

EFFECT OF MICROELEMENTS ON ALFALFA ON A SANDY SOIL

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The effect of 0, 30, 90 and 270 kg·ha<sup>-1</sup> rates of microelements on alfalfa was examined on a calcareous sandy soil during the year 2004 - 2008 in Őrbottyán, Hungary. The salts of the microelements were applied on a single occasion at the start of the experiment in spring 1995 in the form of  $Cr_2(SO_4)_3$ ,  $K_2Cr_2O_7$ ,  $CuSO_4$ ,  $Pb(NO_3)_2$ ,  $Na_2SeO_3$  and  $ZnSO_4$ . The 24 treatments (6 elements×4 application rates) in 3 replications gave a total of 72 plots. The location was prone to drought and was poorly supplied with NPK macronutrients. The ploughed layer contained 0.7–1.0% humus and 2–3% CaCO<sub>3</sub>, and the groundwater was located at a depth of 5–10 m. The whole experiment was given 100 kg·ha<sup>-1</sup> each of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O active ingredients as basal fertilizer each year. The favourable precipitation contributed to the advantageous development and yield of alfalfa during the 5 years period. Pb and Cu loads remained in the ploughed layer. The 0.2-0.4 mg·kg<sup>-1</sup> Pb-content of the control alfalfa hay increased to 0.5-1.4 mg·kg<sup>-1</sup> on the treated soil on average. Cu concentration rose from 5-7 mg·kg<sup>-1</sup> to 9-10 mg·kg<sup>-1</sup> due to high Cu load. Se showed an extreme 3 orders of magnitude accumulation in hay, i.e. from under 1 mg·kg<sup>-1</sup> detection limit to 200-400 mg·kg<sup>-1</sup>. The hay became unsuitable for feeding. However alfalfa can be utilized in phytoremediation. During the 5 years the total yield of 45.5 t·ha<sup>-1</sup> alfalfa hay took up 6-12 kg·ha<sup>-1</sup> Se. In 2006 after the 12<sup>th</sup> year of the experiment the leaching zone of Cr(VI) exceeded 3 meters, and that of Se exceeded 4 meters in 270 kg·ha<sup>-1</sup> treatments. Vertical movement could not be verified in the case of Cr(III), Pb, Zn, Cu.

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## Introduction

In a review of the health risks posed by microelements entering the food chain, the concept of the "soil-plant barrier" was introduced and microelements were classified into four groups. Group number one is comprised of the elements Ag, Cr, Sn, Y and Zr, which pose mild risks, owing to their low solubility in soil and consequently negligible uptake and translocation by plants. Elevated concentrations of these elements in foods usually indicate direct contamination by soil or dust.<sup>1,2</sup>

Group number two includes the elements As, Hg and Pb which are strongly absorbed by plant roots, and not readily translocate to edible plant part and so pose minimum risks to human health. Group number three includes the elements B, Cu, Mn, Ni and Zn. The "soil-plant barrier" may protect the food chain from these elements, which are partly but readily taken up by plants, which are phytotoxic at concentrations that pose little risks to human health. Group number four consists of Cd, Co, Mo and Se, which pose human or animal health risks at plant tissue contents that are not generally phytotoxic.

Out of the element listed above, the elements that give most commonly risk to health concerns about food safety are the heavy metals, such as Cd, Hg and Pb together with anionic metalloids As and Se.<sup>3,4</sup>

The main sources of elevated soil As are the historically widespread use of As compounds as insecticides, herbicides and defoliants for agricultural production. These include inorganic salts and organic compounds of both arsenite As(III) and arsenate As(V). High levels of As occur naturally in some sedimentary rocks and in geothermically active areas. Feed additives for poultry and swine production can result manures with high As pool. Arsenic from phosphate fertilizers, from fossil fuel combustion and from municipal sewage sludge are considered to be significant sources of As pollution in soil. <sup>5, 6, 7, 8</sup>

When metals are added as soluble salts, they generally cause greater plant uptake and toxicity than when applied in forms such as sewage sludge or metal oxides. Metals are not in soluble forms in sludge, but fixed with organic matter binding sites or occluded in  $CaCO_3$  or in other minerals. Sludge organic matter adds metal sorption capacity to the soil and raises the soil CEC and often the pH. So, the source and form of element added may strongly affect the result. This is called the "salt vs. sludge" error in experimentation for evaluating metal responses.

The  $2^{nd}$  error is called the "greenhouse vs. field" error. Greenhouse studies offer greater management and reproducibility, and lower cost than field studies. However, the measured element contents may be increased 2-5 fold over field measurements of the same soil and crop. In pots, the plant roots have small volume of treated soil, plants require abnormal watering. The smaller are the pots, grater are the chances of error. Plant respond differences may originate from the root distribution in the soil with depth, and can only be found correct in field studies. <sup>1,4,9</sup>

Field experiments represent the factual conditions for a given soil-plant system. They are indispensable to follow the transport of elements not only into the various crops, but also into the food chain, their downward migration, and to assess the long-term fate and effects of elements under natural circumstances.

# Methods

A long-term field trial was set up at the Örbottyán Experimental Station of the Institute for Soil Science and Agricultural Chemistry (Centre for Agricultural Research, Hungarian Academy of Sciences) on calcareous sandy soil in 1994. The main characteristics of the soil are as follows: pH<sub>(KCl)</sub> 7.0, clay content about 5%, CaCO<sub>3</sub> 4%, organic C 0.6%. Four levels (0, 30, 90 and 270 kg/ha) of the selected metal salts (Cr(III), Cr(VI), Cu, Pb, Se, Zn, in the form of  $Cr_2(SO_4)_3$ ;  $K_2Cr_2O_7$ ;  $CuSO_4$ ;  $Pb(NO_3)_2$ ;  $Na_2SeO_3$ ;  $ZnSO_4$ , respectively) were added to the 35 m<sup>2</sup> plots once at initiation and were mixed into the 0-20 cm ploughed layer. The rates and forms of microelements applied in the experiment are shown in Table 1. The treatments were arranged in a splitplot design with triplications. The experimental plots were cultivated with commonly used agrotechnics, mineral fertilizers were added yearly (100 kg/ha N, P2O5, K2O as Ca-ammonium nitrate, superphosphate and 60% KCl) to ensure sufficient macronutrient supply. The grown crops were: carrot in 1995, pea in 1996, winter wheat in 1997, sunflower in 1998, garden sorrel in 1999, barley in 2000, rape in 2001, maize in 2002, mustard in 2003, alfalfa in 2004-2008.

Composite *soil samples* consisting of 20 subsamples were collected from the ploughed layer of each plot. Using 20-40

plants or plant parts per plot randomly, plant samples were taken from every cut of alfalfa.

 Table 1. Single rates and forms of microelements applied in the experiment in 1995 (Calcareous sandy soil, Örbottyán)

Element	Rat	es of eleme k	Form of salts		
	1	2	3	4	
Cr(III)	0	30	90	270	$Cr_2(SO_4)_3$
Cr(VI)	0	30	90	270	$K_2Cr_2O_7$
Cu	0	30	90	270	CuSO <sub>4</sub>
Pb	0	30	90	270	$Pb(NO_3)_2$
Se	0	30	90	270	Na <sub>2</sub> SeO <sub>3</sub>
Zn	0	30	90	270	ZnSO <sub>4</sub>

Total number of plots: 6x4 = 24 treatment x 3 replication= 72. Size of plots:  $35 \text{ m}^2$ 

The "total" amount of the elements in homogenized *soil* and *plant samples* were measured after microwave digestion using cc.  $HNO_3+H_2O_2$ . In the soil samples the so-called "mobile" fraction extracted with ammonium-acetate + EDTA <sup>10</sup> was also determined. The composition of prepared samples was analyzed by inductively coupled plasma spectrometry (ICP-AES) detecting 25 elements.

The sowing of alfalfa was done on 5 April 2004 with "Verkó" variety with 30 kg·ha<sup>-1</sup> seed. About the water supply of alfalfa: during the year 2004 and 2008 no extreme draught year occurred therefore the stand could thrive during 5 years. The least precipitation was a total of 466 mm in the year 2007, while highest was 619 mm in the year 2008. The average annual precipitation was 569 mm.

Number of			Yea	Maan	Total		
cuts	2004	2005	2006	2007	2008	Wiean	Total
Green yield t-l	na <sup>-1</sup>						
1.	15	10	16	16	12	14	69
2.	7	13	12	14	10	11	56
3.	3	12	9	5	8	7	37
4.	-	10	9	-	-	4	19
Total	25	45	49	35	30	36	181
Air dry matter	%						
1.	22	19	20	31	32	25	-
2.	23	29	19	27	30	26	-
3.	28	25	23	34	22	27	-
4.	-	22	22	-	-	22	-
Total	24	24	21	31	28	26	-
Air dry hay yie	eld t·ha <sup>-1</sup>						
1.	3.2	1.9	3.3	4.5	3.8	3.3	16.7
2.	1.5	3.9	2.5	3.6	3.0	2.9	14.5
3.	0.8	3.1	2.3	1.7	1.8	1.9	9.7
4.	-	2.1	2.1	-	-	0.8	4.2
Total	5.5	11.0	10.2	9.8	8.6	9.0	45.1

Table 2. The average yield per cuts and years in the 10th-14th years oft he experiment (Calcareous sandy soil, Örbottyán)

**Table 3.** Effect of treatments on the Cr, Pb and Cu content of the air dry hay of alfalfa in the means of the cuts (Calcareous sandy soil, Örbottyán)

Years	Rates of e	lements in spring		LCD	Maar	
	0	30	90	270	LSD <sub>5%</sub>	wiean
Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> treatment, Cr mg·kg	g <sup>-1</sup>					•
2004	0.5	0.8	1.2	1.9	0.6	1.1
2005	0.3	0.6	0.7	0.9	0.5	0.6
2006	0.4	0.5	0.6	0.8	0.3	0.6
2007	0.3	0.4	0.6	0.7	0.3	0.5
2008	0.1	0.2	0.2	0.4	0.2	0.2
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> treatment, Cr mg·kg	-1					
2004	0.4	0.5	0.8	1.4	05	0.8
2005	0.6	0.6	0.8	1.0	0.4	0.8
2006	0.4	0.5	0.7	1.2	0.4	0.7
2007	0.4	0.3	0.7	1.2	0.5	0.7
2008	0.1	0.2	0.4	0.7	0.2	0.3
Pb(NO <sub>3</sub> ) <sub>2</sub> treatment, Pb mg·kg	- <sup>1</sup>					
2004	0.4	0.6	0.8	1.4	0.9	0.8
2005	0.4	0.4	0.6	0.9	0.6	0.6
2006	0.5	0.6	0.6	0.8	0.5	0.6
2007	0.2	0.3	0.5	0.5	0.4	0.4
2008	0.2	0.4	0.4	0.5	0.3	0.4
CuSO <sub>4</sub> treatment, Cu mg·kg <sup>-1</sup>						
2004	5.3	7.3	8.6	9.7	2.3	7.8
2005	4.9	6.8	8.2	9.2	2.2	7.3
2006	6.7	8.6	9.4	10.4	2.8	8.8
2007	7.0	8.0	8.6	9.0	1.6	8.2
2008	6.7	7.6	8.6	9.2	1.8	8.1

Table 4	. Effect of treatments	on the Zn, Se	e and Na content	of the air dry	y hay of alfalfa	in the means	of the cuts (	Calcareous	sandy soil,
Őrbottyá	in)								

Years		Rates of element	kg∙ha <sup>-1</sup>	LOD	Mean	
	0	30	90	270	LSD <sub>5%</sub>	
ZnSO <sub>4</sub> treatment, Zn mg·kg <sup>-1</sup>						
2004	19	25	28	29	8	25
2005	18	20	21	25	4	21
2006	18	20	23	29	5	23
2007	17	20	21	26	4	21
2008	21	24	28	31	5	26
Na <sub>2</sub> SeO <sub>3</sub> treatment, Se mg·kg	-1					
2004	<1	24	131	416	77	190
2005	<1	29	128	249	40	102
2006	<1	27	147	189	40	91
2007	<1	36	187	383	88	152
2008	<1	21	98	223	44	86
Na <sub>2</sub> SeO <sub>3</sub> treatment, Na mg·kg	-1					
2005	350	346	396	429	50	380
2006	386	346	451	488	56	418
2007	408	459	595	622	104	521
2008	388	532	557	706	108	546
Na <sub>2</sub> SeO <sub>3</sub> treatment, S %						
2004	0.36	0.45	0.59	0.66	0.10	0.52
2005	0.35	0.43	0.58	0.55	0.14	0.48
2006	0.34	0.41	0.62	0.53	0.09	0.48
2007	0.33	0.43	0.67	0.67	0.12	0.53
2008	0.24	0.30	0.44	0.43	0.08	0.35

## **Results and discussion**

The yields of alfalfa were not affected significantly by the treatments, so data are presented only in the means of treatments by cuts and years in *Table 2*. The highest green and air dry yields were obtained in the  $2^{nd}$  and  $3^{rd}$  years, when the 4 cuts gave 45-49 t·ha<sup>-1</sup> green, or 10-11 t·ha<sup>-1</sup> air dry hay yield. The number of cuts, and the annual yields remained low in the 1<sup>st</sup>, 4<sup>th</sup> and 5<sup>th</sup> years. The 1<sup>st</sup> and 2<sup>nd</sup> cuts were responsible for most of the yields every year. An exceptional year was 2005, when the  $2^{nd}$  cut gave 12 t·ha<sup>-1</sup> green mass. The air dry mass content of the alfalfa varied between 19-34% depending on the cuts and was at an average of 26%. During the 5 years the sum of the air dry hay yield was 45 t·ha<sup>-1</sup> on calcareous sandy soil.

Effect of Cr(III) treatment on the Cr content of alfalfa hay is shown in *Table 3*. The concentration of Cr rise 2-4-fold in comparison to control as an average of the years and cuts. It is well known that Cr is barely mobile in the soil-plant system. It can accumulate mainly in the root, but its transport is hindered within the plant. On calcareous chernozem soil on Cr-treated plots e.g. the shoot of the young maize contained 3 mg·kg<sup>-1</sup>, while the root had 158 mg·kg<sup>-1</sup> Cr content.<sup>11</sup>

Plant samples were not washed, so external pollution cannot be excluded. The presented data series of 5 years indicate that only a small amount of Cr releases from the applied Cr(III) salt and become available for plants. The Cr accumulation of alfalfa is declining with time.

Cr accumulation of alfalfa was not significant different in Cr(VI) treatment. In this case the Cr content of the shoot is also minor. The alfalfa takes up less Cr in progress of time. However, the effect of treatment becomes relatively enhanced with the years. It is partly resulted by the fact that the water-soluble chromate form leached and accumulated in the 2-3 m soil layer in 2006. So the roots of alfalfa could reach this sphere with time.

Years	Rates of	elements in spi				
	0	30	90	270	LSD <sub>5%</sub>	Mean
$K_2Cr_2O_7$ treatment, Cr g·ha <sup>-1</sup>						
2004	2.8	4.4	6.6	10.4	3.3	6.0
2005	3.3	6.6	7.7	9.9	3.5	6.9
2006	4.1	5.1	6.1	8.2	3.1	5.9
2007	2.9	3.9	5.9	6.9	2.9	4.9
2008	0.9	1.7	1.7	3.4	1.7	1.9
Total:	14.0	21.7	28.0	38.8	7.9	25.6
Pb(NO <sub>3</sub> ) <sub>2</sub> treatment, Pb g·ha <sup>-1</sup>						
2004	2.2	3.3	4.4	7.7	3.0	4.4
2005	4.4	4.4	6.6	9.9	2.6	6.3
2006	5.1	6.1	6.1	8.2	2.1	6.4
2007	2.0	2.9	4.9	4.9	1.4	3.7
2008	1.7	3.4	3.4	4.3	1.2	3.2
Total:	15.4	20.1	25.4	35.0	7.4	24.0
CuSO <sub>4</sub> treatment, Cu g·ha <sup>-1</sup>						
2004	29	40	47	53	13	42
2005	54	75	90	101	24 80	
2006	68	88	96	106	29	90
2007	69	78	84	88	16	80
2008	58	65	74	79	15	69
Total:	278	346	391	427	88	360
ZnSO <sub>4</sub> treatment, Zn g·ha <sup>-1</sup>						
2004	104	138	154	160	44	139
2005	198	220	231	275	44	231
2006	184	204	235	296	51	230
2007	167	196	206	304	49	218
2008	181	206	241	267	43	224
Total:	834	964	1067	1302	174	1042
Na <sub>2</sub> SeO <sub>3</sub> treatment, Se kg·ha <sup>-1</sup>						
2004	<1	0.13	0.72	2.29	0.42	0.78
2005	<1	0.21	1.41	2.74	0.60	1.09
2006	<1	0.35	1.50	1.93	0.80	0.94
2007	<1	0.18	1.83	3.75	0.96	1.44
2008	<1	0.28	0.84	1.92	0.70	0.76
Total:	<1	1.15	6.30	12.63	4.40	5.01

Table 5. Effect of treatments on the microelement yield of alfalfa in the means of the cuts (Calcareous sandy soil, Örbottyán)

**Table 6**. Effect of treatments on the "total" (cc.HNO<sub>3</sub>+cc.H<sub>2</sub>O<sub>2</sub> soluble) and NH<sub>4</sub> acetate+ EDTA soluble element content of the soil in 2008, mg·kg<sup>-1</sup> (Calcareous sandy soil, Örbottyán)

Elements	Rates of el	ements in spring		LSD <sub>5%</sub>	Mean				
	0	30	90	270					
cc.HNO <sub>3</sub> +cc.H <sub>2</sub> O <sub>2</sub> soluble content									
Zn	22	24	28	40	6	28			
<sup>1</sup> Cr	14	18	20	35	6	22			
<sup>2</sup> Cr	11	16	18	29	6	18			
Cu	4	6	13	28	5	13			
Pb	10	11	14	26	5	15			
Se	<1	2	3	3	2	2			
NH <sub>4</sub> -acetate+EDTA soluble co	ontent								
Pb	3.3	3.8	7.5	13.1	2.4	6.9			
Zn	1.9	3.9	6.1	11.7	2.6	5.9			
Cu	1.2	1.8	7.0	20.4	5.0	7.6			
<sup>1</sup> Cr	< 0.1	0.1	0.2	0.4	0.2	0.2			
<sup>2</sup> Cr	< 0.1	0.1	0.2	0.4	0.2	0.2			
Se	< 0.1	0.1	0.2	0.4	0.2	0.2			

Remark: Leaching of Cr(VI) in 2006 exceeded 3 m, while that of Se exceeded 4 m depth. In Cr(III), Pb, Zn, Cu treatments leaching could not be verifiable with  $NH_4$ -acetate+EDTA method. <sup>1</sup>Cr=Cr(III), <sup>2</sup>Cr=(CrVI)

Pb is also hardly available for plants, especially on calcareous soils. Accumulation occurs mainly in roots. According to results shown in *Table 3*, concentration in shoots can increase 2-3-fold on treated plots compared to control. The average Pb content shows a decreasing trend with the years.

**Table 7.**  $NH_4$ -acetate+EDTA soluble element content of the 0-490 cm soil layer on the control and on the 270 kg-ha<sup>-1</sup> treated plots in 2006, in the 12<sup>th</sup> year of the experiment (Calcareous sandy soil, Örbottyán)

Sampling	Co	ntrol soil,	Trea	ted soil,
depth, cm	1	mg∙kg⁻¹	mg	·kg <sup>-1</sup> *
	Se	Cr	Se	Cr(VI)
0-30	0.28	0.05	0.50	0.55
30-60	0.26	0.05	0.53	0.14
60-90	0.17	0.02	0.39	0.19
100-130	0.25	0.02	0.21	0.12
130-160	0.08	0.04	0.86	0.11
160-190	0.15	0.10	1.36	0.14
200-230	0.15	0.09	1.25	0.18
230-260	0.16	0.10	1.93	0.31
260-290	0.26	0.07	2.04	0.59
300-330	0.12	0.10	1.06	0.42
330-360	0.12	0.14	0.64	0.16
360-390	0.14	0.14	0.49	0.13
400-430	0.16	0.10	0.40	0.10
430-460	0.16	0.10	0.12	0.10
460-490	0.16	0.10	0.10	0.10
Mean	0.17	0.08	0.79	0.22

\*The deeper soil layers were not polluted in the Pb, Cr(III), Cu, Zn treatments

In the case of copper the effects of treatments are not considerable, Cu-content of control rise usually with 40-80% due to the load. Copper is also considered as a hardly mobile element. Cu content in plant do not show extreme differences. Copper supply of 6-15 mg·kg<sup>-1</sup> in air dry alfalfa hay considered satisfactory. <sup>12</sup> The applied Cu-load had a positive effect on alfalfa, as it raised its Cu content to the satisfactory level, reaching about 10 mg·kg<sup>-1</sup> values (*Table 3*). In USA, Cu-content under 10 mg·kg<sup>-1</sup> is considered as

low level, and 10-30 mg·kg<sup>-1</sup> as satisfactory level.<sup>13</sup> The highest 270 kg·ha<sup>-1</sup> Zn-load resulted in only 8-10 mg·kg<sup>-1</sup> Zn-content increment on average. The minimum value was 15 mg·kg<sup>-1</sup>, while the maximum was 37 mg·kg<sup>-1</sup> regarding the 1<sup>st</sup> cuts between 2004 and 2006. The other years and cuts were more settled. It is well known that Zn binds and transforms fast to less available ZnCO<sub>3</sub> on calcareous soil. Results shown in *Table 4* reflect this trend. Satisfactory Zn supply of alfalfa hay would be 25-70 mg·kg<sup>-1</sup> of Zn content. <sup>12</sup> So the applied Zn fertilization could only ameliorate the Zn depletion of the stand, bringing it to the lower limit of the satisfactory level.

Se present extremely in hay reaching three orders of magnitude compared to control. As it is shown in *Table 4* the maximum level in plant tissue can be detected in the first year. According to deep drill soil samplings Se similarly to Cr(VI) accumulated in the 2-3 m soil layer in NH<sub>4</sub>-acetate+EDTA soluble form. Presumably the roots of alfalfa could reach this layer and took up Se. Alfalfa is a hyper-accumulator of Se without considerable yield decrease. Se content above 1-2 mg·kg<sup>-1</sup> is physiologically undesirable in long-term. So the hay grown on treated plots cannot be utilized for feeding. Alfalfa can be suitable for phytoremediation purposes in the case of Se pollution or the pollution of deeper soil layer.

The applied Na-selenite can transform to Ca-selenate in the calcareous, well-aerated soil. Na and also the selenite form are very mobile in the soil-plant system, and cation/anion synergism can occur regarding plant uptake. According to *Table 4* Na concentration of hay rises significantly with Se load. This effect is enhanced by the years, in 2008 Na content increases with 80% on heavily Sepolluted plots compared to control. The yearly average Na content also increased with aging from 380 mg·kg<sup>-1</sup> in the 1<sup>st</sup> year to 546 mg·kg<sup>-1</sup> in the 5<sup>th</sup> year. Minimum value was 256, maximum was 883 mg·kg<sup>-1</sup> Na during the 5 years depending on the treatments and cuts. The enrichment of hay in Na could be advantageous for feeding purposes, as Na demand of animals can generally be satisfied only by grasses grown on salt-affected soils. Se load lifted S uptake of plants. A reason for this synergistic effect could be the fact that Se together with S synthesise into amino acids. S content remains at a relatively constant 0.3% level in hay on control plots which increases to 0.6% due to high Se load, in the average of years. Extreme differences can occur as a function of cuts and years. In 2008 0.21% minimum and in 2004 0.92% maximum in the 3<sup>rd</sup> cut means more than 4-fold differences on control soil.

As a member of the oxygen group (O, S, Se, Te, Po) Se has similar characteristics to S. Se enrichment can be found at pyrite rich sites. According to Hungarian geochemical researches greater Se concentration sites are related to sulphide-mineralization. <sup>14, 15</sup> The accumulated Se in plant may cause Se toxicity in grazing animals. Se overload can be moderated by S addition in order to optimize the S/Se rate. In this case the sulphate/selenite antagonism rules and Se uptake is hindered by S-fertilization. Unfortunately Se rich soils are also well supplied by sulphate, so the ameliorative effect of S fertilization cannot develop. The satisfactory S supply of alfalfa is about 0.3-0.6%, similarly to P supply. In this experiment, Se application improved the uptake of S, therefore synergetic effect could be detected as S supply of alfalfa was moderate or poor.

*Table 5* shows the microelements synthesised in the shoot of alfalfa. Uptaken volumes were Cr 14 – 39, Pb 15 – 35, Cu 278 – 427, Zn 834 – 1302 g·ha<sup>-1</sup> depending on the pollution of soils during the 5 years. Considering phytoremediation, to clean the 270 kg·ha<sup>-1</sup> load, for Pb 65 thousand years, for Cr 50 thousand years, for Cu 9060 years, for Zn 2885 years of alfalfa production would be necessary in similar conditions.

The 45 t $\cdot$ ha<sup>-1</sup> hay harvested during the 5 years contained 1.3 kg $\cdot$ ha<sup>-1</sup> Se on 30 kg $\cdot$ ha<sup>-1</sup> Se-treated plots. Thus theoretically 115 years would be necessary to clean this contamination. Similarly on 90 kg $\cdot$ ha<sup>-1</sup> Se-treated plots Se yield was 6.3 kg $\cdot$ ha<sup>-1</sup> which means 56 years of phytoremediation. So phytoremediation can be reasonable on moderately Se polluted sites.

As it is seen in *Table 6* in the year 2008, in the  $14^{th}$  year of the experiment the effect of microelement load is strongly indicated by the "total" (cc.HNO<sub>3</sub>+cc.H<sub>2</sub>O<sub>2</sub> soluble) and the NH<sub>4</sub>-acetate+EDTA soluble element content of the ploughed layer of the soil. A considerable part of Pb, Zn, Cu elements remained in soluble form, but Cr(VI), Cr(III), Se elements can be hardly detected in this form in the upper layer.

According to the results of 5 meters deep drill soil samplings in 2006, in the  $12^{\text{th}}$  year of the experiment, the leaching zone of Cr(VI) exceeded the depth of 3 meters, while that of Se exceeded 4 meters. In Cr(III), Pb, Zn, Cu treatments no leaching could be detected with the NH<sub>4</sub>-acetate+EDTA method.

The soluble contents of Se and Cr are given from every 30 cm layers on control and on 270 kg·ha<sup>-1</sup> treated plots in the examined soil profiles (*Table 7*).

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