

presented dissertation meets the requirements of the ZRASRB and the Regulations of the Agricultural University for its application, which gives me reason to evaluate it POSITIVE.

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award Petya Georgieva Zaharieva the educational and scientific degree "Doctor" in the scientific specialty "Ecology and preservation of ecosystems"

Date: 21.11.2022

Plovdiv

STATEMENT PREPARED BY: .....

(Prof. Dr. Diyan Georgiev)