Evaluation of the metal tolerance and phytoremediation potential of herbaceous and woody species (138806) – Final report

In this PD-OTKA project (grant number: 138806), the previously undertaken research actions were successfully carried out, thus, the following milestone completions can be reported. Results are presented by the three main sub-projects: (i) short-term laboratory test; (ii) mid-term test; (iii) long-term test.

(i) Short-term laboratory test

Results

From March to November 2022, germination tests were run with rapeseed (*Brassica napus* L.), sorghum (*Sorghum bicolor* (L.) Moench), and Sudangrass (*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf. cv. 'GK Csaba') using 0, 10, 50, 100, 500, and 1000 mg l^{-1} concentrations for both Cd and Zn, added to seeds as individual metal loads (Cd or Zn) and also simultaneously (Cd and Zn) in various concentrations, establishing co-contamination patterns. The three species' growth parameter (radicle and hypocotyl length) determination and elemental analysis (Ca, K, Mg, Cd, Cu, Fe, and Zn) were done after 24, 72, and 120 hours of development from December 2022 to January 2023. The data analysis and publication preparations took place between January and May 2023. Then, the interpretation and presentation of results were divided into two parts (done between May 2023 and January 2024).

a) Regarding rapeseed, results indicated that both the single and combined application of Cd and Zn significantly (p<0.05) reduced the lengths of the radicle and hypocotyl. The accumulation intensity of Cd and Zn was influenced by the presence of Cd and the combination of Cd and Zn in the solution, respectively. Furthermore, both metals significantly affected the tissue Mn and had a minor impact on Cu and Fe concentrations. Cd and Zn also significantly influenced macroelement concentrations by decreasing tissue Ca and affecting K and Mg concentrations in a dose- and exposure-time-dependent manner. The short-term results prove the intensive accumulation of elements in rapeseed tissues, indicating the need for additional evaluations during field cultivation and subsequent food processing.

b) Regarding sorghum and Sudangrass, the results showed significant differences in radicle and hypocotyl length by species, contaminant dose, and exposure time. Regarding growth parameters, high-concentration Cd treatments were more restrictive than high Zn doses. Simultaneously, a moderating effect of Zn was observed when applied with Cd. In most instances, Sudan grass exhibited greater radicle and hypocotyl lengths than sorghum. Significant applied treatment- and concentration-dependency were also indicated in the case of tissue element concentrations. Among the comparisons involving single metal doses, two significant interactions were identified: Cu and Fe concentrations were reduced significantly as Cd concentration increased in Sudan grass tissues. The results suggested that further in-depth analyses are required to evaluate influential factors and foster the species' remediation potential (e.g., by using soil amendments).

Additional information

From the beginning of the research process, in addition to Judit Vári, my colleague named in the project, three English-speaking first-year students (Deborah Osariemen Idehen, Jennifer Damilola Osazuwa, John Sule Elias) participating in the Hydrobiology – Water Quality Management MSc program at the University of Debrecen joined the work between February 2022 and May 2023. They all contributed actively to the germination, laboratory sample preparation, and sample digestion procedures. The students defended their theses successfully and were awarded the top grade (5) in June 2023. Due to the research potential within the short-term test, a PhD student (Daniela Isabel Gutierrez Perez) was officially taken under my supervision and has been involved in data analysis and manuscript preparation since July 2024. Further, Zsófi Sajtos and Ágota Zsófia Ragyák made significant contributions to laboratory work and manuscript preparation.

(ii) Mid-term test

Results

After plot designation in April 2023, the sowing procedure of the three species (rapeseed (*Brassica napus* L.)[1], sorghum (*Sorghum bicolor* (L.) Moench), and Sudan grass (*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf. cv. 'GK Csaba') was performed in May. Soil samples were taken in five consecutive months (May to September), while plant samples were collected in four successive months June to September). In October and November, collected samples were prepared in the laboratory; washing, drying, and homogenization procedures were all performed during this period. The elemental analysis of the collected soil and plant samples (sorghum and Sudan grass) was conducted from December 2023 to January 2024. Based on the results, statistical analyses were run to determine the temporal changes (between June and August) in the concentration of three macro- and eight microelements in and among soil and leaf, stem, and root samples from December 2023 to March 2024. The interpretation and presentation of results were completed in one publication, while a manuscript on the comparison of sorghum and Sudan grass has been under preparation since November 2024.

Significant (p<0.05) differences in element (Ca, K, Mg, Al, Ba, Cd, Cr, Cu, Mn, Sr, and Zn) concentrations were assessed by plant organs (leaf, stem, root) and sampling dates (June, July, August) using ANOVA and post hoc Tukey tests. The bioaccumulation factor (BAF) values (C_{root}/C_{soil}) evaluated the accumulation from soil to plants. According to the analyses, Cd, Cr, Cu, and Zn soil concentrations were near or above the legal threshold values. The concentration of Ca varied significantly in stems and roots across sampling periods and among plant organs in July and August. The patterns of Mg and K changed significantly in each organ during sampling periods, with differences appearing only in two months. The concentrations of Cd, Cu, and Mn differed significantly by plant organs and sampling periods. Al, Ba, Cr, and Zn concentrations significantly differed among organs each month, while differences in the selected organ were only occasionally significant. Changes in Sr concentration across sampling periods were significant in stems and roots, and differences among organs were significant in July and August. Root-to-soil concentration ratios exceeded one only for K (ranging from 3.3

[1] Despite the efforts inverted in plot preparation and weeding, one of the three species (rapeseed) showed no sign of development in the study area. Hence, the research plan had to be restricted to the remaining two species.

to 5.2). An elevated accumulation ratio (1>BAF>0.75) was found for Cd in August (0.9) and for Zn in June (0.75).

Additional information

In March 2023, two English-speaking first-year students (Bin Wan Badaruddin Wan Aimran and Munuhe Esther Muthoni) participating in the Environmental Science MSc program at the University of Debrecen joined the project. According to their plans, both students prepared their theses under my supervision. However, only Esther Muthoni Munuhe defended her thesis successfully with the top grade (Wan Aimran Bin Wan Badaruddin had to leave the University training just before the defense due to family issues) in June 2024. Esther Muthoni Munuhe also participated in the TalentUD program under my supervision and was awarded an excellent qualification. In addition, colleagues Judit Vári, Zsófi Sajtos, and Ágota Zsófia Ragyák made significant contributions to laboratory work, data analyses, and publication preparation.

(iii) Long-term test

2.1. Results

In October and November 2021, nine plots for spelt (*Triticum spelta* L.) were selected and prepared through weeding and digging in the Lovász-zug study area. Then, soil samples were taken from the plots, which preceded the sowing and composting processes. From March to May 2022, the same procedures were applied to the Sudan grass plots. Between October and November 2022, soil and plant (root, stem, leaf) samples from the spelt and Sudan grass plots were collected, individuals were harvested, and compost was added to the selected plot soils. Laboratory preparations for the samples began in October 2022, and Siberian elm saplings were planted in halves of the chosen plots. From March to October 2023, iterative maintenance activities were conducted in the study area, with soil and plant samples collected monthly. Additionally, complementary weeding and irrigation were performed weekly to support plant growth. In April 2023, white willow individuals were planted in the cleared spelt and Sudan grass plot halves. During the sampling of woody species (Siberian elm and white willow), individuals planted in 2022 and 2023 were also included. The collected soil and plant samples were stored in the freezer. The soil and plant samples (Siberian elm and white willow planted in spelt and Sudan grass plots) collected during the 2023 growing period were prepared in the laboratory for elemental analysis of 15 macro and microelements from January 2024 to April 2024. Moreover, soil and plant sampling were conducted again in April 2024. Weekly weeding and irrigation of the field individuals were performed between April and September 2024, followed by laboratory preparations and elemental analyses conducted from July 2024 to October 2024. The element analysis data were processed from September 2024 to February 2025.

The results indicated that spelt and Sudan grass plant organs had intensive metal accumulation under multi-metal contamination; however, the uptake was significant only sporadically in a few instances. Siberian elm and white willow individuals planted into the plots of the herbaceous species also showed good accumulation potential. Nevertheless, when results are compared to control, unamended individuals, significant uptake patterns were found only in a few cases for Cd and Zn in leaves and Pb in roots of both species. Additionally, as the growth parameters have also been determined, a moderate, insignificant supporting effect of the green

compost supply was observed. Individuals growing within the amended plots were contrasted to those outside the plots: the comparison revealed greater plant heights for plants growing inside the compost-supplied plots. Based on the results, it was determined that composting does not significantly intensify the element accumulation of Siberian elm and white willow under the given circumstances. However, it was presumed that the wide range and interactions of metal present in the soil disabled the very intensive enrichment of any of the elements (e.g., antagonistic relationship between Ca and Cd). On the other hand, compost buried in the soil had positive effects on plant condition; the researchers involved in this project believe that the better moisture retention and organic matter supply promoted by the presence of Sudan grass and spelt residues hindered the washing out of nutrients, which was a phenomenon of key importance during the summer drought conditions.

As indicated earlier by the application, the results are not allowed to be published in international journals due to the restrictions applied by the maintainer of the study area. However, with the results obtained, the elemental concentration schemes are used as bases for creating lecture materials and stepwise laboratory and field methodological guidance for BSc and MSc students from December 2024 to April 2025. These data will be used in future MSc course *Applied ecology* and PhD course *Phytoremediation* held by Dávid Tőzsér.

2.2. Additional information

Colleagues Judit Vári, Zsófi Sajtos, and Ágota Zsófia Ragyák made significant contributions to laboratory work and data analyses and will be involved in the preparation of lecture materials. Further, field visits were assisted twice by PhD student Daniela Isabel Gutierrez Perez. At the same time, soil and plant sampling were also helped by Szabolcs Mizser and Roland Horváth (both from the Departments of Ecology), who are significant contributors to data analysis.

Publications with reference to this Scholarship in their "Funding" section

(i) Short-term laboratory test (one article published, one article accepted for publication, one Hungarian conference publication)

The paper based on the results of the germination test with rapeseed was published in the journal *Chemosphere* (D1, IF_{2023}: 8.1) on 15 January 2024:

Tőzsér, D., Osariemen Idehen, D., Damilola Osazuwa, J., Sule, J.E., Ragyák, Á.Z., Sajtos, Z., Magura, T. (2024) Early-stage growth and elemental composition patterns of *Brassica napus* L. in response to Cd-Zn contamination. *Chemosphere* 351: 141235.

The paper based on the result of the comparative analysis of sorghum and Sudan grass was prepared and submitted for publication to the Q1-ranked journal *Environmental Geochemistry and Health* (Q1, IF_{2023}: 3.2) (accepted for publication on 28 March 2025):

Tőzsér, D., Damilola Osazuwa, J., Sule, J.E., Osariemen Idehen, D., Gutiérrez Pérez, D.I., Ragyák, Á.Z., Sajtos, Z., Magura, T. Comparative analysis of the short-term germination and metal accumulation patterns of two *Sorghum* hybrids. *Environmental Geochemistry and Health*

A conference publication based on the preliminary results of the three studied species was prepared and presented orally at the XII. Ökotoxikológiai Konferencia on November 25, 2022:

Tőzsér, D., Osazuwa, J.D., Idehen, D.O., Sule, E.J. (2022) Takarmánynövényekkel végzett csíranövény-tesztek összehasonlító értékelése. [Comparative evaluation of germination tests performed with forage crops].

(ii) Mid-term test (one international conference publication, one manuscript under preparation)

A conference publication based on the results of the field evaluation of Sudan grass was prepared and presented orally at the Global Research Conference on Green Energy and Environmental Engineering (GRCGEEE) in Barcelona, Spain, on 24 October 2024.

Tőzsér, D., Muthoni Munuhe, E., Wan Badaruddin, W.A.B., Horváth, R., Mizser, S., Sajtos, Z. (2024) Phytoextraction potential of hybrid Sudan grass in soils with abandoned metal contamination.

A comparative analysis is performed involving sorghum and Sudan grass and a manuscript is being prepared on the novel findings. The paper will be submitted to the Q1-ranked journal *Agriculture* or *Environmental Science and Pollution Research* in 2025.

Tőzsér, D., Muthoni Munuhe, E., Wan Badaruddin, W.A.B., Gutiérrez Pérez, D.I., Horváth, R., Mizser, S., Sajtos, Z. Phytoremediation performance of sorghum and Sudan grass in a former metal-contaminated settling pond.

Other publications related strongly to the research plan, performed as sub-projects (two articles published)

Due to the nature of our experimental/sampling area (Lovász-zug), a manuscript was prepared on the metal uptake characteristics of the abundant earthworm species, complemented by other earthworm species being relevant in metal accumulation. Based on the results, the paper was published in the journal *Environment International* (D1, IF: 11.8) on September 25, 2022.

Tőzsér, D., Mizser, S., Karaffa, K., Málik-Roffa, H., Magura, T. (2022) A meta-analysis-based evaluation of metallic element accumulation in earthworms. *Environment International* 169, 107546.

Further, the composition of the natural vegetation of the research area made it a reasonable and specifically informative step to assess its most abundant herbaceous species. Thus, we evaluated the metal accumulation of amaranths (Amaranthus) – a group of species subject to weeding within our long-term test –with meta-analysis tools. Based on the results, the paper was published in the journal *Environmental Science and Pollution Research* (Q1):

Tőzsér, D., Yelamanova, A., Sipos, B., Magura, T., Simon, E. (2023) A meta-analysis on the heavy metal uptake in *Amaranthus* species. *Environmental Science and Pollution Research* 30: 85102–85112.

Other publications not related to the research plan (five articles published)

A new software was developed for tracking arthropods during laboratory analyses, eliminating some of the significant shortcomings of programs previously launched for this purpose. The paper with the relevant results was published in the journal *Diversity* (Q1, IF: 2.1) on July 8, 2023:

Málik-Roffa, H., Tőzsér, D., Tóthmérész, B., Magura, T. (2023) *BugTracker*: Software for Tracking and Measuring Arthropod Activity. *Diversity* 15: 846.

A bibliometric analysis-based literature review was prepared from the "green" perspective regarding the assessment and use of sustainable building materials. The paper with the relevant results was published in the journal *Resources* (Q2, IF: 3.6) on October 17, 2023.

Boros, A., Tőzsér, D. (2023) The Emerging Role of Plant-Based Building Materials in the Construction Industry—A Bibliometric Analysis. *Resources* 12: 124.

Due to the highly increased supply and demand for green solutions in agriculture, a comprehensive literature analysis was conducted regarding various aspects of sustainable operation in the sector. The paper with the relevant results was published in the journal *Heliyon* (Q1, IF_{2023}: 3.4) on 19 June 2024:

Boros, A., Gordos, B., Tőzsér, D. (2024) A bibliometric analysis-based literature review of the relationship between sustainable water management and green innovations in the agricultural sector. *Heliyon* 10: e33364.

According to the need for green technologies in the agricultural sector, an even more extensive literature review was prepared on the widely used and preferred novelties based on more than 300 references. The paper with the relevant results was published in the journal *Agronomy* (Q1, IF_{2023}: 3.3) on 30 December 2024:

Boros, A., Szólik, E., Desalegn, G., Tőzsér, D. (2025) A systematic review of opportunities and limitations of innovative practices in sustainable agriculture. *Agronomy* 15, 76.

A study on financial institutions' environmental contribution and performance was prepared based on complex text mining and data analysis. The paper with the relevant results was published in the journal *Administrative Sciences* (Q2, IF_{2023}: 3.0) on 14 March 2024:

Tőzsér, D., Lakner, Z., Sudibyo, N.A., Boros, A. (2024) Disclosure Compliance with Different ESG Reporting Guidelines: The Sustainability Ranking of Selected European and Hungarian Banks in the Socio-Economic Crisis Period. *Administrative Sciences* 14: 58.