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DOCTORAL SCHOOL OF ENVIRONMENTAL SCIENCE

Effect of soil heterogeneity on plant uptake of potentially toxic elements

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Introduction and objectives

Potentially toxic elements are in the periodic table that have low or no difference between toxic and beneficial concentrations in living organisms (Kádár 1993).

The direct uptake of potentially toxic elements from soil into animal and human organisms is not significant because human and animal organisms rarely come into direct contact with soil for short periods of time (Kidd et al.2009). The biological cycling of these elements is most likely to occur through plants. This is because plants - are in direct contact with the soil over long periods of time and over a large root surface area - are in intensive metabolism with the soil and take up significant amounts of nutrients and other elements from the soil (Kádár 1993).

The uptake of elements by plants is influenced by a number of parameters (Kabata-Pendias 2011). At present, there is no good theory that models plant uptake well enough to calculate or "predict" the element uptake of plants. Therefore, any experimentally-derived information that can help to understand the uptake of potentially toxic elements by plants is useful.

Taking all this into account, my research was conducted on a heterogeneous field, where the uptake of potentially toxic elements was investigated at several points, with identical treatments and agrotechnical interventions applied across the whole field. With the information and data obtained from experiments on heterogeneous fields, it is easier to estimate how the uptake of potentially toxic elements varies in soils with different exposures, slopes, composition and nutrient supplying capacity, under the same other treatments.

Aims:

- Based on the results of the statistical analyses, I aimed to determine how the measured soil parameters influence the element concentrations in the soil extracts and the element uptake by plants.
- Based on the results, I wanted to draw conclusions on the relationship between the uptake of potentially toxic elements and the measured parameters and nutrient supply of the soil when other external conditions are given, i.e., when the same interventions are applied to the whole field. This will allow us to determine how changes in soil parameters affect the uptake of potentially toxic elements.
- In addition, laboratory experiments were conducted to investigate the effect of the chelating chemical EDTA on the solubility and uptake of potentially harmful elements.

Materials and methods

At first, a field experiment and its results are presented, followed by the results obtained from the analysis of data from a laboratory experiment. The area selected for the field experiment is not only shaped by the topography and the associated environmental conditions (slope effect), but also by the 2

soil types that can be distinguished in the study area. In addition, the three test soil samples were selected on the basis of differences between the basic soil test parameters during the laboratory experiment.

In all cases, statistical analyses were performed to draw conclusions on the impact of soil heterogeneity on potentially toxic elements.

Field experiment

The experimental area is the agricultural area of the DUNA GYÖNGYE 2000" Mezőgazdasági Zrt. in Dunaszekcső. I took soil and plant samples from the experimental area at the following times:

- 2017/11/12;
- 2018/04/23;
- 2018/06/22;
- 2018/10/12;
- 2019/03/29;
- 2019/07/10

The chemical analyses of the plant samples were completed, but after statistical analysis of the results, the plant sample data were not analyzed in this thesis due to their unreliability.

In all cases the soil samples were taken from the upper ploughed layer. From the samples purchased, the necessary soil preparation was determined after:

- Soil physical properties (A_k)
- Calcium content % (by Scheibler calcimeter),
- Humus % (Tyurin method),
- Conductivity,
- pH (from a soil suspension with water and KCl) (Buzás, 1988),
- Total nitrogen including NO_3^- and NH_4^+ concentrations (by Parnas-Wagner steam distillation) and
- Soluble P_2O_5 and K_2O content from ammonium lactate solution (Egner et al. 1960; Sarkadi et al. 1965).

In addition, the concentrations of the following elements were determined from 2 M HNO_3 extract of the samples: Sr, Fe, K, Al, Zn, Cd, V, Ba, Cu, Co, Y, Li, Rb, Cr, Na, Ca, Mg, As. (Száková et al., 2009; Hooda, 2010)

Laboratory experiment

In my experiments, I investigated the effect of Na-EDTA on the mobilizability of 15 potentially toxic elements in three soil types with different binding and adsorption capacity.

Prior to setting up the tests, I weighed 0.5 - 0.5 kg of the experimental soils after proper preparation, and the soils were allowed to mature at 100% water

saturation for 2 weeks at constant room temperature. The control soils were wetted with distilled water, while the artificially contaminated soils were wetted with a 100 mg/kg solution of the potentially toxic element. For this purpose, the solution was prepared from a stock solution with a concentration of 1000 mg/l of the potentially toxic element. The stock solution contained readily soluble salts of the predetermined elements. These included NO_3^- and Cl^- salts.

Translated with www.DeepL.com/Translator (free version) Chemicals used:

$\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$,	$\text{Cd}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$,	$\text{Pb}(\text{NO}_3)_2$,
$\text{BaCl}_2 \cdot 2 \text{H}_2\text{O}$,	$\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$,	ZnCl_2 ,
$\text{FeCl}_2 \cdot 4 \text{H}_2\text{O}$,	$\text{Cr}(\text{NO}_3)_2 \cdot 9 \text{H}_2\text{O}$,	$\text{Ni}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$,
$\text{SrCl}_2 \cdot 6 \text{H}_2\text{O}$,	$\text{Cu}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$,	$\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$,
$\text{Sn}_2\text{Cl}_2 \cdot 2 \text{H}_2\text{O}$,	CaCl_2 ,	$\text{Mg}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$,

After saturation, 2-2 g of the digested and sieved soils were weighed into centrifuge tubes and 20-20 ml of the appropriate EDTA solution was added to the samples. The effect of EDTA extractant was tested at 8 different concentration levels, which were 0; 1; 5; 10; 50; 100; 500; 1000 mg/l. These concentrations, at a ratio of 1:10 (1 g soil to 10 ml of extractant at the appropriate concentration), represent concentrations of 0; 10; 50; 100; 500; 1000; 5000; 10000 mg/kg on soils.

In addition, in soils with high lime content, there are problems in quantifying potentially toxic elements may arise from the reaction of lime and EDTA, i.e. soluble calcium ions complex with EDTA molecules and competition may occur between the potentially harmful element under study and calcium ions. To avoid this (to adjust the pH), NaHCO_3 was added to the soil solutions ($\text{pH} \geq 8$). The concentrations of the potentially toxic element in the soil extracts were then determined using a 4210 MP-AES Microwave Plasma Atomic Emission Spectrometer.

Also analysing the data from the experiment, the thermodynamic modelling program VisualMinteq3.1 is used to model the effect of EDTA on the solubility of potentially toxic elements. The model calculations assume an equilibrium state where all the substances are transferred from the soil particle surface into the solution and then precipitate in insoluble form. Thus, EDTA cause lower concentrations in the solution that are than the model data.

Methods of evaluating data

At first, I analysed the collected data with one-way Anova, which allowed me to detect deviants or outliers that differed from the data sets. In addition, the analysis helped to determine the extent to which each group differed from the other. In this case, I examined groups of time and some potentially toxic elements. In the case of the one-way Anova results, the items were further

grouped by sampling time, so I first used linear regression to further analyse the results. For the laboratory experiment, linear regression proved to be sufficient, as the settings of the experiment made the retention of potentially toxic elements by each soil parameter negligible, and the element concentrations were known and identical for the artificial contamination added to the soils, which resulted in the same variation in the amount of each element in the soil solutions as a result of the treatments. For the field experiment, I also performed linear regression. However, due to the soil and environmental effects and the differences in element contents, the relationships tested did not always show a linear relationship. To address this, I used the SPSS program to perform rank correlations with the data grouped by time. The need for grouping was justified by the differences between years and seasons, and the influence of fertilizer application was also indicated by the analysis of variance.

My last type of analysis is cluster analysis, which I performed only on the field experimental results. The need for this analysis was justified by the distribution of each potentially toxic elemental content by sampling point.

Results and discussion

Results from field experiments

Cluster analysis

As a result of the cluster analysis, it was found that the hilltops are subject to relatively weak erosion forces, which have resulted in a predominance of soil formation processes, i.e. the rate of soil formation is higher than the rate of soil degradation (cluster 3). In contrast, at the "medium altitudes", which are the slopes and elevations, erosional processes dominate and calcareous subsoil has been excavated (cluster 2). The last category is erosion bases, where soil particles from the slopes have been deposited and their calcareous content has started to accumulate (cluster 1)

Linear regression analyses

The correlations between the basic soil parameters from the chemical analysis and the concentration values of potentially toxic elements were investigated using a correlation matrix based on linear regression.

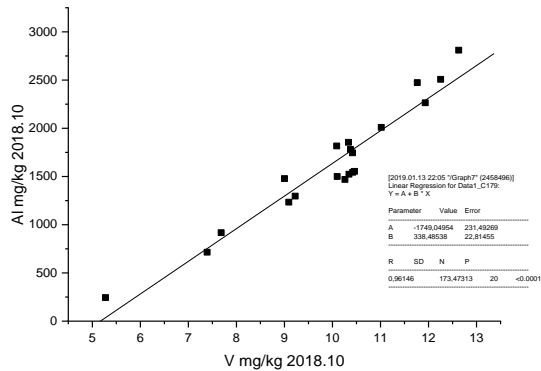


Figure 1. Correlation between Al and V concentrations in soil samples taken in October 2018.

Figure 1 shows the linear relationship between Al and V concentrations in the soil samples taken in October 2018. The concentrations of the two elements are in linear relationship, with Al concentrations being higher in soil samples with higher V content. The coefficient of linear correlation is 0.96. This can be explained by the fact that trivalent Al^{3+} and V^{3+} ions behave chemically similar to each other in both their sorption and complex formation properties.

Second step in the analysis of the relationships, the non-linear relationships were also characterized by other functions. An excellent example is the Ba and Al content of the soil samples taken in 2017 mentioned above. It can be seen in Figure 2, the second-order function fitted to the data for the given parameters fits the data better, here the correlation coefficient $R^2 = 0.95$. In this case, the slope of the correlation is greater for lower concentrations than for higher concentrations, the reason for this is to be found in the other parameters, but in this work, I did not investigate the reasons in more detail, sticking to the description of the phenomenon.

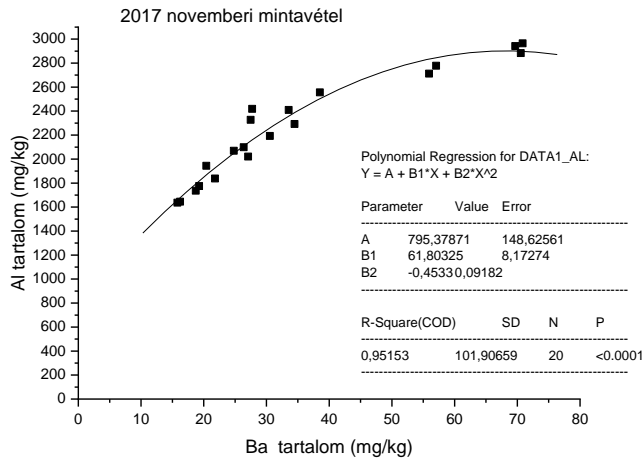


Figure 2. Polynomial correlation of Ba and Al concentrations in soil samples taken in October 2017.

Non-linear correlations were tested by rank correlation at each sampling time. Rank correlation examines the relationship between probability variables based on a monotonic function, ignoring the distribution of the data. In my thesis, I used the Spearman rank correlation coefficient using the SPSS program.

Rank correlation

I did not take monotonicity into account in the evaluation, as I only observed the existence of correlations between the different parameters. In all cases, the data were correlated with each other with an error probability of $P > 0.01$. The correlation coefficients in the correlation tables were averaged to characterise the correlation of the test parameters of the samples taken at different time points and the repetition of the correlation.

I found a correlation between soil pH and lime and organic matter content. Soil lime content leads to an increase in soil pH by binding H^+ ions during dissolution and dissociation of $CaCO_3$ (Fekete 1958). This suggests that soils with a higher lime content have a corresponding increase in pH. In addition, I would like to point out that the relationship between pH and humus content of soils is related to erosion, where the organic matter stock of soils is destroyed faster than it is formed. In this case, the pH of calcareous soils increases while the humus content decreases, i.e., there is an inverse proportionality between the two parameters studied.

The rank correlation showed a weak relationship between different forms of soil nitrogen. In addition, the nitrogen forms did not show a strong correlation with the parameters already studied, which is a consequence of

fertilizer use, since nitrogen is one of the elements that is constantly replenished according to the needs of the crop. This is also true for ammonium lactate soluble K and P, but correlations are already visible for these elements (0.6-0.8). As can be seen in Figure 3, there is a particularly strong relationship ($R^2 = 0.86$) between easily soluble K and the Cu content of soils.

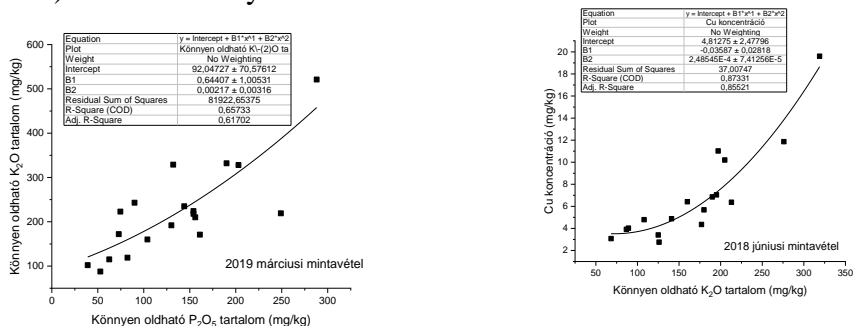


Figure 3. Relationship between ammonium lactate soluble K_2O , P_2O_5 and Cu content of soils.

The non-linearity of the relationships is easier to justify in this case, since the parameters under study have different charges, which cause them to participate in the molecules they form in different proportions. A very good example is K^+ as an ion with a single positive charge and Cu^{2+} as an ion with a double positive charge.

The ranking of the concentrations of potentially toxic elements in soils showed a strong relationship in the following cases (Figure 3).

The correlation coefficients between Ca, Mg, Ba and Sr were close, above 0.8, and only in sample 4 Ba and sample 5 Ca concentrations showed no relationship. This close correlation is due to the similar chemical behaviour of these elements (all elements belong to the alkaline earth metals) and the high lime content, which may result in higher concentrations of these elements in the test soils than in the lime-poor soils.

I found a relationship between Na, Li and Rb element concentrations for samples from sampling dates 1-3 (Figure 4). These alkali metals show a high degree of chemical similarity to each other, with virtually all of their salts being soluble and adsorption on soil colloids being low. The correlation coefficients of the relationships typically range from 0.6 to 0.8.

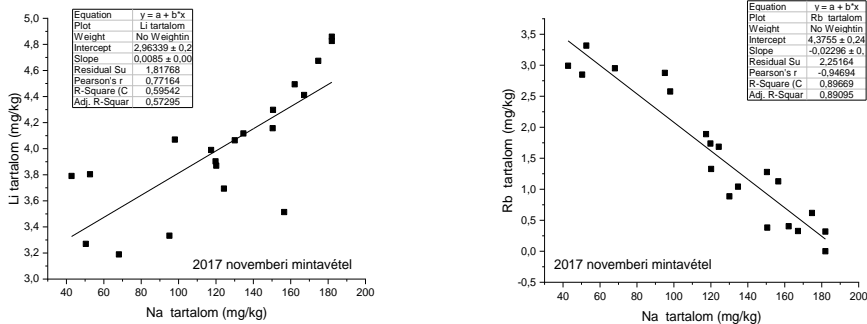


Figure 4. Relationship between soil Na, Li and Ru content.

The relationship between Al and Fe and pH values shows the solubility of these elements, where Al and Fe are found in acidic media in solution, so as pH increases the concentration of these elements in the element's solution decreases. In turn, competition between the elements occurs and H^+ ions are either scavenged from the soil surface (pH increase) or precipitated.

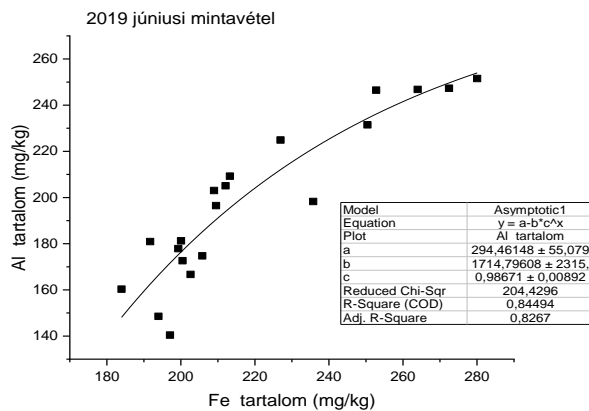


Figure 5. Relationship between soil Fe and Al concentrations.

The data in Figure 5 shows a saturation curve with a fit $R^2 = 0.83$.

Based on the saturation curve, using the rank correlation, a closer correlation between the two parameters under study can be established. Taking all this into account, it can be concluded that the use of a linear correlation for the parameters under study is not appropriate and may produce misleading results. Therefore, the use of rank correlation is recommended in these cases.

In addition, the correlation tables show that there is a close relationship between the lime content/Ca ion concentration, the magnesium concentration and the following elements: Al, V, Co, Y, Cr, Na, V, Fe and Co.

Results from a laboratory experiment

Results of aluminium, iron, tin and lead

The results from the modelling are presented in 3D plots, where the x-axis represents the logarithm of the EDTA concentration, the y-axis the pH values and the z-axis the calculated metal ion concentration (Figure 6).

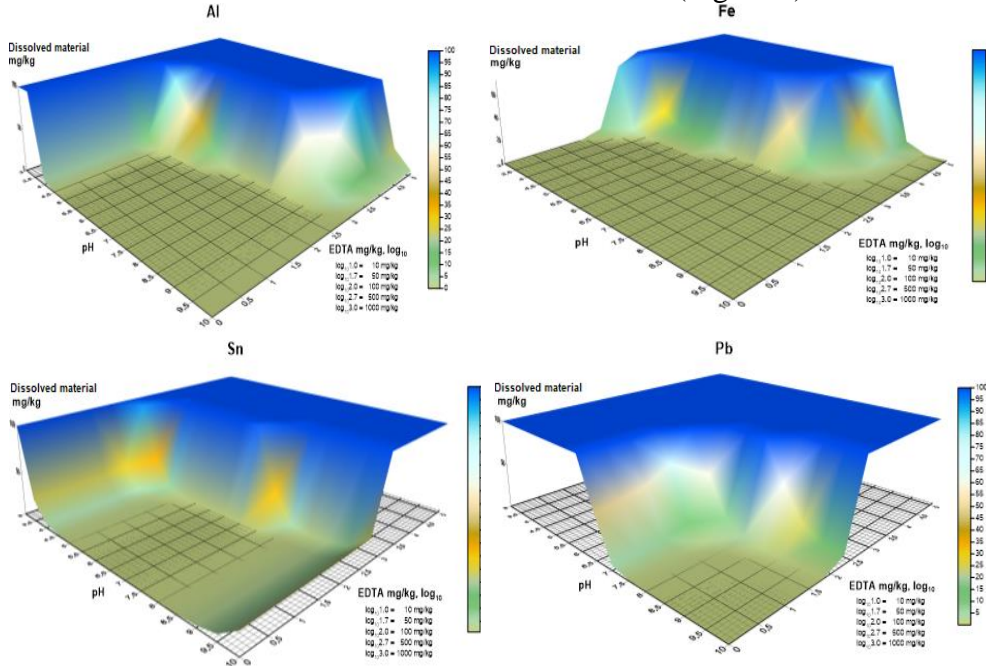


Figure 6. 3D modelled diagram of the solubility of Al, Fe and tin as a function of pH and EDTA concentration.

The 3D plots show the modelled solubility values for Al, Fe, tin and lead. From the plots, it can be seen that for all 4 elements, the respective potentially toxic substances are present in the solution only at low pH or at high EDTA concentrations. This result shows a high similarity with the pH dependence of the different elements, where the 3 elements are mobilised in soil at approximately the same pH (Papp 2011).

Plotting the measured data for Fe and Al yielded curves with similar slopes (Figure 7). Similar concentration increases were then seen for all test soil types and treatments at a given EDTA concentration. As a result of linear regression with the measured data, 9 out of 12 cases show a correlation coefficient of Fe and Al higher than 0.9, i.e. a strong relationship between the two elements.

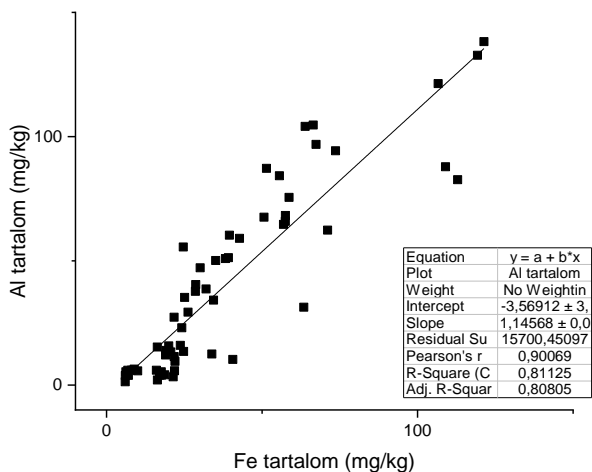


Figure 7. Linear relationship between Al and Fe content in control and treated soils.

The relationship between Pb and Al (Figure 8) was similar, with correlation coefficients ranging from 0.62 to 0.99 also in 9 cases and the correlation coefficient for the relationship between Fe and Pb ranged from 0.7 to 0.99 in 8 cases. However, when plotting the Al and lead concentrations in a coordinate system, it can be seen that the relationship is assumed to be due to an outlier in each case.

In addition, Cu, Ni and Co ion concentrations, presented later, also show a relationship with Fe, but this is only true for artificially contaminated soils ($R^2 = 0.62-0.99$).

The measured tin values were within the limit of measurement, which (maximum measured tin concentration was 4.67 mg/kg) makes them uncertain. This is supported by the high standard deviation values. No change in concentration was observed for Mg, where the soils and treatments tested did not cause any change from the initial values. Furthermore, the correlation matrices show that Mg showed the strongest relationship ($R^2 = 0.72-0.97$) with Fe in only 41% of cases.

Results of copper, zinc, nickel, cobalt, cadmium and chromium

The 3D plots of the model results show that the solubility of Cu, Zn, Ni, Cd, Cr and Co differs greatly (Figure 8). In particular, the stepwise model is different for Co, with no high concentration of Co^{2+} ions in the soil solution over a significant range, independent of pH and EDTA concentrations. In contrast, no stepwise solubility is seen for Zn, Cu, Cd, Cr and Ni. In addition, all but Cd, Cu, Zn, Cr, and Ni, potentially toxic elements, form only insoluble precipitates over a similar range at very high pH (around pH 9) and at

significantly low EDTA concentrations. Cd, based on the model results, becomes insoluble precipitate only in a very narrow EDTA and pH range, which is not a favourable property from the point of view of environmental pollution.

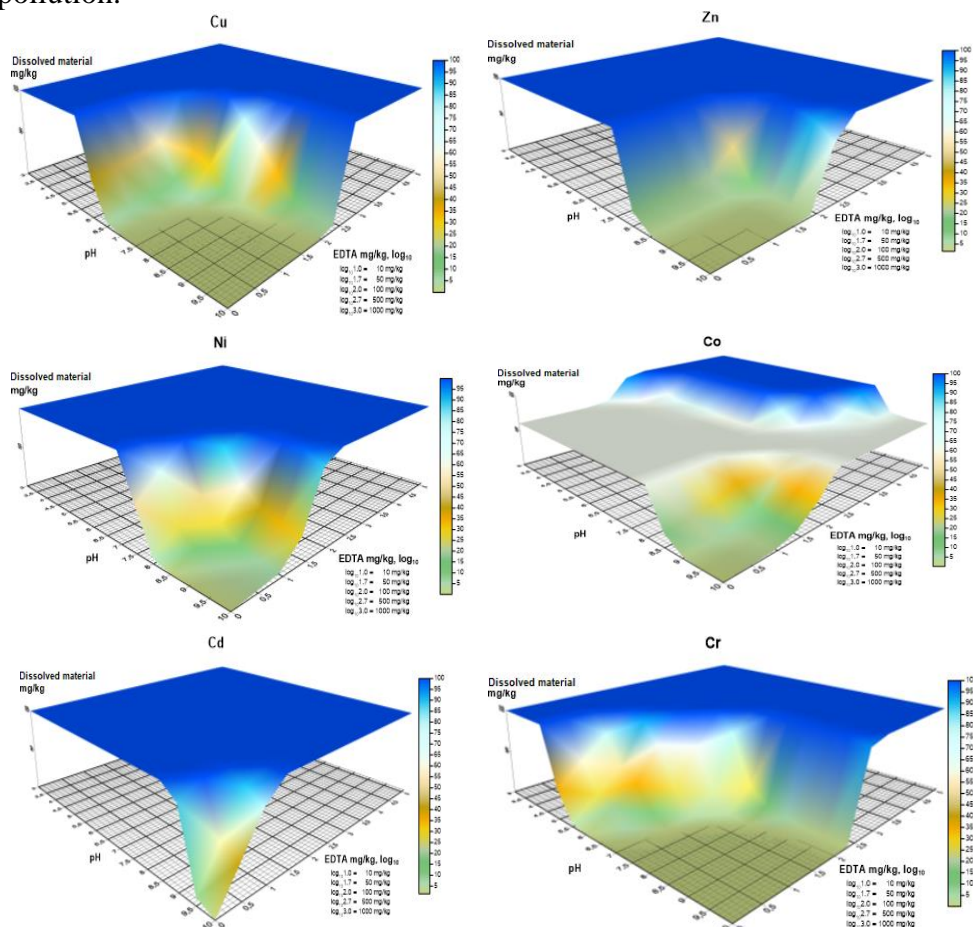
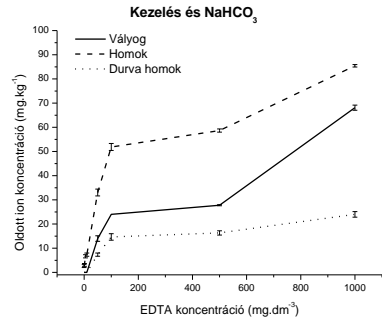
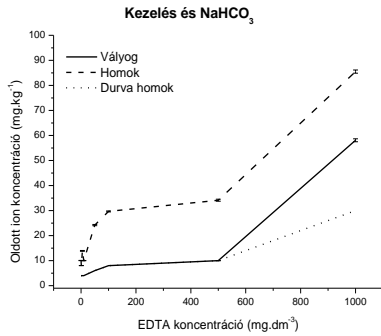


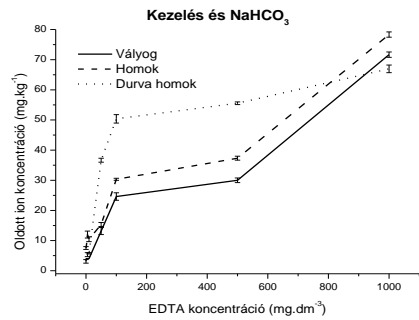
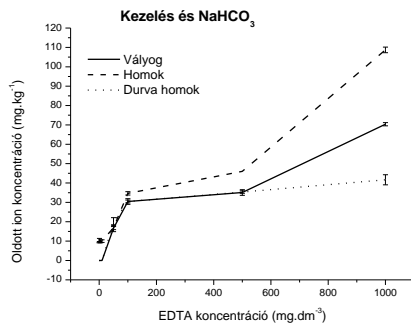
Figure 9. 3D modelled diagram of the solubility of Zn, Ni and Co as a function of pH and EDTA concentration.

The measured results show the same concentration variation at the same EDTA concentration, which I verified by linear regression. However, the correlations observed for these elements were only observed for the artificially contaminated soils, as the original control soils had very low concentrations of these potentially toxic elements. In addition, when looking at the different concentrations, it was found that the addition of NaHCO_3 to the solutions reduced the solubility of these elements. This means that the correlations found for NaHCO_3 and artificial contamination are similar to the results for the contaminated soil solutions, but that sometimes measurement uncertainty may play a role in the data due to the lower concentrations.



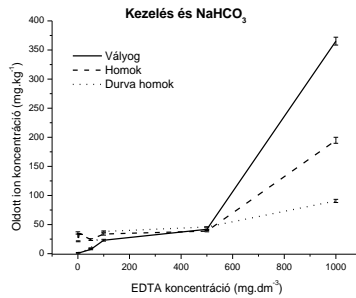
Ni

Co



Zn

Cu



Mn

Figure 10. Results of nickel, cobalt, zinc, copper and manganese.

The experimental results show that Ni, Co, Zn, Cu and Mn can be mobilized in the environment at approximately the same EDTA concentration and pH. This is supported by the linear regression showing that there is a correlation between the 5 elements (for treated soil samples) and in these cases the determination coefficient was higher than 0.8. The measurement results

for Cd and Cr in the modelled values are close to zero, so the concentrations of these elements were not analysed.

Sr and Ba as Co and Cd, no other elements showed a change with treatment. The hydroxide of Sr and Ba is a very strong base, and therefore when dissolved in salts with strong acidity, hydrolysis does not occur, i.e. these cations do not react significantly with the hydroxide ions in solution. It follows that a change in the concentration of hydroxide ions in the solution (thus a change in pH or basic buffering) does not significantly change the concentration of metal ions in solution.

New scientific results

1. By using a combination of cluster analysis and one-way ANOVA, it was possible to find parameters in the study area that are similar in behaviour to many of the potentially toxic elements studied. For example, erosion, which has a significant impact on the mobilizability of many potentially toxic elements, could be identified.

2. Comparing the test results of the measured results, I found many different types of correlations between the element concentrations tested, so I used Spearman's rank correlation tests to determine the monotonic variation of the element concentrations tested with respect to each other. Using this approach, very different types of correlations were identified. Since these relationships cannot always be described by a linear relationship, I used rank correlation.

3. Based on the concentrations of the potentially toxic elements studied, I identified a number of chemical and geochemical properties that can be used to classify potentially toxic elements into types based on their detectable mobility in soil. These include, for example, alkali, alkaline earth metals, triple charged elements with similar behaviour.

4. In my studies, I used thermodynamic modelling to determine the effect of pH and EDTA concentration on the solubility of potentially toxic elements. I investigated the soluble concentrations of potentially toxic elements in three typical soil types and in systems buffered at two different pH values, from which I provided actual measured data on the mobilizing effect of EDTA in soils of different types and pH.

Conclusions and suggestions

This dissertation includes two types of studies (field and laboratory), in which treatments and experiments were used to identify soil-specific relationships.

The data from the field experiments and their evaluation started first with an one-way ANOVA to identify which samples showed significant differences for each parameter tested. By tabulating the data and highlighting the significant differences, it became apparent that a relationship could be assumed for some soil parameters and sample points, and therefore I performed a cluster analysis of the available data using the SPSS program.

In the cluster analysis, I have used the experience from the analysis of variance, based on a careful consideration that the lime content of soils has a significant effect on each of the soil parameters studied. Therefore, when grouping the data, the lime content became the determining factor. However, the topography of the area had a significant influence on the lime content, with erosion processes on the hilltops playing a role of approximately the same magnitude as soil-forming processes. In contrast, erosion forces on slopes resulted in the transport of topsoil into valley reservoirs. Topsoil on the slopes was then displaced and freed to form subsoil with a higher calcium content, and in the valleys. Therefore, I took altitude and lime content into account in the cluster analysis. I then obtained the ideal cluster number, which I used to divide all the study parameters into 3 groups, and subjected the groups to ANOVA statistical analysis. It then became apparent that the 3 groups did not always cover the reality, in that in some cases no significant difference was found between the groups. I therefore carried out a further cluster analysis using SPSS, which predominantly split the data into 2 groups. Significant differences between the groups were then observed and there was still no significant difference between the data within the groups. These relationships suggest that the lime content has a significant effect on the amount of each potentially toxic element, but not to such an extent that the smallest change in lime content becomes an indicator of the different concentrations of each potentially toxic element.

Taking all this into account, I also compared the measured soil parameters with each other using linear regression. After the regression was performed, the resulting correlations were plotted and it became apparent that the relationship between the individual soil parameters is not always linear. Then, also using the SPSS program, I constructed a rank correlation table of the data by separating the samples taken at different sample times. This was prompted by the technical problems mentioned above, and also by the different weather conditions from year to year, in addition to human influences. The rank correlation resulted in a correlation between the concentrations of the different elements. However, in order to make use of these correlations in future work,

further samples and studies are needed to support and complement the findings of this thesis.

A similar correlation was sought in the laboratory experiment to demonstrate the effect of EDTA released into the environment under known conditions. EDTA is a necessary test substance because of its mobilizing effect as a potentially toxic element. In the laboratory experiment, three types of soil samples, differing in their physical properties, were subjected to identical treatments, which allowed a comparison of the effect of each of the soil parameters on the mobilization of potentially toxic elements. In addition, the concentrations of each element were also examined by linear regression, resulting in relationships similar to those obtained for the soil data from the field experiment.

Further experiments involving other soil types and other arable land would be necessary to draw firm conclusions, and would need to be subjected to continuous multi-year testing. The data collected in this way could not only help to predict the impact of climatic factors, but could also be used to help with possible micro-nutrient replenishment.

Related publications

1. Peer-reviewed research articles

1.1. With impact factor (according to WEB OF SCIENCE), in English:

1.1.1. Hungarian publisher:

1.1.2. International publisher:

Czinkota, Imre; Filep, Tibor; Rétháti, Gabriella; Tolner, László; Gulyás, Miklós; Sebők, András; **Dálnoki, Boglárka**. (2021). Derivation and application of a generalised exchange-adsorption isotherm for the adsorption of hydrophobic compounds on soils. SOIL AND WATER RESEARCH 16:2 pp. 67-73. **(18 pont)**

1.2. In English, without IF

1.2.1. Hungarian publisher

1.2.2. International publisher

1.3. In Hungarian, without IF

SEBŐK, András; CZINKOTA, Imre; NYIRI, Balázs; BOSNYÁKOVICS, Gabriella; GULYÁS, Miklós; **DÁLNOKI, Anna Boglárka** (2018). A talaj szervesanyag minőségének vizsgálata UV-VIS spektrumban – az Exponenciális Illesztés Módszere (EFA). NÖVÉNYTERMELÉS 67: 4 pp. 59-71. **(5 pont)**

2. Professional full text article,

2.1. Professional full text article

4. Conference proceedings with ISBN, ISSN or other certification

4.1. Full text article in Foreign language, peer-reviewed

Boglárka, Anna Dálnoki; András, Sebők; János, Grósz; Gabriella, Rétháti; László, Tolner. (2018). Rapid biotest for fertiliser's effects. In: Zoltán, Kende (szerk.) 17th Alps-Adria Scientific Workshop : Abstract book. Gödöllő, Magyarország : Szent István Egyetem Egyetemi Kiadó 172 p. pp. 138-139. **(5 pont)**

4.2. Teljes szövegű közlemény, alkalmi (nem periodika jellegű) kongresszusi kiadványban, magyar nyelven, lektorált formában megjelentetve

Dálnoki, Anna Boglárka; Huszár, Szilvia; Sebők, András; Fekete, György; Czinkota, Imre (2017). Talaj és növény beltartalmi paramétereinek változása trágyakézelések hatására. TALAJVÉDELEM Különszám 2017 pp. 363-370. **(3 pont)**

Sebők, András; Czinkota, Imre; Fekete, György; **Dálnoki, Anna Boglárka**; Grósz, János (2017). Humuszoldat adszorpció és deszorpció kinetikájának meghatározása homokos talajon oszlopkísérlet segítségével. TALAJVÉDELEM Különszám 2017 pp. 251-260. (3 pont)

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