

Liège, September 10, 2021

Evaluation of PhD Thesis – Marc Hanikenne, ULiège, Belgium

PhD candidate: Michał Szopiński

Thesis title: “Characterization of *Arabidopsis* species from metalliferous and non-metalliferous sites in Southern Poland”

During his PhD project, Michał Szopiński characterized two *Arabidopsis* species, *Arabidopsis halleri*, a known Zn/Cd hyperaccumulator species, and *Arabidopsis arenosa*, that both live on metal contaminated and non-contaminated sites in Poland. The experimental work included the characterization of field and lab samples for metal tolerance and accumulation, photosynthetic parameters, the accumulation of chlorophyll and metabolites, as well as gene expression.

The manuscript is structured in 5 main chapters: an introduction, 3 chapters of results in the form of 3 (to be) published articles and a conclusion. By nature, this format results in some repetitions between the general introduction and the articles. However, I find that the general introduction could have offered a more in-depth description of what is known (and unknown) about the mechanisms of metal hyperaccumulation in plants. This section of the document mostly cites reviews and it is my opinion that it would be preferable to cite original reports. The introduction also contains unprecise or incorrect information. Indeed, when describing the contribution of different transporter families in Zn/Cd hyperaccumulation, I suggest to be much more careful in the presentation. For instance, pg 18, IRT1 was suggested to contribute to Zn or Cd uptake in *A. halleri*. This was never shown. It is the same for the putative function of NRAMP3/4 in mobilizing metals from the vacuoles in *A. halleri*. Pg 18-19 are also not precise, as the presented information is a mix between knowledge that is specific to *A. halleri* or that is more generic on metal transporters in *A. thaliana*. Some other statements are incorrect: (i) the sentence on the function of HMA2 in *A. halleri* (pg 18), (ii) FRD3 does not transport metals chelated by citrate (pg 19) and (iii) ZIF1 does not transport Zn-NA (pg 19). The 2 latter errors are also present in Fig. 6 (pg 76).

Chapters 2 and 3 are presented as published articles in international peer-reviewed journals, that are among top journals in plant science. This is a recognition of the quality and originality of the work.

Chapter 4, on the comparative analysis of metal homeostasis gene expression in *A. halleri* and *A. arenosa* is presented as a draft manuscript. It presents interesting data, and upon further improvement in the presentation, it will represent an additional fine contribution to the field.

Chapter 5, as a general conclusion, is very short. Discussing more extensively how this work contributes to our understanding of metal hyperaccumulation in a broader context that the comparison between the two species would be appreciated.

Altogether, I find that the work is of quality and original and represents a fine contribution to our scientific knowledge.

I also have a few minor comments:

- The manuscript still needs some editing (typos, formatting, grammar), in the non-published sections.
- I spotted some errors in references:
 - Please check the reference Words and Krämer. It is wrong. Krämer is the only author.
 - Motte et al. 2013 does not exist.
- For the results chapter, I would suggest to add a small paragraph describing the contribution of the candidate among all co-authors.
- I believe it would be good to include the supplemental material of papers as annexes to the thesis manuscript (to have all info in one document).

In my opinion, the doctoral dissertation submitted for review meets all the requirements set out in Article 13 of the Act of 14 March 2003 on Scientific Degrees and Academic Title and Degrees and Title in Art (Journal of Laws of 2017, item 1789) and in Article 179(1) of the Act of 3 July 2018 Introductory provisions of the Act - Law on higher education and science (Journal of Laws of 2018, item 1669). In view of the above, I hereby apply to the High Council of the Institute of Biology, Biotechnology and Environmental Protection of the Faculty of Natural Sciences of the University of Silesia in Katowice to admit Mr. Michał Szopiński, M.Sc. to further stages of the doctoral procedure.”

Liège,



M. Hanikenne

Marc Hanikenne