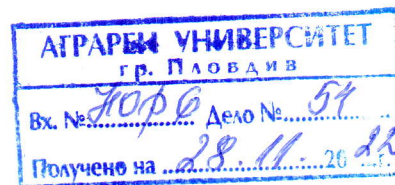


REVIEW



On a dissertation for obtaining the educational and scientific degree "Doctor" in: field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional field 4.4. "Earth Sciences", scientific specialty "Ecology and ecosystem conservation"

Author of the dissertation:

Petya Georgieva Zaharieva, regular PhD student at the Department of Agroecology and Environmental Protection, Agrarian University, Plovdiv

Topic of the dissertation:

"Heavy metal content in fish and their parasites from the Danube River - ecology and bioindication"

Reviewer:

Assoc. Prof. Dr. Penka Stancheva Zaprianova-Alexieva, Agrarian University; field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.4. Earth Sciences, scientific specialty Ecology and Ecosystem Conservation, appointed as a member of the scientific jury by Order No. RD-16-1118/31.10.2022 of the Rector of the University of Applied Sciences.

1. Brief Biographical Information about the candidate.

Petya Zaharieva was born on 29.03.1992 in Plovdiv.

In 2010 she graduated from the Private Vocational School of Economics and Trade, Plovdiv. She graduated from Plovdiv in 2010. She obtained a Bachelor's degree in Economics in 2014.

After obtaining a Master's degree in Ecology of Settlement Systems, in 2019 Petya Zaharieva won a competition for a regular PhD in Ecology and Ecosystem Conservation at the Department of Agroecology and Environmental Protection, AU-Plovdiv. On 07.07.2022 she was dismissed with the right to defend.

2. Relevance of the problem.

The issue of environmental pollution, respectively of food products with heavy metals and other toxic elements is dominant in science. In the food chain, heavy metals enter living organisms through soil, water and air.

In water bodies, heavy metals accumulate in all vital organs and tissues of fish. Toxic heavy metals have negative effects on fish and can affect their growth, reproduction, and mortality. Heavy metals can damage and disrupt the normal functioning of the brain, lungs, kidneys, liver and other important organs of fish. The highest concentrations of heavy metals are found in the liver, kidneys and gills of fish and, in some, in the gut. Toxic elements also enter the muscle tissue of fish that are

consumed by humans, posing a toxicological risk to human health. All this makes the relevance of the research in Petya Zaharieva's thesis undeniable. Such research has been identified as a priority area in a number of national and EU documents.

3. Purpose, tasks, hypotheses and research methods

The formulated five research tasks are a logical consequence of the literature review and are subordinate to the main goal: to carry out scientific research on the content of heavy metals in fish and their parasites from the freshwater ecosystem of the Danube River.

In the "Material and methods" section, a brief natural-geographical characterisation of the Danube, the Danube basin and the biotope under study, as well as a characterisation of the heavy metals and metalloid (Cu, Cd and As) investigated are presented.

The field studies carried out, the ways of sampling water, sediments and fish from the Danube, Kudelin biotope are described in great detail. Validated BDS and ISO standardised methods were used for this purpose.

Sampling methods for water, sediment and fish are detailed. The laboratory tests were carried out with modern equipment ensuring their precision and reliability. The section "Material and methods" reflects national and international legislation (Dutch Target and Intervention Values; World Food Organisation / FAO (<http://www.fao.org/home/en/>); World Health Organisation / WHO (<https://www.who.int/>); COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs). The results obtained from the chemical analyses carried out for Cu, Cd and As content in the samples of biological elements (freshwater fish), water and sediment are discussed in relation to the mentioned regulatory documents.

Statistical data processing was performed using MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece et al., 1997) and Statistica 10 (StatSoft Inc., 2011). The Materials and Methods section provides information on the following metrics that were used in the statistical processing of the data: bioconcentration, bioconcentration factor (BCF), bioaccumulation, and bioaccumulation factor (BAF).

4. Visualization and presentation of the obtained results.

The dissertation is written in 250 pages. It contains 42 tables and 106 figures. The tables present the research results in a very clear and understandable way. The figures are excellently designed and very accurately orient the reader to the content within them.

The dissertation is structured as follows: introduction - 2 pages, literature review - 58 pages, aim and objectives - 1 page, material and methods - 21 pages, results - 127 pages, summaries and conclusions - 5 pages, scientific and applied contributions - 2 pages, recommendations - 1 page, references - 27 pages.

The presentation of the experimental data occupies a major part of the thesis. The Results section includes 9 parts. The first part deals with the content of Cu, Cd and As in tissues and organs of *Alburnus alburnus*, *Abramis brama* and *Schondrostoma nasus* from the Danube River, Kudelin biotope. The second one presents data on concentrations of the three elements in helminths of *Alburnus alburnus*, *Abramis brama* and *Schondrostoma nasus* from the study area. This is followed by a discussion of the copper, cadmium and arsenic contents of waters and sediments from the Danube River after which a detailed and consistent element-by-element comparative consideration of their contents in fish tissues and organs, helminths, waters and sediments is made.

Another important part of the presentation of the results is the seasonal and annual changes that occur in the concentrations of the studied elements in tissues and organs of *Alburnus alburnus*, *Abramis brama* and *Schondrostoma nasus*.

The last parts of the Results section include analyses of the circulation of Cu, Cd and As in water and sediment, tissues and organs of fish by season and by year. Overall, the dissertation results are presented in a comprehensive, detailed, logical sequence and are well illustrated. The style of presentation is scientifically and grammatically sound.

5. Discussion of the results and literature used.

The results of the large number of studies conducted are in full agreement with the objectives set in the dissertation. The professional, intelligent and thorough interpretation of the data makes a very good impression. A discussion of each set of results is carried out within the individual parts of the dissertation. The discussion is well argued and supported by up-to-date literature sources, which shows the PhD student's excellent awareness of the issues under investigation. The experimental results obtained in the thesis are compared with data from studies conducted in other countries and those conducted in Bulgaria. On the basis of these comparisons, the novelty found by the doctoral student in the process of the research is indicated.

Three hundred and forty-nine literature sources were used in the dissertation, including 28 in Cyrillic and 285 in Latin, 6 normative documents, 10 standardized methods (BDS and ISO) and 20 online databases. The literature sources are from the period 1975 - 2022, with a predominance of those from recent years.

As a result of the analysis of the experimental results, 12 conclusions were drawn and 8 recommendations were made.

6. Contributions of the dissertation.

The conclusions and contributions of the dissertation fully meet the set tasks and the realization of the main goal. The contributions can be grouped into 2 groups.

Scientific contributions

The field studies and laboratory investigations have provided valuable information and enriched the scientific literature on the Cu, Cd and As content of Alb tissues and organs. Alburnus, Abr. Brama and Ch. Nasus, parasites (*P. laevis*, *Contracaecum* sp.), and in waters, and sediments of the freshwater ecosystem of the river. Danube.

New data are presented on: the Cu, Cd and As content in liver, skin and muscle of Ch. Nasus and its parasites *P. laevis* and *Contracaecum* sp.; for the Cu content in liver, skin and muscle of Alb. Alburnus; of Cu and Cd in *P. laevis* of Alb. Alburnus; of Cd in *P. laevis* from Abr. Brama; of Cd in liver, skin and muscle of Abr. Brama from *P. laevis* Danube and Danube basin in Bulgaria.

For the first time, a comparison of Cu, Cd and As contents in liver, skin and muscle of *Alburnus alburnus*, *Abramis brama* and *Shondrostoma nasus* was made. Seasonal variations of Cu and Cd contents in liver, skin and muscle of *Alburnus alburnus* and *Abramis brama*; annual variations of Cu, Cd and As contents in liver, skin and muscle of Alb. *alburnus* and Abr. *brama*; the seasonal and annual variations of Cu, Cd and As contents in the liver, skin and muscle of *Shondrostoma nasus* from the Bulgarian section of the river Danube.

New data are presented on: the Cu and Cd cycling in liver, skin and muscle of *Alburnus alburnus* and *Abramis brama*, and the Cu, Cd and As cycling in liver, skin and muscle of *Shondrostoma nasus*, their parasites, water and sediments from the river Danube in Bulgaria.

For the first time, bioconcentration factor values are reported for Cu in liver, skin and muscle of *Alburnus alburnus* relative to water and sediment; for Cd in liver, skin and muscle of *Abramis brama* relative to water and sediment; for Cu in liver, skin and muscle of *Abramis brama* relative to water; for Cu, Cd and As in liver, skin and muscle of *Shondrostoma nasus* relative to water and sediment; for Cu and Cd in *P. laevis* of *Alburnus alburnus* and *Abramis brama* relative to waters; for Cu, Cd and As in *P. laevis* of *Shondrostoma nasus* relative to waters; for Cu, Cd and As in *Contracaecum* sp. of *Shondrostoma nasus* relative to water and sediment; and for bioaccumulation factor values for Cu and Cd in *P. laevis* of *Alburnus alburnus*; for Cd in *P. laevis* of *Abramis brama*; for Cu, Cd and As in *P. laevis* and *Contracaecum* sp. of *Shondrostoma nasus* from *P. laevis* and *Contracaecum* sp. Danube in Bulgaria.

It is proposed to use the liver of *Shondrostoma nasus* and *Alburnus alburnus* as bioindicators of Cd content; the liver of *Abramis brama* as a bioindicator of As content; *Contracaecum* sp. as a bioindicator of Cd content; *P. Laevis* as a bioindicator of As content.

Updated data on: Cd and As content in liver, skin and muscle of *Alburnus alburnus*; Cu and As content in liver, skin and muscle of *Abramis brama*; As content in *P. laevis* of *Alburnus alburnus* and *Abramis brama*; Cu content in *P. laevis* of *Abramis brama*; Cu, Cd and As content in water and sediments from the Bulgarian section of the river Danube with results from the Kudelin biotope; seasonal changes in As content in liver, skin and muscle of *Alburnus alburnus* and *Abramis brama*.

Applied scientific contributions

Arsenic, copper, and cadmium exceedances were detected in water and sediments of the study reach of the river. Danube pose a potential risk of elevated concentrations of toxic elements in other fish and parasites.

In my opinion, a specific scientific and applied contribution is made by some of the recommendations included in the thesis on the need for increased monitoring and continuous research (Ministry of Environment and Water, Executive Environmental Agency, Ministry of Agriculture, Bulgarian Food Safety Agency) on the water and sediment quality, as well as on the heavy metal content in tissues, organs and parasites of fish in the study section of the river. Danube; the need to optimise transboundary cooperation to limit negative impacts from industry and agriculture; improving the state of the freshwater ecosystem and preserving species diversity.

In relation to the protection of human health and the reduction of toxicological risk, it is necessary to limit the consumption of the three fish species investigated (*Alburnus alburnus*, *Abramis brama* and *Shondrostoma nasus*) due to the reported exceedances of Cd and As concentrations in muscle samples compared to the acceptable limits; removal of fish skin prior to consumption; removal of internal organs of smaller fish such as redfish due to the highest concentrations and exceedances of the three elements tested in liver samples.

7. Critical remarks and questions.

I have no critical comments and recommendations. I believe that in terms of the scope, validity and execution of the experiments and originality of the results, the proposed material significantly exceeds the requirements for obtaining the Ph.

Questions:

1. Which of the elements, the subject of the dissertation, is necessary for the normal vital activity of flora and fauna?
2. Does the PhD student have information about rivers in Bulgaria that are polluted with heavy metals and pose a potential risk of contamination of hydrobionts?

8. Published articles and citations.

Publication activity of the PhD student includes 2 full text publications in WEB of Science (Q4). In both publications she is first author. Doctoral student Zaharieva has participated in 3 scientific international conferences (The proceedings of the conferences are published in WEB of Science publications).

All this gives me a reason to assume that the PhD student's contribution to the development of the dissertation, the description and interpretation of the results, as well as their formulation as scientific publications is substantial.

The submitted abstract is written in 34 pages and objectively reflects the structure and content of the dissertation.

CONCLUSIONS:

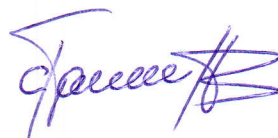
The peer-reviewed dissertation illustrates in an indisputable way Petya Zaharieva's theoretical knowledge and ability to conduct independent research. My overall assessment of the dissertation, based on its relevance, structure, content and theoretical generalizations, gives me reason to assume that it contains the results stipulated in the Law on the Development of Academic Staff in the Republic of Bulgaria, the PPPADRB and the Regulations of the Agrarian University, which constitute a contribution to science and practice. On the basis of the analysis I give a **POSITIVE** evaluation of the dissertation.

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 field of higher education: 4. Natural sciences, informatics and mathematics; professional direction: 4.4. Earth Sciences, Science Major: Ecology and Ecosystem Conservation.

Date: 16.11.2022

Plovdiv

Reviewer:



(Assoc. Prof. Dr. P. Zaprianova-Alexieva)