Master's Thesis Engineering Technology

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Uranium Toxicity to the Freshwater Plant *Lemna minor* in an Environmentally Relevant Metal Mixture: Selection and Validation of Potential Uranium-Biomarker Genes

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Introduction & objectives

Historical **uranium** (U) mining operations resulted in the contamination of large areas, including surface waters. The effects of U on the freshwater plant ***Lemna minor*** (*L. minor*) have been investigated, although they were mainly studied on individual and cellular level by measuring, for example, the growth inhibition whereby the number and the surface of the leaves, called fronds, were determined. Sequencing of the whole *L. minor* genome and transcriptome [1] gives possibilities to study the effects on molecular level.

The aim of this study is to select and validate genes that can be used as **potential U-biomarker** for the prediction of **U toxicity** on population level as well as on ecosystem level. In addition a previously discovered potential U-biomarker (primer 33) will be further tested. Since organisms growing in contaminated waters are not only exposed to U, but also to multiple co-contaminants, it is necessary to find biomarkers that react specifically to U exposure and not to the co-contaminants. Therefore, an **environmentally relevant metal mixture** is used, based on concentrations that were measured in the Beaverlodge Lake in Canada [2]. The most important co-contaminants (Cu, Ni, Zn and Pb) were selected and used in this research.

Materials and methods

Contaminants

- U0, U2 or U10 (0, 2 or 10 µM U)
- M0, M1, M10 or M100

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0.0157 µM Cu
0.0170 µM Ni
0.0765 µM Zn
0.0145 µM Pb

Exposure

1, 4 or 7 days

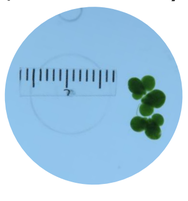


Figure 1: L. minor at the start of the experiment

Harvest

U0-M0 U10-M100

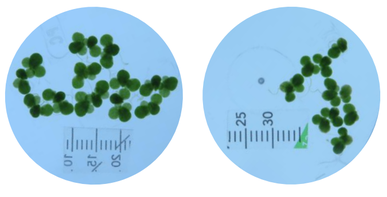


Figure 2: L. minor at the end of the experiment

Growth analysis and gene expression

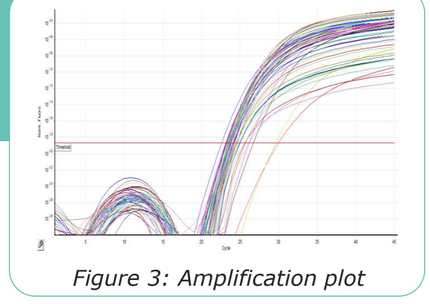
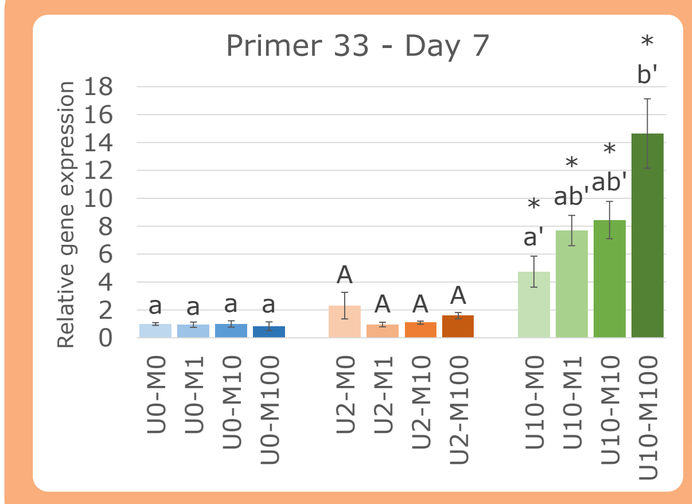


Figure 3: Amplification plot



U0 and U2: no significant effects between the different metal concentrations

U10: metal dependent response but only significant increase at M100

Strong upregulated U-dependent response

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Non-significant at 2 µM U
Significant at 10 µM U

Figure 4: Gene expression levels of potential U-biomarker primer 33 for L. minor relative to control condition (U0-M0) after exposure for 7 days to U (0, 2 or 10 µM) and/or metal mixtures (M0, M1, M10 or M100)

U0, U2 and U10: no significant effects between the different metal concentrations

Downregulated U-dependent response

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Significant at 2 µM U
Significant at 10 µM U

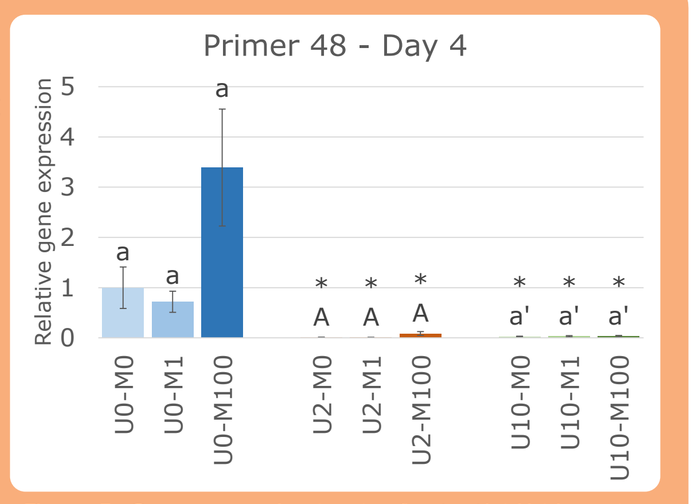

Figure 5: Gene expression levels of potential U-biomarker primer 48 for L. minor relative to control condition (U0-M0) after exposure for 4 or 7 days to U (0, 2 or 10 µM) and/or metal mixtures (M0, M1, M100)

Results

Table 1: Overview of the selected potential U-biomarker primers

	Exposure dose-response relationship	Exposure time-response relationship	Sensitivity	Chemical specificity
Primer 33	✓	✓	✓	✓
Primer 44	✗	✓	✗	✗
Primer 46	✗	✗	✗	✗
Primer 48	✗	✓	✓	✓
Primer 52	✗	✓	✓	✓

10 potential U-biomarkers were tested.

A selection of 5 primers was made based on a first screening (Table 1).

Primer 33 meets all the defined criteria for a good biomarker. Also primer 48 and primer 52 have the potential for being U-biomarkers.

U0 and U10: no significant effects between the different metal concentrations

U2: M100 is significant lower than M0

Downregulated U-dependent response

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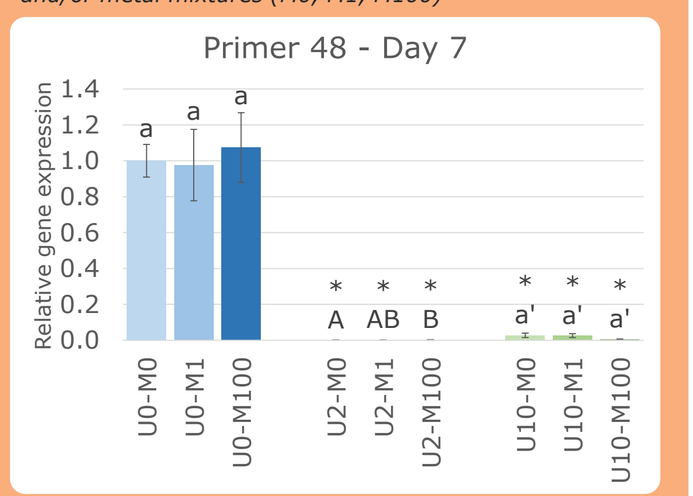
Significant at 2 µM U
Significant at 10 µM U



Conclusion

The earlier discovered primer 33 was validated as biomarker for U exposure. In addition, based on gene expression analysis two new potential U-biomarkers were selected within this project, i.e. primer 48 and primer 52, since their related genes reacted specifically to U already after 4 days of exposure and to the lowest tested U concentration (2 µM). Future research will focus on further testing the selected genes under different environmental relevant scenarios.

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[1] A. Van Hoeck, N. Horemans, P. Monsieurs, H. Xuan Cao and H. B. R. Vandenhove, "The first draft genome of the aquatic model plant Lemna minor opens the route for future stress physiology research and biotechnological applications," Biotechnical Biofuels, vol. 8, no. 188, pp. 1-13, 2015.

[2] S. Lofts, L. Fevrier, N. Horemans, R. Giblin, C. Bruggeman and H. Vandenhove, "Assessment of co-contaminant effects on uranium and thorium speciation in freshwater using geochemical modelling," Journal of Environmental Radioactivity, vol. 149, pp. 99-109, 2015.

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