UNIWERSYTET IM. ADAMA MICKIEWICZA W POZNANIU
Wydział Biologii

Dr hab. Aneta Piechalak
Laboratory of Genome Biology
Faculty of Biology
Adam Mickiewicz University
Uniwersytetu Poznańskiego 6
61-614 Poznan

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Evaluation of the doctoral dissertation

Ms. Arlindy Cakaj, M Sc.

Titled „Alternative heavy metals garden – evaluation of perspectives to use new bioindicators for air pollution"

The review of the doctoral thesis of Ms. Arlinda Cakaj, M.Sc. was prepared on the basis of the resolution of the Scientific Council of the Discipline of Environmental Engineering, Mining and Power Engineering of the Poznań University of Life Sciences dated 20.06.2024, as well as the letter from the Chairman of the Scientific Council of the Discipline of Environmental Engineering, Mining and Power Engineering, Prof. Dr. hab. Mariusz Sojka, dated 27.06.2024, in accordance with the requirements set out in Article 20(5) of the Act of 14.03.2003 on Scientific Degrees and Academic Title and Degrees and Title in Art (Journal of Laws of 2003, No. 65, item 595, as amended) and Introductory Provisions of the Act - Law on Higher Education and Science of 20 July 2018 (Journal of Laws of 2018, item 1668, as amended) .

The presented doctoral dissertation of M.Sc. Arlinda Cakaj was prepared under the supervision of Prof. UPP dr hab. inż. Maria Drapikowska from the Department of Ecology and Environmental Protection, Poznań University of Life Sciences, and Assistant Promoter: Dr inż. Marta Lisiak-Zielińska from the Department of Ecology and Environmental Protection, Poznań University of Life Sciences. The dissertation concerns the subject matter in the field of technical sciences in the discipline of environmental engineering, mining and power engineering.

The dissertation submitted for evaluation by Ms. Arlinda Cakaj, M.A., consists of three thematically consistent scientific articles. The papers were published between 2023 and 2024 in the journals: *Scientific Reports* (2023), *Sustainability* (2023) and *Environmental Research* (2024). All papers were published in journals listed in the *Journal Citation Reports* (JCR) database, with a combined impact factor IF: 17.8 and a total score of 340 points according to the Communication from the Minister of Science dated January 5, 2024, regarding the list of scientific journals and peer reviewed proceedings of international conferences.

The series in question includes the following papers:

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. DOI: 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. DOI: 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. DOI: https://doi.org/10.3390/su15097325

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The problems that Ms. Arlinda Cakaj, M.Sc., undertook to solve, as specified in the Title and Section 2: Research hypothesis, aim and detailed aims of the study, are in line with the topics of research conducted by Prof. Maria Drapikowska's team. In recent years, increasing attention has been paid to assessing the health and well-being of species, populations and ecosystems. Scientific institutions, ecologists, government agencies and non-governmental organizations are all involved. Due to the huge increase in the level of biotic and abiotic factors that can affect the environment, ways are being sought to study, assess and monitor the state of ecosystems and their components. Since ecosystems consist of thousands of living species, it is very difficult to assess the condition and monitor changes in it. Organisms that provide both qualitative and quantitative information are called biological monitors or biomonitors, and the process of measuring toxic substances in an organism is known as biomonitoring. It is extremely important to develop appropriate markers that can be used to assess status and monitor changes. Various organisms, such as plants, plankton, animals and microorganisms, can be used to determine the state of the environment and will therefore be considered bioindicators.

The work constituting the doctoral thesis concerns the search for suitable bioindicators of heavy metal pollution (such as Cd, Pb, Cu, Zn or Ni), among the widely distributed and common weeds - rough amaranth (*Amaranthus retroflexus* L.), meadow clover (*Trifolium pratense* L.), wood sorrel (*Rumex acetosa* L.), plantain (*Plantago lanceolata* L.) and pink mallow (*Alcea rosea* L.).

Formal evaluation of the dissertation

The submitted dissertation includes the previously mentioned three thematically consistent articles of an experimental nature. All papers are multi-authored, and the PhD Candidate is the first author in them. According to the declarations, Ms Arlinda Cakaj own contribution to the creation of the articles, and more generally to the research work conducted, consisted of collecting the material, conducting experiments, analyzing the results, contributing to the scientific conception of the research, reviewing the literature and preparation of the manuscripts (including their visual aspect). In papers published in *Sustainability* and *Environmental Research*, the PhD student also serves as a correspondence author. The statements of the other authors, especially regarding the articles published in *Scientific Reports* and *Environmental Research*, contain very similar descriptions of their contributions, i.e. co-authorship of the research concept, analysis of the material, preparation of the manuscript, which makes it somewhat difficult to assess the scientific skills of the PhD student.

The submitted documentation also includes a 90-plus page paper including title page, abstracts in Polish and English, list of used abbreviations, Introduction, Research Hypothesis and Objectives, Materials and Methods, Results, Discussion and Conclusion, as well as pdfs of the three articles forming the basis of the dissertation and the authors' statements.

The title of the dissertation was formulated correctly, and the research problem presented in the dissertation is appropriate to the title. The dissertation has been written correctly in terms of language and is generally stylistically correct. Sentences are formulated correctly with logical continuity. In addition, there are a few errors resulting from the use of laboratory jargon, which in no way affects the positive formal evaluation of the thesis.

It should be emphasised that, in addition to the manuscripts included in the PhD thesis, Ms Arlinda Cakaj's scientific output includes 6 scientific articles published in *JCR* journals with a total IF: over 26. I therefore believe that the scientific output of the PhD Candidate (not counting her numerous conference reports) is very good.

Summarising the formal assessment, I conclude that, on the basis of the prepared statements, I assess Ms Arlinda Cakaj's contribution to the preparation of the articles and the conduct of the research work as significant. The dissertation form presented by the PhD Candidate is complete, structured and readable and therefore meets the requirements for PhD theses according to the relevant legal basis.

Substantive evaluation of the doctoral dissertation

The main part of the presented dissertation, entitled '' Alternative heavy metals garden – evaluation of perspectives to use new bioindicators for air pollution" by Ms Arlinda Cakaj, consists of three thematically coherent articles published in very good scientific journals, which were positively evaluated by reviewers as well as editors from the field of Plant Science.

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. DOI: 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. DOI: https://doi.org/10.1016/j.envres.2024.119222
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However, out of the reviewer's duty, I will refer to the most important achievements and their synthetic discussion in the presented dissertation. Evaluating the dissertation from the substantive point of view, I state that both the planned research tasks and their implementation, the choice of research methods and discussion of the obtained results do not raise any doubts. The Introduction contains all the information necessary to introduce the problems of the dissertation.

The PhD student's brief Introduction outlined the problem of heavy metal pollution in the environment and the need to monitor it, which was a bit of a repetition of the information provided in the accompanying publications and therefore I believe this section could have been significantly shortened. I would have been more interested to read more and more detailed information about the plant species included as bioindicators, especially weeds and ruderal species, a table listing such species, and which factors they respond to would have been a good addition. As the author herself emphasised, this is a dynamically changing area of knowledge where there are constantly many question marks.

Purpose of the study

The author has clearly formulated research hypotheses, assuming that selected, commonly occurring plants: *Amaranthus retroflexus* L., *Trifolium pratense* L., *Rumex acetosa* L. and *Plantago lanceolata* L. have the potential to be used as bioindicators of heavy metals, both present in soil and air. The PhD candidate hypothesised that the level of metal accumulation in the organs of selected plants is dependent on the HM concentration in the soil and hence could be an indicator of environmental contamination and the physiological stress response is relevant and dependent on the level of accumulated metal.

Arlinda Cakaj, M.Sc., set herself the task of evaluating selected species in terms of their suitability as bioindicators and potential use in a so-called 'alternative heavy metal garden', where they would help assess air and soil pollution levels. To this end, four specific aims have been formulated. These tasks included assessing the ability of selected plant species to: accumulate heavy metals (Cd, Pb, Cu, Ni, Zn) in leaf and root tissues; long-distance transport of heavy metals; and bioconcentration and translocation of HMs. In addition, experiments were planned to investigate physiological stress responses, including the assessment of oxidative stress parameters and experiments conducted in situ on test sites typical for urban areas.

The research topic undertaken in the work of Arlinda Cakaj, is extremely important and relevant because of the great ecological potential of the obtained results. The PhD Candidate's research concerned the possibility of using common plants for environmental monitoring and there is very little research conducted on non-cultivated plants, which increases the difficulty of the experiments. The research area covered both controlled conditions and natural sites and, particularly noteworthy, the research was conducted on metal mixtures. This makes it necessary to take into account not only the uptake capacity of the plants, but also the interaction between the individual metals, which is known to be an extremely difficult task. Interpretation cannot be based on literature reports alone, because the nature of the interaction is dynamic and depends on many factors like the concentration of the metals themselves, the properties of the soil, the presence of other macro- and micro-elements such as calcium and magnesium, and the species and even the variety of plant. The question is, therefore, whether the PhD student checked the uptake, accumulation and transport of metals in the plants under study, if they are applied individually? If so, do you see changes of trend in the mixtures and what kind of interactions are present in such systems.

In publication number 1, Ms Arlinda Cakaj published the results from Experiment 1 where pots with 60-day-old plants were moved from greenhouse conditions to selected urban locations and remained there for a further six weeks. After this time, the samples taken were analysed for absorption and accumulation of heavy metals and physiological parameters such as photosynthetic activity, relative water content, antioxidant enzyme activity or hydrogen peroxide levels. The results obtained by Ms Arlinda Cakaj show that different species showed specific responses to heavy metal contamination, but a very similar and consistent trend in metal uptake regardless of species or location, where essential metals were taken up in higher amounts than non-essential metals such as cadmium or lead. Particularly interesting are the results regarding bioconcentration factors (BCF), where it was shown that for zinc and cadmium in all plants the BCF values exceeded 1. How can this trend be explained, is this related to Zn and Cd sharing, at least in part, the same transport system, as they are congeners. Another extremely interesting result is the high TF values obtained for lead, especially in *A. retrofexus* and *P. lanceolata*. This is all the more surprising as this element in most plants is quite poorly transported between roots and aboveground parts. Is it possible that some of the Pb in the leaves came from the atmosphere? Or perhaps the PhD student has another theory explaining this effect (which is also described in Publication 2).

The paper also contains the results of the physiological reactions of the studied plants, and, for me, it is extremely difficult to assess whether these reactions are a response to metals or to other environmental factors present in the locations, such as UV radiation, mechanical damage, pathogens or pests. Was this type of analysis undertaken when evaluating the results?

Publication 2 describes the results of Experiment 2, in which five plant species, i.e. *Amaranthus retroflexus* L., *Lolium multiflorum* L., *Plantago lanceolata* L., *Rumex acetosa* L. and *Trifolium pratense* Lupus grown under greenhouse conditions were treated with metals at selected concentrations after 77 days and the dosage was repeated after a further 10 days. This time, instead of copper, nickel was given as the fourth metal. As I understand it, due to literature data indicating that *Amaranthus retroflexus* L., *Plantago lanceolata* and *Trifolium pratense* L., have already been analysed as bioindicators of this element. In selecting HM concentrations, the PhD candidate was guided by data from the Finnish Ministry of the Environment (2007) for industrial areas, i.e. high limit values, and for other land uses, i.e. low limit values. It is not entirely clear to me why these data were chosen, and I will ask for clarification. Especially considering the high concentrations of non-essential elements. The results obtained allowed the PhD student to confirm that *Amaranthus retroflexus*, *Trifolium pratense* and *Plantago lanceolata* have the highest potential as possible bioindicators of heavy metal pollution.

Publication 3 contains the results of Experiment 3, where soil and *Trifolium pratense* L, a plant that was selected as a potential bioindicator, were sampled from a variety of locations. I believe that the sites were very well selected and show a great diversity of metal contamination. Publication 3 contains the results of Experiment 3, in which soil and *Trifolium pratense* L, a plant selected as a potential bioindicator, were sampled from different urban locations. I believe that the sites were very well selected and show a wide variety of metal contamination. As in publication 2, the obtained values of the bioconcentration factor BF remain for cadmium, lead and zinc for most of the samples at a level below 1. Similarly, the level of the translocation factor TF for these metals remains at a similar level. Interestingly, *Trifolium pratense* L. showed good uptake and accumulation of chromium and partly nickel. However, with regard to these results, I would ask the PhD student to interpret the chromium levels in the roots and leaves in relation to the elemental levels in the soil (especially at POZ 05/07 and 08 locations). I also do not fully understand why zinc analysis was omitted from the experiment. Especially since, as the PhD student showed in Experiment 1, cadmium and zinc accumulation are linked. I agree with Ms Arlinda Cakaj's conclusion that the selected plant *T. pratense* shows potential as a bioindicator of heavy metal pollution in urban areas. However, further studies under controlled conditions as well as in other urban areas and in different seasons are necessary.

However, observations on morphological symptoms were missing from all publications. Very often, plant bio-indicators play a key role in detecting environmental stressors by showing observable changes such as damage, colour changes, changes in leaf morphology or modifications in growth patterns. The doctoral student has completely omitted this aspect from her dissertation, although I think she has made such observations by even keeping photographic records.

At this point, I would like to stress that all posted questions or comments on the work of Mrs. Arlinda Cakaj and the attached study only indicate the need to develop the discussion on some very interesting issues and themes of the work. However, they do not diminish the high level of the research, and the value of the results obtained.

Final conclusion

In conclusion, I conclude that the doctoral thesis of Ms Arlinda Cakaj, M.Sc. submitted for review represents an original and novel solution to a scientific problem and testifies to the extensive

research skills of the PhD student. The reliably documented results are of high cognitive value and contribute important information on the possible use of plants referred to as weeds in bioindication. Reading the dissertation convinces that Ms Arlinda Cakaj is a mature researcher, very well prepared for further work.

I believe that the doctoral dissertation presented to me for review entitled. 'Alternative heavy metals garden - evaluation of perspectives to use new bioindicators for air pollution' meets the formal requirements for doctoral dissertations by the Act of 20 July 2018 (Journal of Laws of 2018, item 1668 with later amendments) and the customary conditions for doctoral dissertations. I therefore apply to the Council of the Faculty of Environmental Engineering, Mining and Energy of the University of Life Sciences in Poznań to admit Ms Arlinda Cakaj to further stages of the doctoral dissertation.

Poznań, 23.08.2024

dr hab. Aneta Piechalak, prof. AMU.