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Review of the doctoral dissertation of M.Sc. Kalisa Amarsingh Bogati, entitled “The impact of simulated drought on changes in microbial biodiversity and soil biological activity”

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The basis for the review is the letter from prof. dr hab. Justyna Rogalska, Dean of the Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun informing about resolution of Scientific Council of the Discipline of Biological Sciences appointing me as a reviewer of the doctoral dissertation. The basis for applying for a doctoral degree is a series of three thematically related scientific articles published in years 2022-2023 in journals from the JCR database. The doctoral dissertation consists of a summary of research, one review publication and two research publications. All these publications constitute a coherent whole. The manuscript includes the following chapters: Abstract, Introduction, Aim of the study, Hypothesis, Research objectives, References, Contributions and declarations, Publication list, Conclusions, Summary and Curriculum Vitae.

List of publications constituting the doctoral dissertation:

1. Bogati, K., & Walczak, M. (2022). Review - The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy* 12(1):189. doi: <https://doi.org/10.3390/agronomy12010189>.
2. Bogati, K.A., Golińska, P., Sewerniak, P., Burkowska-But, A., Walczak, M. (2023). Deciphering the Impact of Induced Drought in Agriculture Soils: Changes in Microbial Community Structure, Enzymatic and Metabolic Diversity. *Agronomy*, 13, 1417. doi:<https://doi.org/10.3390/agronomy13051417>.
3. Bogati, K.A., Sewerniak, P., Walczak, M. (2023). Effect of changes in soil moisture on agriculture soils: response of microbial community, enzymatic and physiological diversity. *Ecological Questions*, 34(3). doi: <https://doi.org/10.12775/EQ.2023.043>.

The total score of the published manuscripts is 220 points, and their total Impact Factor is 8.21.

The subject of research undertaken by the author is up-to-date and very important, and it results from the research issues that are being carried out at the Department of Environmental Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University in Toruń.

In recent years, climate change has become one of the most serious challenges facing humanity. The changes we are already experiencing will threaten, among others, agriculture and its indispensable element, which is the soil environment. It is expected that, in addition to global warming, a significant threat will be the lack of precipitation, limited availability of water and associated with it drought. This will threaten the agricultural production and food safety in many parts of the world. It is worth to remembering that soil properties depend not only on its physical and chemical characteristics, but to a significant extent on the living organisms inhabiting it, including microorganisms. Soil microorganisms play an important role in biogeochemical cycling of macro- and micronutrients and other elements which are vital for plant growth. Changes induced by climatic change in soil enzymes produced by soil microorganisms can radically modify biogeochemical cycles and functioning of the whole agroecosystem.

The introduction to the three publications is written clearly and includes information of current climate changes in Central Europe, and its effect on microbial activity. This part well introduces reader to the dissertation topic. Research presented in the thesis concerns explanation of long-term drought impact on the biodiversity of microbial communities, activity of soil microbial enzymes and changes in the physiological diversity of soil microorganisms in agricultural soil. Author has presented three hypothesis and three research objectives. The research methodology was described in detail in the publications constituting the dissertation and its description was omitted in summary of research which I consider reasonable.

The first publication is a literature review. In this review 183 relevant and timely sources are cited. The manuscript is well structured and demonstrates current knowledge and understanding of the drought impact on soil microorganisms. The author focused on the effect of drought stress on soil microbial community abundance, structure and enzyme activities. Moreover, the impact of drought on plants growth and development as well as the role of soil microorganisms in these processes was discussed. Also the methods of mitigation the drought effect on diversity of microbial community and its abundance and activity was presented. An important part of the review is chapter explaining the importance of plant growth-promoting rhizobacteria and fungi in mitigating the adverse effects of drought stress on plants.

The aim of the second publication was the determination of the effect of prolonged drought on the microbial community, including bacteria, fungi and actinomycetes, their

enzymes, and metabolic diversity in selected soils samples collected from agricultural areas near Torun, Poland. The experiment was well designed. Four different types of soils were chosen to reflect average soil conditions in this part of Poland. The methods used in the experiment do not raise any objections and enables verification of the formulated hypothesis. Chemical properties of soil used in the experiment was analysed in laboratory with standard methods. Determination of the number of bacteria, actinomycetes and fungi in soil samples, as well as soil enzymatic activities was done with widely accepted methods. The Biolog EcoPlate Technique was applied for assessing the metabolic diversity of soil microorganisms. The 16S rRNA and ITS amplicon sequencing technique were employed to identify and compare bacteria and fungi present within analysed soil samples. Advanced statistical methods were used to analysed the data, e.g. the principal component analysis (PCA) was performed to assess variations in the impact of drought stress on analysed parameters.

The results of these experiments showed that:

- prolonged drought conditions decreased the number of bacteria, and significantly increased the number of Actinomycetota but not affected the number of fungi,
- prolonged induced drought led to an overall reduction in the metabolism of carbohydrates, carboxylic and acetic acids, and amino acids, polymers, and amines,
- the relative abundance of Actinomycetota and Bacillota increased, while that of Pseudomonadota and Bacteroidota decreased after prolonged drought conditions.

An especially interesting result obtained by the author is the increase of relative abundance of the genus *Fusarium* in soil samples after prolong drought. It means that drought stress may increase pressure from soil borne pathogens to agricultural plants.

The third publication included in the doctoral thesis is based on the same experiment design and research methods as in the previous one. In the first experiment the soil sampling and further analyses were carried out in spring season whereas in the second experiment in autumn. The Author aim was to include the season factor in the analyses.

The results of these experiments showed that:

- prolonged drought conditions decreased the number of bacteria and Actinomycetota,
- the enzyme activities were significantly decreased under drought stress conditions,
- changes in soil moisture can cause overall reduction in metabolism of carbohydrates, carboxylic and acetic acids, amino acids, polymers, and amines,
- decrease in relative abundance of Actinobacteriota, Bacteroidota, and Acidobacteriota after prolonged drought conditions was observed.

The author also discussed the possible methods that can be used to mitigate the adverse effect of drought on soil microorganism.

After publications constituting the doctoral dissertation the section “Conclusions” and “Summary” is included in which the author summarizes the results and formulates three conclusions and make some recommendation for future research. The doctoral thesis ends with section “Curriculum Vitae” containing information about student’s work experience, publications and other scientific achievements.

Reviewer’s remarks and questions:

- Information about crops cultivated in the fields at the time of soil sampling should be provided as the plant cover may affect the microbial composition and activity.
- I would like to know if soil samples were taken at exactly the same area in both experiments? Information provided on maps in second and third publication suggest that not.
- There is no data provided about the temperatures during the experiments carried out in containers (pot experiment). There is only information that “... soil samples were protected against rainfall at ambient temperature, ...”. It seems that such information is relevant, as not only the season of soil sampling and soil moisture is important but also the temperature conditions during the experiment.
- In my opinion, the chapter "Conclusions" should be renamed to “Summary” and the next chapter “Summary” should be called “Conclusions”.
- Which of the tested parameters of microbial activity could be according to the author’s opinion the best indicator of drought-induced changes in the soil?
- What are the author recommendations for prevention of soil drought and how we can mitigate the negative effect of drought stress on soil microbiome and plants.
- In which season there is the highest risk of drought in Poland? Furthermore, there is a question arising why the experiment was not carried out in other seasons?

The overall achievements of the Ph.D. student are significant and deserve to be emphasized. In addition to papers included in her doctoral dissertation, she is a co-author of 5 other publications in journals from the JCR database. These publications have been published in such journals as: *Frontiers in Plant Science*, *Continental Shelf Research*, *Zootaxa*, *Environmental Monitoring and Assessment*, and *Symbiosis*. The total IF of these publications is 16,876. The author has participated in many workshops and conferences. It is worth mentioning that PhD student has received 5 grants.

Final conclusion

Summarizing I must emphasise that the research presented in the dissertation constitutes a significant and valuable contribution to understanding the effect of drought on soil microorganisms, especially their diversity, physiological biodiversity and their enzymatic activity. The results were published in well-known journals from the JCR list and student is the

first author of the manuscripts. In addition, the content and quality of the reviewed dissertation demonstrates student ability to design and conduct scientific research, as well as analyse and report the results. My overall assessment of the thesis is positive. The aim of the research was achieved.

In my opinion the doctoral dissertation “The impact of simulated drought on changes in microbial biodiversity and soil biological activity” by M.Sc. Kalisa Amarsingh Bogati is original scientific contribution and meets all requirements set for PhD thesis in Article 187 of the Act of July 20 2018 Law on Higher Education and Science (Dz. U. z 2018 r. poz. 1668 z poz. zm.).

In conclusion, I submit a request to the Scientific Council of the Discipline of Biological Sciences of Nicolaus Copernicus University in Toruń to admit M.Sc. Kalisa Amarsingh Bogati, to further stages of the doctoral procedure.

Dariusz Bpelt