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REVIEW

by

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member of the scientific committee for awarding the educational and scientific degree "doctor" (PhD), pursuant to Order No. 22/07.03.2025 issued by the Director of the Institute of Biodiversity and Ecosystem Research – BAS.

About a dissertation for awarding the educational and scientific degree "doctor" (PhD) by:

Field of higher education: 4. Natural sciences, mathematics and informatics professional direction, 4.3. Biological Sciences, doctoral program "Ecology and ecosystem conservation"

Author: Blagovesta Dimitrova Dimitrova

Title: Impact of Pesticides on Amphibian Species from Water Bodies with Varying Degrees of Anthropogenic Influence in Central Bulgaria

Scientific advisor: Assoc. Prof. Simeon Lukanov, PhD

This review examines the dissertation thesis presented for acquisition of the educational and scientific degree "doctor" (PhD) in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, doctoral program "Ecology and ecosystem conservation". Blagovesta Dimitrova Dimitrova is a full-time PhD student in scientific specialty 4.3. Biological Sciences ("Ecology and ecosystem conservation"), to the "Ecosystem research, ecological risk and conservation biology" department at the the Institute of biodiversity and ecosystems research, Bulgarian Academy of Sciences. The thesis was developed as part of a full-time doctorate at the IBER - BAS. The PhD student's documents were prepared, deposited and checked according to the accepted requirements of the Scientific Council of IBER at the Bulgarian Academy of Sciences.

Short biography of the candidate

Blagovesta Dimitrova holds a Master's degree in Ecology, Evolution, and Systematics from LMU Munich and a Bachelor's degree in Zoology from the University of Nottingham. Her professional experience includes working as a biologist at the Bulgarian Academy of Sciences and as an environmental consultant in a private company. She actively participates in national and international scientific projects focused on amphibians, pesticides, invasive species, and biodiversity. She is a recipient of the UNESCO MAB "Young Scientist Award" and the "Lehre@LMU Award" for scientific research. She has authored numerous publications in renowned scientific journals related to environmental conservation and amphibian ecology. Blagovesta has also taken part in various conferences across Europe, including in Italy, Germany, Serbia, and Bulgaria. She is fluent in English, German, and Spanish. She possesses excellent organizational and communication skills, developed through involvement in international initiatives and volunteer work. Her interests include hiking, photography, cultural exchange, and science communication.

General characteristics of the dissertation thesis

The content, structure, and format of the dissertation comply with the established requirements and include all necessary sections. It comprises 122 pages and is structured as follows: "Acknowledgements" (1 page), "Summary" (2 pages), "List of Figures" (2 pages), "List of Tables" (2 pages), "Introduction" (2 pages), "Literature Review" (15 pages), "Objectives and Tasks" (2 pages), "Materials and Methods" (17 pages), "Results" (23 pages), "Discussion" (21 pages), "Conclusion and Key Findings" (2 pages), "Declaration of Originality" (1 page), "References" (21 pages), and "Appendices" (6 pages). The dissertation includes 14 tables and 20 figures. The reference list comprises 203 unique sources (1 in Cyrillic and 202 in Latin script).

The thesis summary is made according to the generally accepted requirements, contains 29 pages, contains basic information from the research and follows the basic structure of the dissertation.

I have not detected plagiarism in the dissertation and the thesis summary presented to me for review.

Literature awareness and theoretical preparation of the candidate

The PhD student knows perfectly the issues involved in the dissertation thesis. She emphasizes on all the major aspects of the study. There are a significant number of literary sources in the work - 203 unique titles, most of which are in English language, in my opinion no significant works have been missed. The PhD student has cleverly used the accumulated knowledge of the problem, which is successfully applied in the interpretation of the results. She demonstrates free handling of the specific terminology. In the course of the work, his good practical preparation and a professional approach to the subject matter is evident.

Methodical approach

The study was conducted according to a modern and adequate, well-developed methodology, properly applied, which allows the achievement of the objective and solving the tasks.

The doctoral candidate demonstrates a complex and scientifically grounded methodological approach that combines spatial analysis, field observations, and laboratory experiments. The research is structured into three main areas.

The first area involves a spatial-ecological analysis based on a large volume of secondary data collected through the SmartBirds Pro system, GBIF, and personal observations. The data were filtered and processed using ArcGIS software, employing CORINE Land Cover (CLC 2018) maps. To assess species richness and similarity between land cover types, the Rényi diversity index and Morisita cluster analysis were applied using the PAST software, version 4.07. This stage is critically important for defining the ecological significance of agricultural areas as amphibian habitats and provides a broader picture of the national distribution of the studied amphibian species.

The second area focuses on field studies, including monitoring of water bodies, acoustic observation, and analysis of water samples. Acoustic devices, AudioMoth, configured for nighttime recording, were used, and the data were processed on the Arbimon platform using Random Forest models. These models demonstrated high accuracy in detecting target species, with the analysis validated and statistically supported through non-parametric tests (Shapiro-Wilk, Kruskal-Wallis, Spearman). Additionally, the influence of meteorological factors was considered, providing further reliability to the interpretations made.

The third area involves a series of laboratory experiments aimed at determining the effects of ammonium nitrate and a fungicide (Ortiva® TOP SC) on the development and behavior of larvae of several amphibian species. Tests for acute and chronic toxicity, as well as behavioral analyses for predator avoidance, were conducted. All experiments were strictly controlled and repeated multiple times, with variables such as mortality, locomotor activity, growth, and body condition index recorded. Analyses were performed using Statistica v10.0, applying non-parametric methods appropriate to the data characteristics.

The methodological implementation is characterized by clearly defined objectives, carefully selected indicator species, and a high degree of scientific precision. The tools and techniques used are modern and well-validated, and the approach aligns with international best practices in ecology, conservation biology, and ecotoxicology.

What is striking is the huge amount of field and laboratory work done by the PhD student, as well as the large amount of time invested in the processing of the results, which also requires specific preliminary knowledge and skills, from which comes the significant difficulty in this kind of research.

Significance and importance of the results, interpretations and conclusions

The results presented in the dissertation are based on a comprehensive empirical approach encompassing spatial analyses, field observations, and laboratory experiments. The significance of the established relationships between agricultural areas and the distribution of five amphibian species in Bulgaria is indisputable. Four of the five target species are found to a significant extent precisely in agricultural regions, with over 50% of observation points for some species located within such territories. This underscores the crucial role that agricultural landscapes play in maintaining biodiversity, especially for species with limited habitat preferences and vulnerability to chemical pollution.

The doctoral candidate applied diverse and well-founded statistical models to predict species distribution in situ on one hand, and laboratory tests assessing the effects of ammonium nitrate and fungicide under ex situ conditions on the other. The robustness of the results stems from a clearly justified methodology and carefully applied and correctly interpreted statistical tests. No attempts were observed to exaggerate effects, which contributes to the objectivity of the conclusions. Validation of observations through multiple repetitions strengthens the reliability of the data.

The experimental part confirms the harmful impact of commonly used agrochemicals. The effects of ammonium nitrate and the fungicide Ortiva® TOP SC are presented through precise quantitative assessments of mortality, locomotor activity, and growth. In some cases, a clear dose-dependent response is noted, such as in Rana dalmatina, where high concentrations result in significant differences compared to control groups. Behavioral analyses, including locomotor activity in the presence of predator cues and avoidance behavior, provide additional depth to the interpretation of toxicological effects.

The interpretations in the discussion section of the dissertation are logical and well-argued. The doctoral candidate clearly acknowledges the limitations of the models used and demonstrates maturity in handling complex statistical data. Supporting the main conclusions with up-to-date literature sources adds to their credibility.

The results in the dissertation are both statistically valid and interpreted in a scientifically precise and well-reasoned manner. Their significance lies not only in contributing to the fundamental ecology of amphibians but also in their potential application in the management of

agricultural territories. The doctoral candidate demonstrates analytical competence, methodological consistency, and a high level of scientific ethics in presenting the data and conclusions.

This dissertation effectively represents the first study of its kind for the territory of Bulgaria, making the topic highly relevant and underscoring the crucial need for similar research in the future.

Critical notes to the dissertation thesis

- I recommend avoiding foreign loanwords in the Bulgarian text, such as “associated”, “intensification”, “initiation”, and others.
- It is a good idea to number the bibliography, which makes it much easier to work with.
- The Latin names of species are conventionally written in italics. In the reference list, they are not formatted accordingly.

Scientific contributions

The formulated scientific contributions are divided into two categories: scientific and scientific-applied contributions. All of them accurately and correctly reflect the main achievements of the doctoral candidate.

This is the first comprehensive study of its kind conducted in Bulgaria on this topic. Within the research, a clear link was established between agricultural practices and the distribution of five amphibian species, revealing that a large part of the populations inhabit agricultural areas and are exposed to the effects of agrochemicals.

Among the significant scientific contributions are the results demonstrating the higher sensitivity of the Agile frog (*Rana dalmatina*) to ammonium nitrate compared to the common toad (*Bufo bufo*). Differences in the behavior of the two species upon pesticide exposure were shown, including their ability to recognize chemical signals from predators - an aspect that has not previously been investigated in similar studies in the country. Another important contribution is the demonstrated lack of avoidance behavior in the larvae when exposed to ammonium nitrate, which questions the effectiveness of protective mechanisms in certain species under pollution conditions.

The work contributes to expanding knowledge in toxicology by demonstrating direct and indirect effects of a specific fungicide (Ortiva® TOP SC) on the development, locomotor activity, and metamorphosis of anuran larvae. Differences in species richness and amphibian activity between rice fields and natural water bodies were documented, allowing well-founded conclusions to be drawn about exposure pathways of chemicals in different habitats. The scientific significance of the results is further emphasized by the fact that this study presents the first toxicological data for the investigated pesticide, which can serve as an argument for reevaluating its use.

From the perspective of the scientific-applied contribution, the dissertation offers results with tangible practical value. They can be used in the development of sustainable agricultural practices and in the assessment of ecological risks posed by agrochemicals. The collected data are applicable for designing future monitoring programs and environmental assessments, as well as for formulating measures to protect amphibians in agroecosystems. The study provides a scientific basis for including agricultural water bodies in conservation strategies. Additionally, the developed methodology for laboratory experiments with fire-bellied toads can be successfully applied in toxicity assessments of other chemical substances, and the results of the work are suitable for use in educational and awareness campaigns aimed at environmental protection.

In summary, the dissertation contains theoretical, methodological, and applied scientific contributions. The study is distinguished by its originality, interdisciplinary approach, and high degree of applicability. The presented results make a significant contribution to the advancement of science in the fields of ecology, toxicology, and biodiversity conservation, and deserve high scientific recognition.

Scientific publications reflecting the dissertation thesis

A total of three publications have been produced based on the dissertation research. The works are co-authored, with the doctoral candidate serving as the lead author on one of them. It is important to note that research of this nature would be extremely difficult to conduct by a single individual, so teamwork is a common and entirely normal aspect of contemporary scientific studies. One of the publications was printed in a Bulgarian journal, while two were published in international peer-reviewed journals, all in English. All three articles have an impact factor ("Ecological Frontiers" (formerly Acta Ecologica Sinica) – Q1, $IF_{2023} = 4.7$; "Animals (MDPI)" – Q1, $IF_{2023} = 2.7$; and "Acta zoologica bulgarica" – Q4, $IF_{2023} = 0.4$). All three publications fully address the dissertation topic and accurately present the obtained results.

Additionally, the doctoral candidate has participated in two international scientific conferences and one national conference. The fact that shortly after its publication, one of these articles (the one in "Ecological Frontiers") has already been cited in another international peer-reviewed journal indicates that the published results are current and generate interest within the scientific community.

Conclusion

I do not know the doctoral candidate Blagovesta Dimitrova Dimitrova personally, so I assess her qualities as a scientific specialist and herpetologist solely based on the dissertation submitted for my review and the scientific articles published to date. In this regard, I can say that Blagovesta Dimitrova Dimitrova is establishing herself and proving to be a very good specialist, a meticulous researcher and analyst, an exceptionally well-prepared herpetologist and ecologist, whose name is already well regarded by the scientific community.

During the doctoral period, the candidate has conducted extensive field and laboratory work, as well as thorough analysis and processing of the obtained results. A substantial amount of material has been collected based on modern, up-to-date methods. Valuable original results have been obtained, on the basis of which she makes numerous contributions of both original and confirmatory nature.

The dissertation shows that the PhD student Blagovesta Dimitrova Dimitrova possesses in-depth theoretical knowledge and professional skills to obtain the scientific and educational degree "doctor" (PhD) in professional field 4.3. Biological sciences, in the scientific specialty "Ecology and ecosystems conservation" by demonstrating qualities and skills for independent conduct of scientific research. The critical remarks I have given above are in the nature of recommendations for the future work of the PhD student and in no way detract from the overall contribution of the dissertation.

Due to the above, I confidently give my **positive assessment** of the conducted research, presented in the dissertation thesis, the abstract, achieved results and contributions. Therefore, I propose to the members of the Scientific Council of the Institute of biodiversity and ecosystems research at the BAS to vote **positively** for the acquisition of the scientific and educational degree

“doctor” (PhD) in professional field 4.3. Biological sciences, in the scientific specialty “Ecology and ecosystems conservation” of Blagovesta Dimitrova Dimitrova.

Plovdiv, 21.05.2025.

Reviewer:

(Assoc. Prof. Ivelin Mollov, PhD)