UNIVERSITY OF DEBRECEN
CENTRE FOR AGRICULTURAL SCIENCES
FACULTY OF AGRONOMY

INSTITUTE OF FOOD SCIENCE, QUALITY ASSURANCE AND MICROBIOLOGY

DOCTORAL (PhD.) SCHOOL OF CROP PRODUCTION AND HORTICULTURE

Leader of doctoral school:
Dr. Győri Zoltán DSc.

Supervisor:
Dr. Győri Zoltán DSc.
and
Dr. Kovács Béla

„THESES OF DOCTORAL (PhD) DISSERTATION"

Studying of change of selenium species in soil and plant samples from a long-term field experiment

By:
Éva Széles

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1. Introduction

Nowadays, selenium (Se) is one of the most investigated microelement. It has an important proven role in many vital processes. In direct or indirect way selenium deficiency can play a role in evolvement of many diseases (cancer, health diseases, etc). Selenium deficiency also has a role in some special diseases e.g. Down-syndrom, Alzheimer-disease or AIDS.

It is important to deal with the role of this element in the dietetics, because the soil has deficiency in selenium in some parts of the world (e.g. England, Finland, Carpathian basin, China).

Although, the main area of the selenium research is selenium deficiency, because it is a real problem in the human health, dealing with selenium contamination is also important. It is toxic if taken in excess. Selenium is a naturally occurring trace element that can be released in the waste materials from certain mining, agricultural, petrochemical, and industrial manufacturing operations. Once in the aquatic environment, it can rapidly attain levels that are toxic to fish and wildlife because of bioaccumulation in food chains and resultant dietary exposure.

The quest, study and remediation (extinguish of the contamination, recovery of the original state of soil and living world) of such contaminated areas is very important and serious exercise for scientists. A high environmental contamination influences human diet and health.

Although, selenium is an essential trace element, it is toxic if taken in excess. The narrow gap of dietary intake between necessary and toxic concentrations of selenium is 0.04–4 mg kg^{-1}.

For the selenium supplementation in human diet soil fertilization with selenium was introduced in some countries of Europe. It is not completely known, how different plants uptake, transform and pass forward necessary selenium for human.

It is important to study, which form of selenium is applicable to selenium supplementation to animals and human by fertilization. There are also questions to be answered; what is the suitable dose, which change processes happen in soil and plant, which selenium forms enter to human body, what kind of effects have selenium forms in human body, etc.?

It is also essential to study the environmental effect (contamination) of selenium; the moving of selenium in soil (leaching effect) and its toxic effect in plants. Therefore, measurement of the total selenium content is not enough; determination of selenium forms in soil and plants is also necessary. These measurements can show, which selenium species are necessary to human and in which dose.

In Hungary, Prof. Dr. Imre Kádár (the professor of Hungarian Academic of Science, Research Institute of Pedology and Agrochemistry) set up an open-field experiment in Nagyhörcsök in 1991 to study the long-term effects of 13 heavy metals and also selenium. This time the calcareous chernozem formed on loess was artificially contaminated with different doses of Se as Na_2SeO_3. In the experiment effect of the artificially contaminating elements to cereals and soil life was studied. The open-field experiment was set up in Nagyhörcsök in 1991 on calcareous chernozem soil.

Experiences showed, that toxic effect of selenium increased in the first years of the experiment. During following 10 years the toxicity decreased. This experience indicates, that selenite changed to other forms and leached to deeper soil layers. This way, toxicity of top-soil layer to plants decreased.

This open-field experiment is exceptional in the world; it is appropriate to base many experience about behaviour of heavy metals and also selenium in soil. The applied high doses of elements permit good traceability of the effects of toxic elements in soil and plants.

In my PhD work I studied the selenium-treated samples of this open-field experiment. From the obtained results I aimed to answer the questions in the topic of selenium supplementation in human diet with selenium fertilization, possible solution of the selenium deficiency, selenium adsorption, change and moving in soil and remediation of selenium contaminated soils.

2. Materials and methods

2.1. Samples

The investigated soil and plant samples came from the Hungarian open-field experiment from Prof. Dr. Imre Kádár. During 1991 this open-field experiment was set up in Nagyhörcsök to study the long-term effects of 13 heavy metals. This time the calcareous chernozem formed on loess was artificially contaminated with 0, 30, 90, 270 or 810 kg ha^{-1} Se as Na_2SeO_3.
The investigated samples were taken from the soil and from the cultivated plants of the treated areas which samples originated from the years of 1991, 1992, 1994, 1997 and 2000. The plant types and parts were in corresponding order with the years: corn (stalk), carrot (root), pea (stalk), winter wheat (straw+glume) and barley (straw+glume). I could analyse also the spinach samples from the year of 1996. In the case of this plant it was possible to analyse different parts (stalk and leaves) of the plant separately.

Samples from deeper soil layers were taken at each 3 and 5 years in the open-field experiment. The sampling was carried out by 0.3 m, until 3 m depths. In this work the samples from 3 m depths and from the year 2000 were studied.

The investigated soil is calcareous chernozem (pH_{KCl} 7.1-7.4, loamy texture, clay+silt content 75-85%; humus: 3-3.5%; $CaCO_3$ equivalent 3-5%; CEC: 30-32 $cmol_c\ kg^{-1}$), the fundamental rock is loess with 15-20 m thick.

Samples were taken at every year in parcels of the experiment from the top-soil (20 cm).

The sampling was the same in the case of plant samples too. 20-20 randomly taken samples were collected excluding boundary rows. Samples were dried (40 °C) and homogenized. After the sampling and before the homogenization the plant samples were cleaned from possible contamination.

2.2. Sample preparation method for the determination of total selenium content and total element content

For determination of total selenium and element content of samples nitric acid – hydrogen peroxide wet digestion sample preparation method was applied. 1 g of soil or plant sample was added to glass digestion tubes (25x420 mm, calibrated to 50 cm^3 and 100 cm^3, heat-resisting quartz tubes, Hungary). Samples were dissolved in 5 ml by soil and 10 ml by plants of HNO_3 (65 m/m %, Scharlau Chemie, Spain) and kept for a night. On the next day samples were heated at 60 °C for 30 minutes by plant and 60 minutes in case of soil samples in a block-digester system (Labor MIM OE 718/A, Hungary). Then 3 ml for plants and 5 ml for soil of H_2O_2 (30 % m/m %, Merck, Germany) was added to the samples. The digestion was continued at 120 °C for 90 minutes for plants and 4.5 h for soil samples. After cooling down of samples they were washed up with Milli-Q water (18MΩ cm^{-2}, Millipore Corporation, USA, column: Quantum™, EX Milli-Q) to a total volume of 50 mL, and finally filtered. All filtrations were carried out in folded filters (MN 619 G1/4, Macherey-Nagel, Germany). In case of all sample-digestion a blank sample was also prepared to observe the possible contamination.

2.3. Sample preparation method for selenium speciation

To determine selenium species in soil and also plant samples I applied water extraction methods in 1:10 ratio with cool water.

Beforehand, I studied some parameters of the extraction procedure, namely extraction ratio (1:5, 1:10, 1:20), extraction time (2, 4, 24 h), using ultrasonic bath and hot water extraction.
With the pre-experiment I could determine the optimal sample preparation method: 0.5 g powdered and homogenized samples were measured to plastic tubes (15 ml, PP, Hungary) than added to 5 ml deionised water for soil and 10 ml for plant samples. In the case of plant samples 1:20 extraction ratio was needed, because of the big volume of the plant tissues.
After that, the tubes were taken to ultrasonic bath and 10 minutes long sonicated. After the ultrasonic bath, samples were left stand for 2 h during shaken at intervals. Before filtration samples were taken again to the ultrasonic bath for 5 minutes. Finally samples were filtered (MN 619 G1/4, Macherey-Nagel, Germany). For the analysis 1 ml of each sample was necessary.

2.4. Instrument, parameters of the measurements and standard materials for development of total element content in the samples

After the acidic sample preparation, an inductively coupled plasma optical emisson spectrometer (ICP-OES) (Perkin Elmer Ltd., Optima 3300 DV, USA) was used for the measurement of total element content from soil and plant samples.
The instrumental operation conditions and the parameters of measurements are given in Table 1 and Table 2.

Table 1: Parameters of the inductively coupled plasma optical emission spectrometer

ICP-OES instrument	
Type	Optima 3300 DV
Company	Perkin-Elmer Ltd.
Optical system	Echelle grating-based, Ar flushed
Wavelength range	160-782 nm
RF generator	40 MHz
Detector	Segment-array Charged-Coupled deviced Detector, SCD
Plasma view	Axial
Nebulizer type	Meinhard Type A
Type of peristaltic pump tube	black-black
optical system resolwing power	normal
Spectral bandpass	0.007 nm

Table 2: Instrument set-up and operating parameters

ICP-OES instrument	
changeable parameters	**values**
Rf power	1300 W
Nebulizer gas flow rate	0.95 dm^3 min^{-1}.
Cooling gas flow rate	15 dm^3 min^{-1}.
Auxiliary gas flow rate	1,0 dm^3 min^{-1}.
Sample introduction rate	1 cm^3 min^{-1}.

The software of the ICP-OES instrument is: Perkin Elmer ICP Winlab^{TM} (Instrument Control Software, 1997) version number: 1.42. The obtained data (ICP-OES signal (cps)) were evaulated in Microsoft Office Excel 2003 software.

Standard solutions for the calibration curve were prepared. The 100 % standard solution was prepared from 1000 mg l^{-1} concentration atomic absorption acidic standard solutions (with 0.5 mol l^{-1} HNO_3, Scharlau Chemie, Spain). Elements were in different concentration in the 100 % std. solution; specifically for plant and soil samples. To the development of calibration curves, a dilution row was used (0.2; 1; 5; 20 %). The acid concentration of the standard solutions was 3 mol l^{-1} of HNO_3.

2.5. Development of the total selenium content in the water extracts and acid digested samples with ICP-MS (inductively coupled plasma mass spectrometer) system

The inductively coupled plasma mass spectrometer (ICP-MS) (X series, earlier Thermo Elemental, England, today Thermo Fisher Scientific, Germany) with collision cell technology (CCT) was used by the measures of total selenium content in soil and plant samples. The software of the ICP-MS instrument is: Plasmalab, 2.3.0., version number: 161. The instrumental operation conditions are given in Table 3.

Table 3: Set-up and measure parameters of the ICP-MS instrument

Rf power	1400 W
Plasma gas flow rate,	14 l min^{-1}
Nebulizer gas flow rate	0.8 l min^{-1}
Auxiliary gas flow rate	0.95 l min^{-1}
Sample introduction rate	1 l min^{-1}
Pole Bias*	- 3.1 V
Hexapole Bias*	4.5 V
Extraction*	-118 V
Focus	3 V
Analogue detector	2500 V
PC detector	3850 V
CCT gas (H_2:He) flow rate (7% H_2 + 93% He)	5.9 ml min^{-1}
Integration time	0.1 s
Stabilization time	35 s
Monitored selenium isotopes	^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se
Sample pump tube (white/white) (Anachem Ltd., Anglia)	1.02 mm

For the measurements to reduce the interferences (polyatomic adducts) collision cell technology (CCT) was used.

The standard solutions to the calibration curve were prepared from 1000 mg l^{-1} of concentration atomic absorption acidic standard solution (with 0.5 M HNO_3, Scharlau Chemie, Spain) in a concentration of 0.1; 1; 10; 100 µg l^{-1} (with 2 % HNO_3).

Samples were diluted with distilled water to 2 % of acidic content.

2.6. Development of selenium species using IC-ICP-MS (ion exchange chromatography-inductively coupled plasma-mass spectrometer) system; measurement parameters and standard materials

IC-ICP-MS coupled system was used for development of selenium species. The Meinhard nebulizer (cooled to 2 °C) was connected to an anion exchange chromatography column (100 mm x 4.6 mm × 12µm, Polyspher, IC-AN1, Merck, Germany) with a peristaltic pump tube (65 cm long, 0.38 mm i.d.). The solution eluting from the column was introduced on-line to ICP-MS.

An Merck, Hitachi L-6200A intelligent high-performance liquid chromatography (HPLC) pump (Germany) was used in the coupled system (flow rate: 1 ml min^{-1} pressure: 30 bar) with a Rheodyne injector valve (CA, USA) fitted with a 100 µL sample loop (PEEK, 0.5 mm i.d., Alltech, USA). Anion-exchange HPLC was carried out using a Merck IC-AN1 column.

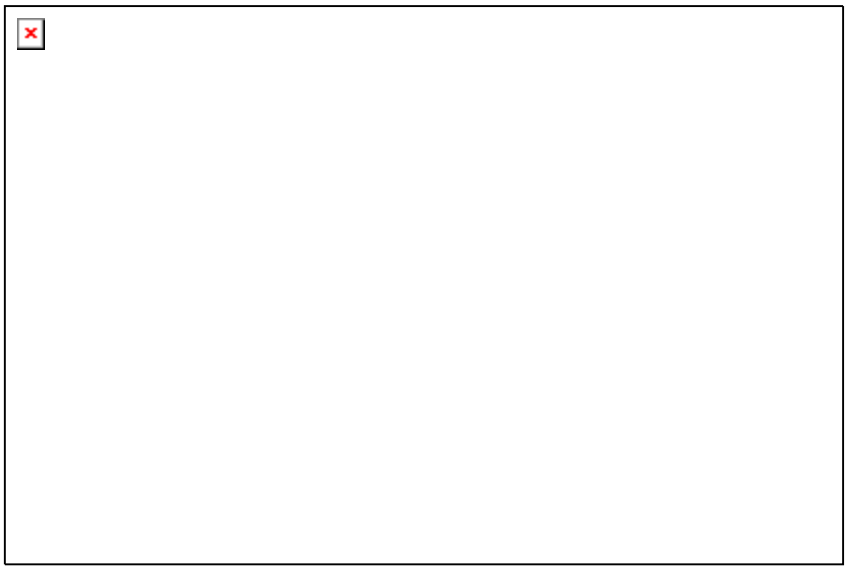
Use of anion-exchange column enables analysis of anionic selenium forms (inorganic compounds). The ICP-MS instrument and the operating parameters were the same as in the chapter 2.5. The obtained data (ICP-MS signal (cps)) were evaulated with Windows NT Office Excel 2002 software. At ICP-MS measurements ^{80}Se isotope was monitored.

The applied 1000 mg l^{-1} (calculated to selenium) selenium standards for speciation analysis were: seleno-L-cystine ($SeCys_2$; Fluka Chemie, Switzerland); seleno-DL-methionine (SeMet; 99%+, Fluka Chemie, Switzerland), Se(IV) ($Na_2SeO_3.5H_2O$; 99%; Sigma-Aldrich, Switzerland) and Se(VI) (Na_2SeO_4; 98%; Aldrich Chemical Company, WI, USA).

Standard materials were solved in Milli-Q water. The 100 µg l^{-1} concentration Se(IV), Se(VI) and SeMet standard solution was prepared from the 1000 mg l^{-1} standards fresh every day.

The eluent was a Tris: Phthalic acid solution (0.171 g Tris (Tris(hydroxymethyl)aminomethane, Sigma, Germany) and 0.249 g phthalic acid (Sigma, Germany) filled up to 1 l, with Milli-Q water, filtered after 15 minutes ultrasonic bath (HF-frequency: 35 kHz, Bandelin Electronic, Sonorex Super RK 103H, Germany).

Picture 1 shows the IC-ICP-MS system.



Picture 1: IC-ICP-MS system

2.7. Development of adsorption isotherms

For studying adsorption isotherms, control chernozem top-soil (0-20 cm) samples from Nagyhörcsök were used. 15 ml of selenite and selenate solution in different concentration (calculated to selenium) were added to 1.5 g soil. After that samples were shaken (Sklárny, Avalier, Czech Republic) for 2 h than filtered (MN 619 G1/4, Macherey-Nagel, Germany). Concentration of filter-liquor was measured by ICP-MS.

2.8. Stability experiment

In this experiment, control soil sample was also used. 66 ml (75 % of solution from Arany-cohesiveness number) of selenite solution was added to 2*200 g soil samples. The Se standards were the same as in the chapter 2.7. Selenium concentration was 10 mg l^{-1}.
Soil samples were moistened and mixed with selenium solution than dried in oven (Labormim, Hungary) at 40 °C for 24 h. After that soil samples were grinded (Retsch, Germany) and homogenized.
5-5 samples (1 and 0.5 g) were collected randomly. Acidic digested sample from 1 g soil and water extracts from 0.5 g soil samples were prepared as in the chapters 2.2. and 2.3.
Total selenium concentration of acidic digested samples was measured with ICP-MS and selenium species in water extracts were developed with IC-ICP-MS system. With this method homogeneity of the treated samples was checked.
The stability experiment was carried out at 3 different temperature (-20 °C, 4 °C and 25 °C) for 5 months in 3 repeats. After 5 months all samples were analysed at the same time by IC-ICP-MS coupled system according to that in the chapter 2.6.

3. Results and discussion

3.1. Development of the selenium-speciation method; identification of selenium species by standard materials

Before the selenium speciation measurements individual peaks of standard materials with their retention times and peak heights were determined. Through this, identification of peaks of selenium species in soil and plant samples was made possible. Chromatogram of standard solution with 3 Se forms; Se(IV), Se(VI) and SeMet in concentration of 100 µg l^{-1} was also analysed. Before measuring of inorganic standard materials, two organic compounds of selenium were analysed to study the separability of organic selenium forms using anion-

exchange column. In accordance with the expectations, at the applied pH the separation of organic compounds was not possible. The Se-Cys and Se-Met came in the same retention time. The separation of organic selenium species was not aim of this work. The change of selenite-selenate in soil and the content of these species next to the organic Se compounds in plant samples were important for the study.

3.2. Studying of soil samples of the long-term field experiment in Nagyhörcsök

3.2.1. Speciation analysis of the soil samples

Studying of the acidic digested control soil 20 µg kg^{-1} total selenium concentration was determined. By analysis of water extracts of control soil selenium peaks were not obtained. Consequently, the selenium content of control soil of the open-field experiment is low.

Studying of water extracts of soil samples from 1991, the increment of the dose of selenite is good traceable. The obtained results already show the change of selenite to selenate and organic forms in the first year of the experiment, in the studied calcareous chernozem soil. The obtained chromatograms can be seen in Fig. 1.

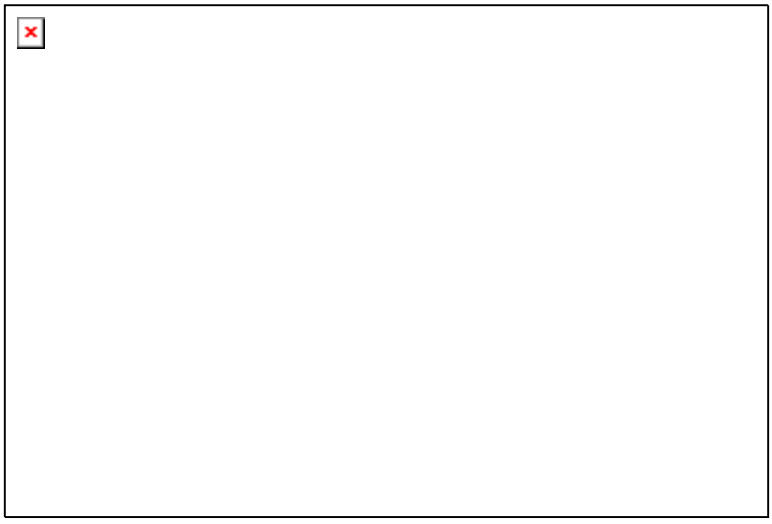


Fig. 1: Selenium species in soil samples from 1991
and comparison of their contents (I: 1. repeat, 1,2,3,4: doses: 30, 90, 270 and 810 kg/ha,
peaks: a = organic Se comp.; b = Se(IV); c = Se(VI))

The results of the speciation analysis of soil samples in respect of time show that the most part of selenite transformed to selenate during the 10 years of the experiment. Organic forms are also present in low content but its presence is not remarkable to selenite-selenate change. Fig. 2 shows the obtained results in column-diagram.

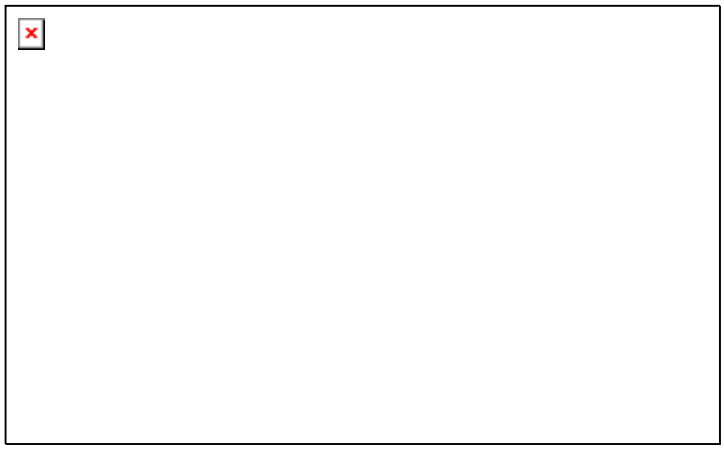


Fig. 2: Change of the content of selenite and selenate in the top soil in the different years drawn in column diagram

Moving of selenium forms in soil is traceable with study of the deeper soil layers.

Selenite is in higher concentration in the top 0-0.6 m soil layer. Bellow 0.6 m selenite almost disappears. The organic selenium compounds behave in similar way. However, high concentration of selenate could be measured in the deepest soil layer (at 3 m depths). These results show our supposition; in the investigated soil the majority of selenite transforms to selenate and it moves towards deeper soil layers (leaching-effect).

Figure 3 shows the adsorption and leaching effect in the deeper soil layers till 3 m in soil samples of 2000.

a) SeO_3^{2-} b) SeO_4^{2-}

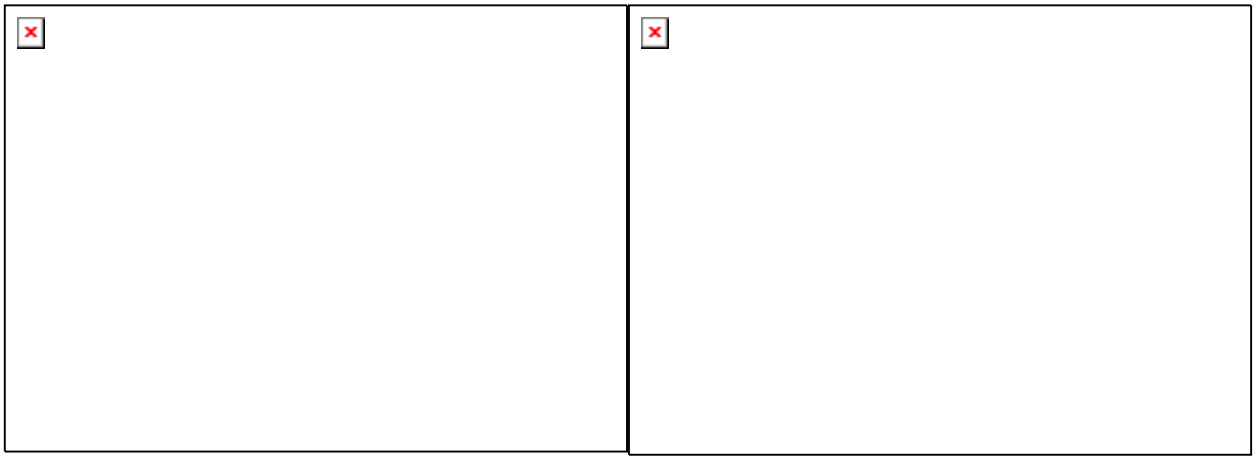


Fig. 3: Adsorption and leaching effect of selenite a) and selenate b) in the deeper soil layers of the studied calcareous chernozem soil from the year of 2000

3.2.2. Adsorption of selenium in soil samples from Nagyhörcsök; study of the adsorption isotherms

The adsorption isotherms give the functional relationship between the equilibrium solution (C_e [mg l^{-1}]) and content of the adsorbed material. From the measured equilibrium concentration of solution and the known basic concentration of the solution the adsorbed content of selenium (q_e [mg g^{-1}]) was measured for the studied control calcareous chernozem soil. The calculated adsorption isotherms apply to room temperature. These can be seen in Fig. 4.

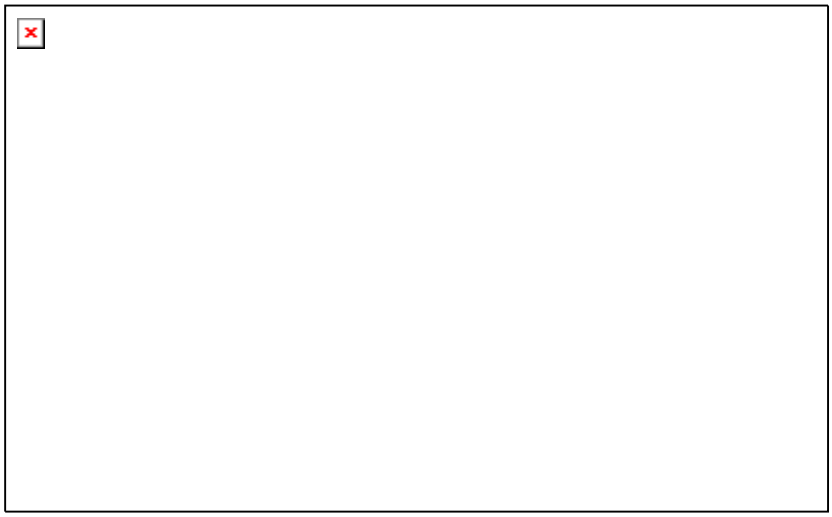


Fig. 4: Adsorption isotherms of selenium species
(q_e content of the adsorbed Se in soil in equilibrium (mg g^{-1}),
C_e solution concentration in equilibrium (mg l^{-1}))

The adsorption of selenite and selenate in the top-soil was simulated with adsorption-isotherms. Selenite adsorption appeared to be stronger on the studied chernozem soil than selenate adsorption. In this soil selenate is more mobile than selenite and selenium in this form moves toward deeper soil layers.

For simulation of adsorption of selenium forms also the linearised Langmuir-adsorption isotherms were used. The linearised Langmuir-isotherms can be seen in Fig. 5.

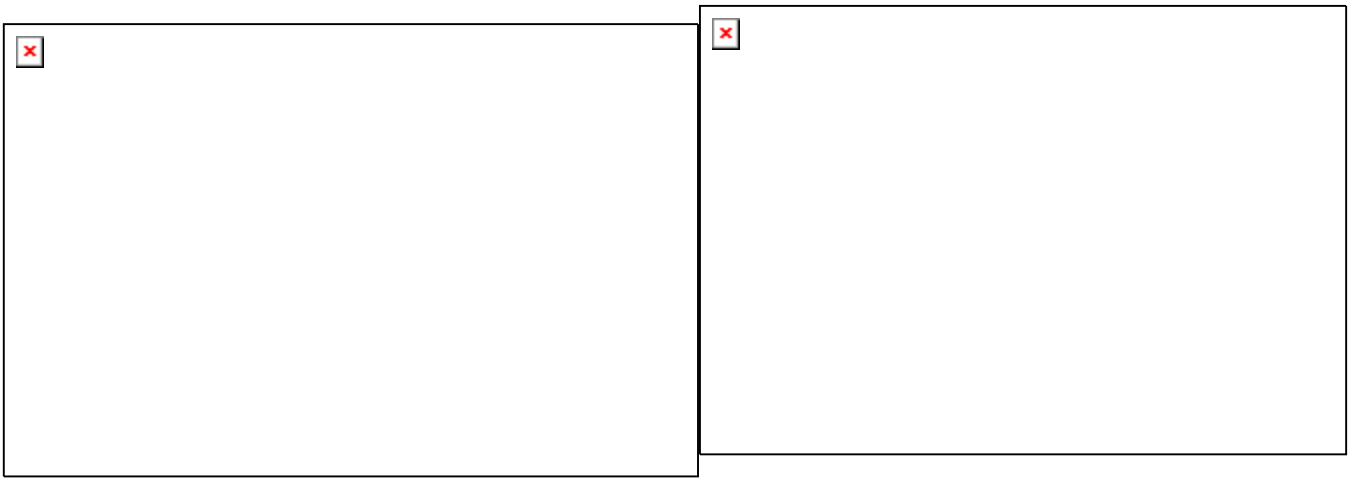


Fig. 5: The linearised Langmuir-isotherms of seleniteand selenate adsorption

Parameters of the Langmuir-equation can be calculated from the crossing point and slope of the axes. Table 4 shows the obtained results.

Table 4: Parameters of the Langmuir-isotherm calculated from the crossing point and slope of the axes. The r^2-values in the Table are the regression coefficients of the lines' fitting

sample	Se(IV)			Se(VI)		
	k_L [mg l^{-1}]	Q [mg g^{-1}]	r^2	k_L [mg l^{-1}]	Q [mg g^{-1}]	r^2
calcareous chernozem soil from Nagyh.	0.702	17.9	0,997	21.8	20.5	0,9996

3.2.3. Development of the content of total water soluble selenite and selenate calculated from the adsorption isotherms and comparison with total and soluble selenium content in soil

ICP-MS instrument was used to determine total selenium concentration in water extracted and acidic digested soil samples.

The next figure shows the comparison of total selenium content in the acidic digested and water extracted soil samples from the year of 1991, which were treated with different doses of Na-selenite. (For the better visibility of the results logarithmic scale was applied).

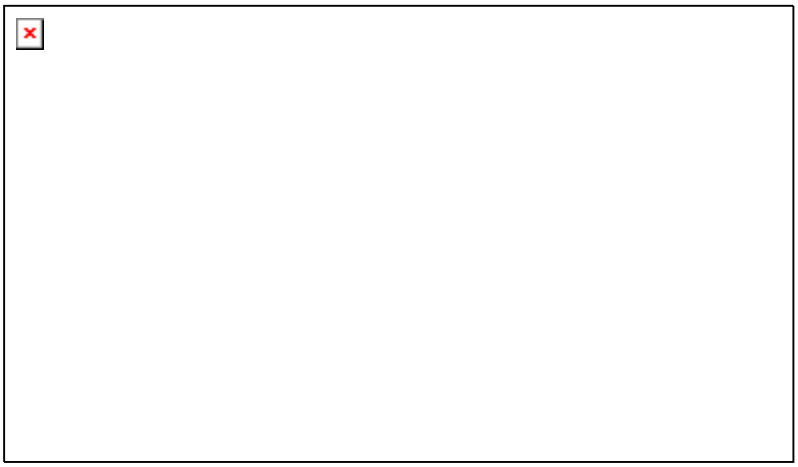


Fig. 6: Comparison of the total Se concentration in the acidic digested samples and the water extracts

Fig. 7 shows, that selenite content is the highest in the first year of the experiment but low concentration of selenate and organic forms of selenium are also present.

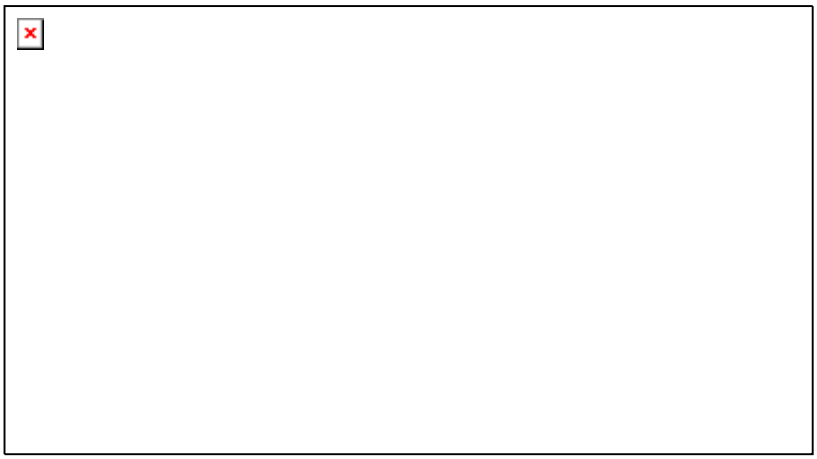


Fig. 7: Comparison of content of selenium species in water extracts of soil samples from 1991

After the speciation analysis total selenium concentration of the water extracts and acidic digested samples were also measured. Efficiency of the water extraction was calculated; it was between 20-30%. It showed to be higher than previous published efficiency values (~10%). The reason of this high extraction yield is that selenium is mostly in two forms (selenite and selenate) in the studied soil and these forms are water soluble.

Total water soluble concentration of selenite and selenate was calculated from the adsorption isotherms. It means the summa of two concentrations, that of selenite and selenate in the water solution (soluble content) and the adsorbed concentration in soil. The obtained concentration is comparable to the total selenium content in the acidic digested samples.

Through this vehicle, the adsorption of the water soluble selenium species and the fixed selenium forms in soil can be estimated more precisely.

From the equation-parameters of linear adsorption isotherms the k_L values were calculated. It is for selenite: 0.702 and for selenate: 21.8. Through transformation of the equations and respecting the dimension change the content of the adsorbed water soluble selenite and selenate in soil can be calculated with the next formulas:

$$c_{ads(selenite)} = 1/(17.9+39.2/c_{measured\ in\ water\ extracts\ (selenite)} \times 10) \times 1000$$

$$c_{ads(selenate)} = 1/(20.5+1066.8/c_{measured\ in\ water\ extracts\ (selenate)} \times 10) \times 1000$$

The obtained results and dimensions are in Table 5

Table 5: Measured and calculated total selenium concentration and their ratio by soil samples from 1991 and 2000 (doses: 1 = 30, 2 = 90, 3 = 270, 4 = 810 kg ha^{-1})

	Concentration (mg kg^{-1})				
1991	**Total Se, acidic A[a]**	**Total meas. solubl. Se B[b]**	**Ads. Se in soil, calculated rem. C[c]**	**Meas. total solubl. Se B+C[d]**	**Ratio of total solubl. and total Se in solution (B+C)/A*100[e]**
1	2.70	0.795	1.84	2.64	97.5
2	13.1	3.78	7.93	11.7	89.3
3	50.7	17.7	24.7	42.4	83.6
4	118	34.0	33.7	67.8	57.2

[a] A = total selenium content in the acidic digested soil samples (mg kg^{-1})
[b] B = total selenium content measured in the water extracts (water soluble Se) (mg L^{-1})
[c] C = calculated adsorbed Se content in soil (mg kg^{-1})
[d] (B+C) = total water soluble Se = total Se content in water extracts + adsorbed Se content in soil (mg kg^{-1})
[e] (B+C)/A*100 = Ratio of total water soluble Se and the total Se content in acidic digested soil samples

The obtained results show that 80-90 % from the total selenium is water soluble in soil and only 10-20 % is fixed. The reason of this fact is, that the treatment was carried out with water soluble selenite, which form changed to selenate in soil, which form is also water soluble. Only little amount of the selenium transforms to organic compounds through microbiological activity of soil.

3.2.4. Stability experiment; studying of stability of selenium species in the stored soil samples

The stability experiment aimed to study weather the change of selenite to selenate and organic forms came from open-field circumstances or do they also transform under storage in the laboratory.

Before the studies homogeneity of the samples was checked. The samples were prepared according to the chapter 2.8. 5-5 samples were collected randomly. Means and p-values of the one way analysis of variance confirmed that the samples were homogenous.

The stability experiment was carried out at 3 different temperature (-20 °C, 4 °C and + 25 °C) for 5 months in 3 repeats. With the speciation analysis of water extracted samples the stability of selenium forms in samples kept in refrigerator (-20 °C) and fridge (4 °C) was determined. However, a part of selenite changed to organic forms at room temperature but this change was negligible (0,001 %). Consequently, the transformation of selenite to selenate and organic forms was really due to open-field circumstances.

3.3. Studying of the plant samples from the long-term filed experiment in Nagyhörcsök

3.3.1. Speciation analysis of the plant samples; studying of the selenium take-up and estimation of the selenium species in the plant types

After studying soil samples, plant samples from the treated areas were analysed. Selenium species and their concentrations in water extracts of plant samples were studied. The selenium in plants occurs principally in two forms: organic forms and selenate. In some plants really only these two forms were present, but in certain plants all the three forms occurred (Se(IV), Se(VI and also organic forms). Ratio of selenite was low in all samples. The selenate content was high in carrot, winter wheat and barley and especially in pea. Fig. 9 shows the comparison of the amount of selenium species in corn-stalk samples from 1991.

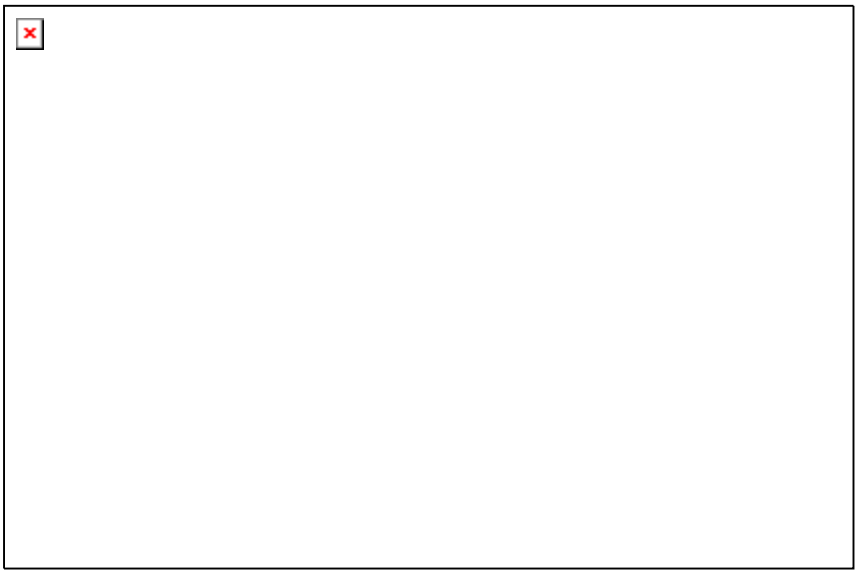


Fig. 8: Obtained selenium species by measure of corn samples originated from 1991 (II: 2. repeat, 1, 2, 3, 4: doses: 30, 90, 270 and 810 kg ha^{-1}, peaks: 1 = organic Se forms; 2 = Se(IV); 3 = Se(VI))

Fig. 8 shows that the peaks of selenium species increase with the increment of Na-selenite doses. It is also visual, that in corn-stalk samples only organic forms and selenite were present.

The next figure shows the ratio of selenium species in pea stalk samples. High content of selenate in this plant is well visible.

One cause of the presence of high concentration selenate in plants can be that the prior take up form of selenium is selenate. This form is easily uptakeable for plants, because it is similar to sulphate. The possibility of selenate uptake even increases by the years because ratio of selenate to selenite also increases in soil.

Another cause of the high selenate concentration in plants could be, that plants also take up both selenite besides selenate, but it quickly transforms to the latter form.

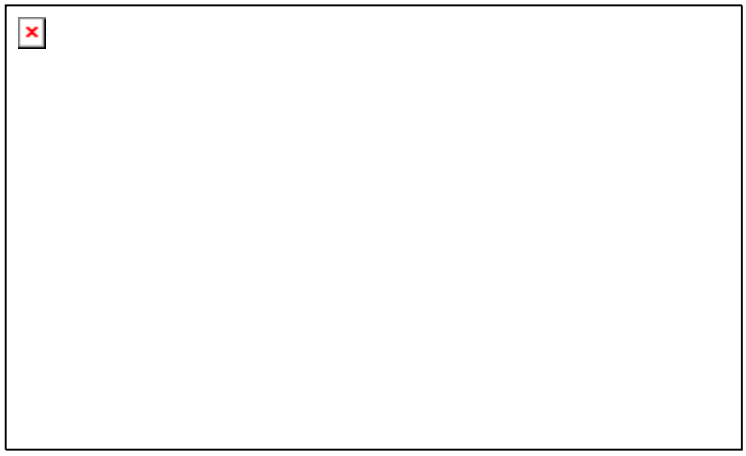
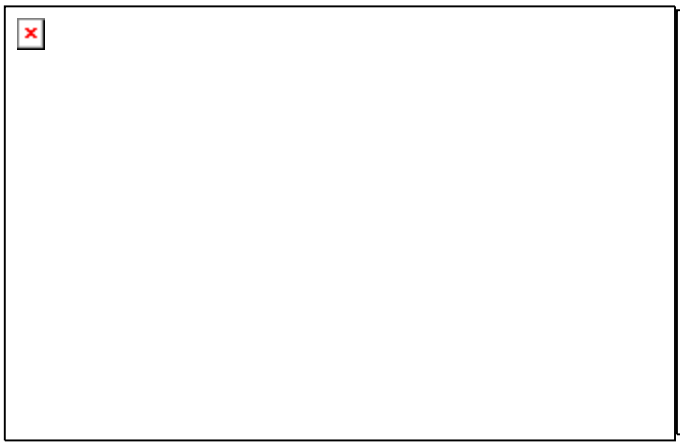


Fig. 9: Ratio of the content of selenium forms in pea stalk samples from 1994

In the part a) and b) of the next picture ratio of selenium species in further two plant samples from Nagyhörcsök was shown.

a) carrot root



b) winter wheat (straw+glume)

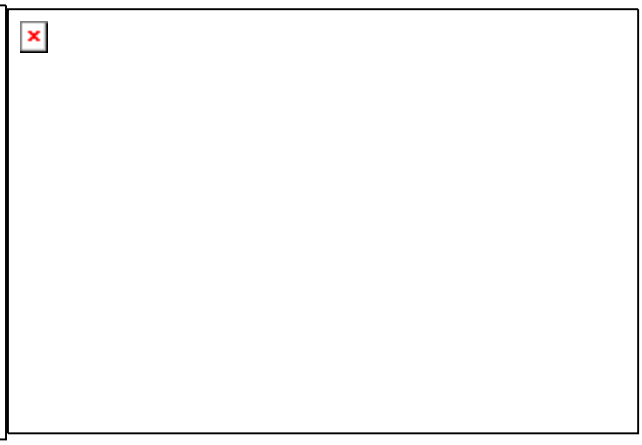


Fig. 10: Ratio of selenium species in plant samples from Nagyhörcsök; a) carrot (root), 1992; b) winter wheat (straw+glume), 1997

3.3.2. Studying of the total selenium content in the plant samples and its comparison with the selenium content in their water extracts. Studying of the efficiency of the water extraction

Fig. 11 shows the comparison of total selenium content in acidic digested and water extracted corn-stalk samples. It can be seen in Fig. 11 that the ratio of total selenium content in the water extracts is lower than the same ratio of the soil samples. Efficiency of the water extraction could be calculated; it was between 13-15%, which agrees with the previously published efficiency values (~10%). This is reasonable, because selenium is in organic (not water soluble) form in plants.

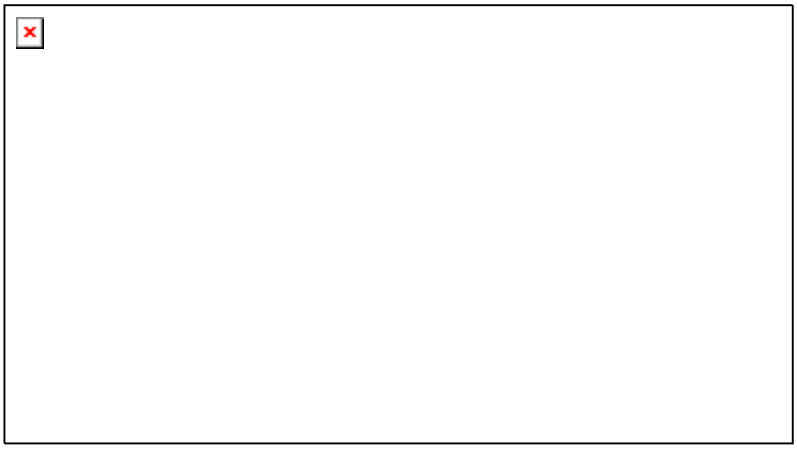


Fig. 11: Comparison of the total selenium content in the acidic digested and water extracted samples of corn from 1991

3.3.4. The main processes in soil and plants related to selenite and selenate

Fig. 12 shows the main processes of selenite and selenate in the studied chernozem soil and seeds cultivated on. It can be seen in Fig 13 that selenite mostly adsorbes in this soil strongly and changes to selenate. Selenate adsorbes less and moves to deeper soil layers (leaching). Plants take up both of two forms but selenate uptake is stronger. Selenite changes to organic forms in plants. The change of selenate was not considerable in the studied seeds. However, some well-known plants can transform selenate to other forms, e.g. Brassica and Allium crops.

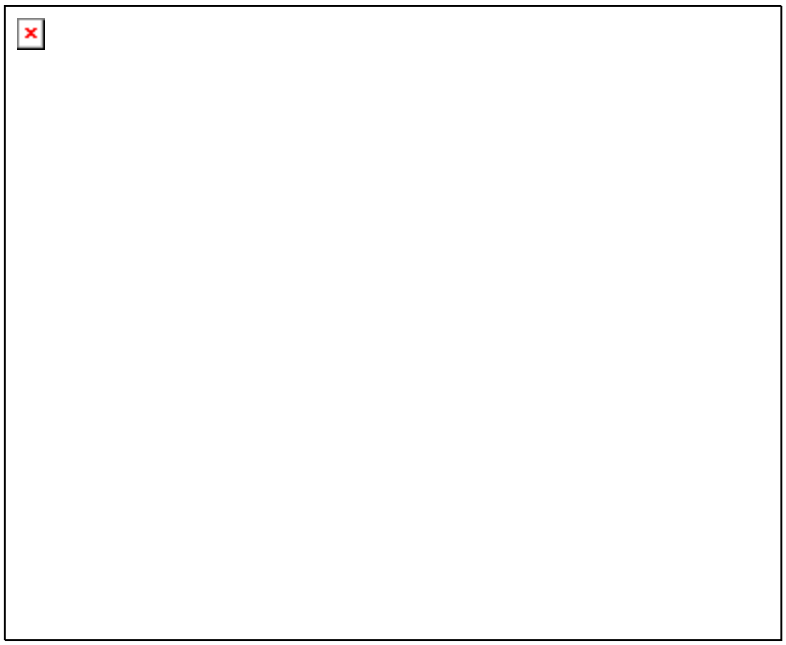


Fig. 12: The main change and move process of selenite and selenate in the studied soil and the cultivated seeds

New and novel scientific results

- I determined that the given out selenite oxidize to selenate form in calcareous chernozem soil in time. The transformation is not sudden, it needs more years. Also organic forms appear in low content but their presence is not remarkable compared to selenite-selenate change.

- On calcareous chernozem soil in case of selenite fertilization one must calculate with the possibility of considerable leaching in the form of selenate. This latter form is more mobile than selenite in alkaline soils. Selenate is in soil at 3 m depth in high

content even after 10 years. Selenite adsorbs in the top-soil and it stays in the surface layer. Selenate moves toward deeper soil layers.

- I determined the constant values of adsorption isotherms of selenite and selenate in soil profile. They are necessary for modelling of leaching effect.

- I could calculate the total water soluble concentration of selenite and selenate from the equation-parameters of linear Langmuir-isotherms. It means the summa of two concentrations, that of selenite and selenate in the water solution (soluble content) and the adsorbed concentration in soil. Through this vehicle, the adsorption of the water soluble selenium species and the fixed selenium forms in soil can be estimated more precisely.

- I experienced high selenate content in case of carrot, winter wheat, barley and especially pea. One cause of the presence of high concentration selenate can be that plants take up the selenium mostly in selenate form. This form is easily uptakeable for plants, because it is similar to sulphate. The possibility of selenate uptake even increases by the years because ratio of selenate to selenite also increases in soil. Another cause of the high selenate concentration in plants could be, that plants also take up both selenite besides selenate, but it quickly transforms to the latter form.

- On the basis of my results I can firmly state, that selenite is not applicable to selenium supplementation in high doses in respect of soil-manuring. In concern of soil fertilization a maximum of about 30-50 kg ha^{-1} concentration could be advisable. However, high doses are not applicable on calcareous chernozem soil. Selenite to selenate transformation is very slaw, so this mechanism can insure a continuous selenium supply for plants. The adsorption of selenite is stronger on chernozem soil so its leaching possibility is lower.

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