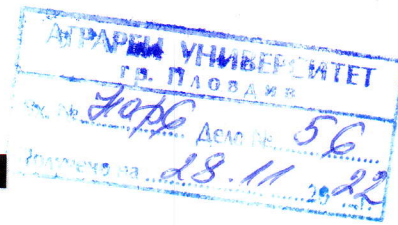


OPINION



on a dissertation for obtaining the educational and scientific degree "doctor" by: field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.4 Earth sciences, the scientific specialty Ecology and ecosystem protection.

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

Dissertation topic: Content of heavy metals in fish and their parasites from the Danube River - ecology and bioindication.

Reviewer: Prof. Dr. Vladislav Haralampiev Popov, Agricultural University (AU)-Plovdiv, region of higher education 4. "Natural sciences, mathematics and informatics", professional direction 4.4 "Earth sciences", the scientific specialty "Ecology and ecosystem protection", designated as a member of the scientific jury by order No. RD -16-1117/31.10.2022 of the Rector of the AU-Plovdiv.

1. Relevance of the problem

The topic of the dissertation is relevant and related to the study of the effect of the harmful effects in the aquatic food chains of pollutants such as heavy metals on different types of fish in the Danube River. Fish are suitable for bioindication and they are important food for humans. Toxic heavy metals have a negative impact on fish, as they can affect their growth, reproduction, mortality. The dissertability of the topic is also emphasized by the fact that there are insufficient data on the concentrations of heavy metals and metalloids in tissues and organs of fish and their parasites from the Bulgarian section of the Danube River, as well as on the circulation of heavy metals in the system fish-parasites- water-sediments in the section immediately after the Danube River enters Bulgaria. This argues the interest and necessity of conducting the research.

2. Purpose, tasks, hypotheses and research methods

The purpose of the scientific research, as well as the set scientific tasks for its achievement, are clear and well defined. They reflect the author's scientific approaches to test the scientific hypothesis, which suggests the discovery and accumulation of new data, regarding the content of heavy metals in fish and their parasites from the freshwater ecosystem of the Danube River, and more specifically determining the content of copper (Cu), cadmium (Cd) and arsenic (As) in tissues, organs and helminths of *Alburnus alburnus* (Linnaeus, 1758), *Abramis brama* (Linnaeus, 1758) and *Chondrostoma nasus* (Linnaeus, 1758), waters and sediments from the Danube, Kudelin biotope (Vidin region) for the period 2019-2021, incl. seasonal dynamics; trace the circulation of Cu, Cd and As, incl. the influence of seasonal and annual changes in it, in the water-sediment system- *Chondrostoma nasus* - *Pomphorhynchus laevis* - *Contracaecum* sp. These approaches are based on a well-conducted and extensive literature review and the identified gaps in scientific data and information on the subject.

The "Materials and methods" section provides sufficiently detailed and accurate information about the complex nature of the study - suitable objects of study and a methodology consistent with the direction of the study, a wide range of indicators for the study of biodiversity, modern methods of analysis and statistical processing of the data, strengthening the reliability of the obtained results and conclusions. The dissertation student has mastered to a good extent the proposed standard methodology for conducting research (field and laboratory).

3. Transparency and presentation of the obtained results.

In terms of volume and structure, the dissertation meets the requirements for a dissertation for the ONS "Doctor". The dissertation covers 250 pages and contains 42 tables and 106 figures. 349 literary sources were used, of which 28 in Cyrillic and 285 in Latin, 6 normative documents, 10 standardized methods (BDS) and 20 online databases. The dissertation includes all the required sections and sub-sections, which allows the complex studies to be followed in their interrelationship. The dissertation is well illustrated with tables, figures and diagrams that support the perception of information, data and conclusions. Statistical treatment of the results and regression and correlation dependences are specifically and clearly presented. The dissertation uses a clear scholarly style that is both scientifically sound and comprehensible.

4. Discussion of the results and used literature.

The literature review covers the period from 1975 to 2022 and is based on 193 scientific publications that provide data on the concentrations of heavy metals and metalloids in waters and sediments, freshwater fish and their parasites from the Danube River and the Danube Basin. The literature review is sufficiently detailed, systematic and meets the goal of conducting a complex study of the subject.

The dissertation student systematically and consistently presents the achieved results and the obtained data in the "Results and Discussion" section, making a well-founded discussion on them, following the sequence of the scientific tasks set for implementation. The results of the research on the content of Cu, Cd and As in liver, skin and muscles of dominant species of fish from the Cyprinidae family (*Alb. alburnus*, *Abr. brama* and *Ch. nasus*) from the freshwater ecosystem of the Danube River (biotope Kudelin), which differ in their way of life and nutrition. It was also studied with the content of Cu, Cd and As in helminths of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus* from the Danube River, Kudelin biotope. The content of Cu, Cd and As in water (mg.l⁻¹) and sediments (mg.kg⁻¹ air dry weight) from the Danube River (Kudelin biotope) for the period 2019-2021 was examined as well as the excesses of the three elements in waters and sediments in relation to national and international legislative documents. Seasonal and annual variations in the content of Cu, Cd and As in tissues and organs of *Alb. alburnus*, *Abr. brama* and *Ch. nasus*, in waters and sediments were examined. The excesses of the 3 investigated elements in liver, skin and muscles, in water and sediments compared to documents from the national and international legislation by seasons and years are indicated.

5. Contributions of the dissertation work.

The presented contributions of the dissertation can be divided into:

Scientific contributions

The first studies for Bulgaria and the Bulgarian section of the Danube River on the content of Cu, Cd and As in the liver, skin and muscles of *Ch. nasus* and its parasites *P. laevis* and *Contracaecum* sp. For the first time, the content is compared, incl. seasonal and annual variations of Cu, Cd and As in liver, skin and muscle of *Alb. alburnus*, *Abr. brama* and *Ch. nasus* in waters and sediments from the Bulgarian section of the Danube River with the results from the Kudelin biotope. The scientific literature on the content of Cu, Cd and As in tissues and organs of has been enriched *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. Data on bioconcentration factor values for Cu in liver, skin and muscle of *Alb. alburnus* are reported for the first time relative to waters and sediments; for Cd in liver, skin and muscle of *Abr. brama* vs. waters and sediments; for Cu in liver, skin and muscle of *Abr. brama* versus waters; for Cu, Cd and As in liver, skin and muscle of *Ch. nasus* vs. waters and sediments; for Cu and Cd in *P. laevis* from bream and bream versus waters; for Cu, Cd and As in *P. laevis* from carp versus waters; for Cu, Cd and As in *Contracaecum* sp. from a stapler to waters and sediments; as well as for the values of the bioaccumulation factor for Cu

and Cd in *P. laevis* from uklay; for Cd in *P. laevis* from bream; for Cu, Cd and As in *P. laevis* and *Contracaecum* sp. from a carpenter from the Danube River in Bulgaria.

Scientific and applied contributions

Data on bioconcentration factor values for Cd and As in liver, skin and muscle of *Alb. alburnus* have been updated relative to waters and sediments; for As in liver, skin and muscle of *Abr. brama* vs. waters and sediments; for Cu in liver, skin and muscle of *Abr. brama* versus sediments; for As in *P. laevis* from bream and bream versus waters; as well as bioaccumulation factor values for Cu in *P. laevis* from bream; for As in *P. laevis* from bream and bream from the Danube River in Bulgaria with the results from the Kudelin biotope. Prominent bioindicators are: liver of carp and uklay for Cd content; bream liver for As content; *Contracaecum* sp. for Cd content; *P. laevis* for As content.

On the basis of the contributions, **recommendations** related to limiting the consumption of the three investigated fish species (bream, bream and bream) due to the reported excesses in the concentrations of Cd and As in the muscle samples compared to the norms in Ordinance No. 31, as well as regular monitoring of indicators and enhanced control (MOEW, IAEA, RIOSW) on the quality of water and sediments in the studied section of the Danube in connection with elevated concentrations of Cu, Cd and As, and optimization of cross-border cooperation in connection with limiting the negative impact of industry and agriculture; improving the state of the freshwater ecosystem and preserving species diversity.

6 . Critical notes and questions.

A clearer and more specific differentiation of the scientific and scientific-applied contributions is recommended, as well as a differentiation of the practical benefit of the research and the direction of the recommendations, for example, to the bodies of the IAEA, those concerning the monitoring of heavy metals. The interrelationships between seasonal and annual fluctuations, dynamics of heavy metal content, bioconcentration and environmental or anthropogenic causes could be presented more clearly. The large number of investigated indicators , based on comparative analyzes of the obtained data, could be formulated by using statistical tools, such as multifactor analysis of variance, which will convey statistical significance of the differences found by fish species, heavy metals, parasite species, seasons and so called

7. Published articles and citations.

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 2393-2260; ISSN-L 2285-5750

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.

The presented abstract reflects objectively the structure and content of the dissertation work.

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 ŽRASRB and the Regulations of the Agrarian University - Plovdiv for its application, which gives me the reason to evaluates **POSITIVELY** .

I take the liberty of proposing to the honorable Scientific Jury to also vote positively and award Petya Zaharieva the educational and scientific degree " **Doctor** " in the scientific specialty Ecology and Ecosystem Protection.

Date: 10.11.2022
city of Plovdiv

AUTHOR OF THE OPINION:
(Prof. Dr. Vladislav Popov)