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Review of the Doctoral Dissertation

The review of the doctoral dissertation of Arlinda Cakaj M.Sc., entitled „Alternative heavy metals garden – evaluation of perspectives to use new bioindicators for air pollution".

1. Formal basis for the preparation of the review

The formal basis for the preparation of the review is a letter from the Chairman of the Council of the Scientific Discipline of Environmental Engineering, Mining and Energy, prof. dr hab. inż. Mariusza Sojki of the Poznan University of Life Sciences (WI.4000.8/2024) of 27.06.2024 informing about the resolution of the Scientific Council entrusting me with the duties of the reviewer and the preparation of the opinion of the doctoral dissertation as formulated above. The opinion was prepared based on the statutory requirements specified in par. 187 of the Act of 20 July 2018 Law on the Law on Higher Education and Science (Journal of Laws 2020, item 85 as amended).

2. Candidate description

The author of the dissertation, Arlinda Cakaj M.Sc. is the author or co-author of 9 publications, she actively participated in 21 scientific conferences in various countries, she paid a visit to the ARGANS Institute twice in the framework of an internship. Moreover, she took part in the "Innovator" program (no. 02/2022/INN).

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3. Subject of the review

The subject of the review is the study entitled "Alternative heavy metals garden – evaluation of perspectives to use new bioindicators for air pollution" by Arlinda Cakaj M.Sc., written under the supervision of Prof. UPP dr hab. Maria Drapikowska and co-advisor Dr inż. Marta Lisiak-Zielińska. Taking into account that all publications contained in the dissertation I have been presented with for assessment were earnestly compiled and positively assessed by reviewers in the publication process, as well as being published in very good scientific journals with a broad international scope and high Impact Factor, I will focus on a formal and substantive assessment of the prepared dissertation. The main part of Msc Arlinda Cakaj's, dissertation is a cycle of three publications that are consistent in terms of the subject matter, published in the years 2023-2024. The submitted body of works includes the following publications:

1. **Cakaj A.**, Lisiak-Zielińska M., Hanć A., Małecka A., Borowiak K., Drapikowska M. 2023. Common weeds as heavy metal bioindicators: a new approach in biomonitoring. *Scientific Reports*, 13(1), 6926. https://doi.org/10.1038/s41598-023-34019-9

2. **Cakaj A.**, Drzewiecka K., Hanć A., Lisiak-Zielińska M., Ciszewska L., Drapikowska M. 2024. Plants as effective bioindicators for heavy metal pollution monitoring. *Environmental Research*. https://doi.org/ 10.1016/j.envres.2024.119222

3. **Cakaj A.**, Hanć A., Lisiak-Zielińska M., Borowiak K., Drapikowska M. 2023. *Trifolium pratense* and the heavy metal content in various urban areas. *Sustainability*, 15(9), 7325. https://doi.org/10.3390/su15097325

In the case of the doctoral dissertation based on the co-authored cycle of publications, a significant element of the review is the assessment of the individual contribution of the PhD student in the publications which make up the dissertation, which can be carried out on the basis of the co-authors' declarations. The substantive contribution of the Candidate to the publications is significant, which indicates a leading role in caring out the studies

contained in the doctoral dissertation. The statements of all co-authors of the works are attached to the dissertation, based on the analysis of which it can be confirmed that the individual contribution of the PhD student to the works serving as the subject of the doctoral dissertation meets the required criteria when analyzed, serve as confirmation of this fact.

The publications comprising the cycle are prepared very well and carefully, with their total IF amounting to 17.8, whereas points determined in the communication from the Minister of Science dated January 5, 2024, regarding the list of scientific journals and peer-reviewed proceedings of international conferences is 340. The publications that make up the cycle were published in renowned journals from the JCR list with high Impact Factor (IF) coefficients (Environmental Research IF=8.3; Scientific Reports IF=4.6; Sustainability IF=3.9). All works were prepared in accordance with the guidelines of the journals which they had been published in, and underwent a substantive and editorial review process by each publisher.

The summary of academic achievements - a personal research paper of the PhD student amounting to 92 pages - contains a list of abbreviations used, a summary in Polish and English, a theoretical introduction, research hypotheses, main aim and detailed aims of the study, a description of the assumed research methodology, characteristics of the obtained results, discussion, conclusions, references thematically connected with the carried out research, articles forming a coherent cycle of publications and a statement by the PhD student and coauthors of the publication.

The summary starts off with a 3-page Introduction, which is very important in terms of the rationalization behind the research topic within the selected discipline and formulating the research problem. The manner in which it was prepared proves that the PhD student possesses the ability to study and analyze scientific works, which can be stated based on the appropriately selected references. In the subchapter, the PhD student formulated the research hypotheses as well as the main aim of the dissertation,

i.e.: "Explore the heavy metal bioindicator potential of various plant species". This aim comprises four detailed aims, which are:

- Evaluation of heavy metal accumulation: assessment the ability of selected plant species, including *T. pratense* L., *R. acetosa* L., *A. retroflexus* L., *P. lanceolata* L., *A. rosea* L., to accumulate heavy metals (Cd, Pb, Cu, Ni, Zn) in their leaves and roots tissues under controlled conditions.

- Assessment of the ability to translocate heavy metals between plant organs: determination of the ability of studied species to bioconcentrate and translocate HMs, along with the assessment of their physiological responses to stress, including evaluating oxidative stress parameters and antioxidative enzyme activity.

- In situ plant and soil research: detection variations in heavy metal contamination in plants and soil at research sites representing the different land uses typical of urban areas.

- Establishment of bioindicator recommendations: provide recommendations for the use of the studied plant species as bioindicators in environmental monitoring, considering their effectiveness in assessing heavy metal pollution in both urban and non-urban areas.

Individual research aims contained in the summary of academic achievements have their cross-references in publications included in the dissertation and have been realized, as confirmed by the result chapters of all the works. The dissertation is also characterized by a practical dimension, and thus a setting out a utilitarian purpose would do much to enrich the reviewed achievement. The author of the dissertation indicates that the carried out research aimed to fill a critical research gap involving the study of physiological reactions of selected weeds to different levels of heavy metal contamination along with an explanation of the mechanisms which lie at the foundation of their potential as bioindicators. The Methodology chapter was constructed in a coherent manner, by first describing the plant species which

were used in the research. Next, the carried out experiments were described in a general manner, and the parameters used in the statistical research described. It is the Reviewer's opinion that the approach to this element should have been more meticulous and detailed. The Results chapter was presented in a systemized and comprehensible manner. The PhD student referred to a specific publication each time in the description of this chapter, listing the most important results derived from the carried out research. The Discussion chapter is clearly formulated, and was divided into appropriate subchapters in reference to specific results. However, a more extensive interpretation of why such results were obtained is missing in this part of the summary of academic achievements.

4. Most important scientific achievements of PhD candidate

The research subject undertaken by Arlinda Cakaj M.Sc. is very interesting and important, both in terms of the cognitive as well as practical aspect, due to which the choice of research topic ought to be considered appropriate and fully justified by the latest scientific achievement and practical needs. The search for ever more effective strategies of monitoring individual components of the natural environment, and thus the selection appropriate bioindicators of contamination with potentially toxic elements (PTEs), is extremely important due to the increasing risk of contamination of the natural environment by these elements. Bioindication takes advantage of the ability of organisms to intrasystemically accumulate, e.g. heavy metals and is thus increasingly frequently used in order to expand the comprehensiveness of environmental research. It is important to highlight, at this point, the importance of in-situ experiments carried out by the Candidate, where controlling the level of individual elements of the environment affecting the plants is made somewhat more difficult. Based on physiological characteristics and bioconcentration values, candidate indicates that *Trifolium pratense* and *Plantago lanceolata* stand out as the most suitable bioindicators for heavy metal pollution, displaying consistent efficacy across multiple contaminants. *Trifolium pratense* demonstrated remarkable adaptability and

efficient uptake of Zn, Ni, and Cd, while *Plantago lanceolata* excelled in accumulating high concentrations of Zn and Cd.

Thanks to the review of literature, the PhD candidate planned the realization of the assumed research task in a correct manner, and carried out the necessary research along with preparing and analyzing results. She thus proved her maturity and independence in solving such research problems. The results presented in the dissertation can serve as the basis for achieving further scientific progress and practical application.

5. Detailed remarks and discussion

In terms of the substantive aspect, the work merits a positive opinion, though certain detailed objections are worth noting, with some of them subject to discussion; however, I hope that these remarks will help the PhD student to aptly refine her research workshop.

When familiarizing myself with the contents of the dissertation, a few general questions arise:

- Please explain your understanding of the term "heavy metals gardens" in the title. How does the structure of this experiment reflect the assumptions of the research conducted ?

- Please indicate which criteria were used to select plant species for this research ?

Moreover, when reading the dissertation, the following detailed remarks arise:

- While appreciating the scientific value of the cycle of publications, it is the reviewer's opinion that the Polish summary was prepared with inadequate diligence, especially in terms of making use of specialized wording.

- I had some difficulty connected with the lack of a clear reference to the research hypotheses posed in the conclusions. As the research paper progressed, the Author should have carried out their verification.

- Despite the Author pointing to the possibility of the most suitable bioindicators for heavy metal pollution in practice, the summary of

academic achievements is missing information to what extent the studies will contribute to development of the disciplines of Environmental Engineering, Mining and Energy production. A request is also made to familiarize the reader with further research perspectives.

- "Supplementary materials" have not been attached to the prepared dissertation, both in the case of the first as well as second publication.

- I recommend replacement of the term "heavy metals" with scientifically defensible terms like "potentially toxic elements", "trace metal elements".

- A few important aspects of information are missing in the materials and methods section: how many repetitions were carried out for the plant growing experiment, according to which classification is the soil texture determined, at what level was the moisture content of the soil maintained over the course of the experiment. On page 15 of the dissertation, the Author states that "Controlled heavy metal contamination was applied through irrigation with metal nitrate solutions at two levels: low and high" – on what basis were the "low and high" ranges determined?

6. Final Assessment

I thus affirm that the presented doctoral dissertation of PhD candidate Arlinda Cakaj M.Sc. titled "Alternative heavy metals garden – evaluation of perspectives to use new bioindicators for air pollution" fulfills the formal and customary requirements set forth for doctoral dissertations in the Art. 187 of the Act of 20 July 2018 on the Law on Higher Education and Science, which states that:

1. The doctoral dissertation presents general theoretical knowledge of the candidate in the discipline or disciplines as well as the ability to independently carry out scientific or artistic work.

2. The topic of the doctoral dissertation is an original solution to a scientific problem, an original solution in terms of applying the results of own scientific research in the economic or social sphere, or an original artistic accomplishment.

Taking into account the above as well as my positive assessment of the doctoral dissertation in terms of the possessed theoretical and practical knowledge, the specificity of the issues under research, the ability to interpret results and the appropriate application of terms and names along with specialist terminology, I confirm that Arlinda Cakaj M.Sc. possesses the ability to independently conduct scientific research in the field of engineering and technical studies.

I hereby call for the Council of the Science Discipline of Environmental Engineering, Mining and Energy to admit Arlinda Cakaj M.Sc. to subsequent stages of the doctoral programme.

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Prof. dr hab. inż. Maja Radziemska