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Reviewer's opinion on the Ph.D thesis

Reviewer: Irena Brányiková, Ph.D.
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Thesis: **Beneficial and toxic effects of REE in algae and plants**

Author: **Nermeen Ashraf, M.Sc.**

The present Ph.D. thesis deals with an interesting and up-to-date topic of impact of rare earth elements on microalgae and plants.

The thesis consists of an Introduction (Chapter 1), which provides a current overview of related fields, Aims and Hypothesis (Chapter 2), followed by three chapters representing two published articles and one submitted article, and Chapter 6 – Summary, which concludes the thesis. The overall structure and page count are usual and in line with expectations, the number of references is rather high standard. On the other hand, in my opinion, the text of chapters 1, 2 and 6 is not well formulated, the level of English is not always sufficient and combined with extremely long sentences, the reader is sometimes prevented from understanding the message. I also find the annotation very imprecise and not informative enough. I consider the core of the thesis to be the paper published in the journal Aquatic Toxicology entitled "Effect of nanomolar concentrations of lanthanum on *Desmodesmus quadricauda* cultivated under environmentally relevant conditions", which is well structured, clear and written in good English.

Questions:

Pg. 18, sentence: "The degree to which lanthanides can be transported from soil to plants is almost 20% and much of this is concentrated in roots and less in stems and other vegetal tissues including reproductive structures or parts." Can you please explain what this 20% means? What do they refer to? Can you give me an example?

Pg. 20, sentence: "These plants have exceptional mechanisms through which they can accumulate metals up to a maximum of 10% of their dry weight (Baker et al. 2020)" The stated concentration of 10% metals in the dry weight of the plant is very surprising, so I looked up the article and tried to verify it. I found the article of the same name published as a chapter in a book from 2000 (not 2020). I quickly looked through the article, but I did not find this information. Can you please tell us what kind of metal can be concentrated in what kind of plant and by what mechanisms to such an extent?

On the page 45, the main aim of the thesis is defined as: "The main aim of the thesis was to evaluate REE phytoremediation using microalgae and plants that can predict the toxicity response of these elements to microalgae or in plants under environmental conditions." Can you please briefly and succinctly summarize your findings on this main objective?



Comments and questions to Chapter 4:

To my knowledge, a chemostat is characterized by being a steady-state bioprocess in which a microbial culture is continuously supplied with nutrients at a fixed rate and harvested at the same time to maintain a constant culture volume and biomass concentration. To achieve this steady-state, the culture must be limited by at least one essential nutrient, the supply rate of which determines the growth rate of the culture. In heterotrophic cultures, this is in principle easy to achieve, since the sources of energy and nutrients are usually dissolved in the supplied medium. In steady state, the specific growth rate of the microorganism is equal to the dilution rate and the cell concentration is given by the substrate concentration in the supplied medium. On the other hand, in the case of autotrophic growth (microalgae), it is more complicated to achieve a chemostat condition because the nutrients are partially dissolved in the medium (source of N, P, S and micronutrients), partially derived from the gas supplied to the culture (CO_2 – source of C) and the most important – source of energy is represented by the incident light.

Can you please explain how (by what means) you managed to achieve a constant concentration of biomass in your “chemostats”? What was the limiting factor? If you call these cultures “chemostatic”, why are there different cell concentrations in “week1” and “week2” (pg. 62, fig. 1.C)? Is this not evidence that the cultures were not in steady state, i.e., not chemostatic? Can you please share a record of the time-courses of the cell counts of each “chemostat”?

Do I understand page 69 Fig. 1.A and 1.B correctly, that all these cultures grew under the same conditions and you consider the 64% difference in cell number (at day 5) as not significant? What would be a minimal difference in cell count that you would consider significant?

Illumination certainly has a significant effect on photosynthetic parameters and growth of the cultures. Since the illumination had a sigmoidal time-course, was this taken into account during sampling? For example, were samples taken e.g. exactly the same hour of each day?

Did you measure the concentration of La in the biomass of *Desmodesmus*? Was it higher/lower compared to the culture medium? If not, what would you expect and why?

Although in my opinion the above parts of the thesis text could be improved, a considerable amount of experimental work has been delivered and the results have been successfully published in international scientific journals of good standard, therefore I recommend the submitted thesis for a public defense.



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Evaluation of the PhD manuscript of Ashraf Nermeen

The PhD thesis of Ashraf Nermeen "Beneficial and toxic effects of REE in algae and plants" presents novel and original information about the effects of REE (Lanthanum) in the green microalgae *Desmodesmus quadricauda* and in plants.

Three publications are included in the manuscript, for two of those Ashraf Nermeen is the first author. Page VI clearly specifies the contribution of the candidate to each publication.

Overall, the PhD manuscript fulfills the necessary criteria to be presented at the public defense. The discussion presents a nice synthesis of the contributions of the thesis in comparison with the literature on the subject.

I have two minor concerns:

1. The language in some (non-published) parts of the thesis would deserve attention.
2. In the chapter 2 « aims and hypothesis », the candidate starts with a summary of the different chapters rather than with the aims of the PhD. I recommend changing the order of the information and to improve the writing of the "principal goals" (page 46)

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