Theses of doctoral (PhD) dissertation

CADMIUM SENSITIVITY OF MAIZE AND SUNFLOWER HYBRIDS, THE POSSIBILITY OF REDUCING THE HARMFUL EFFECTS

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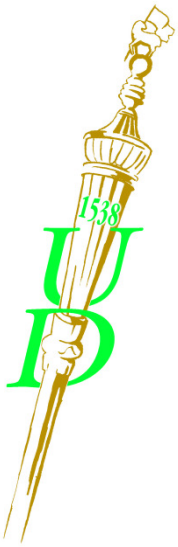

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1. ANTECEDENTS AND GOALS OF THE DOCTORAL DISSERTATION

Nowadays agricultural plants are continuously exposed to toxic chemicals, including heavy metals. Heavy metals contaminate the environment and do harm to human health. In recent years heavy metal content of our soils significantly increased on the one hand due to mining, the industrial activities and urbanisation, while on the other hand due to the effects of pesticides and fertilizers used in agriculture. It is known that toxic heavy metals (lead, cadmium, mercury) are able to enter the living organisms and can hinder many important metabolic processes. Presence of toxic heavy metals do harms in the homeostasis of plant cells, in transpiration and in several other physiological processes (Di Tioppi and Gabbrielli 1999). However, plants are able to survive this abiotic stress, due to their defense mechanism (Supalkova et al. 2007). Plants can live and grow in contaminated environment, even they fix and detoxify the heavy metal ions in their tissues. Therefore using the harvested plants as food can threathen to both animal and human health (Zehnalek et al. 2004). According to the recommendation of the FAO/WHO Joint Expert Committee on Food Additives the tolerable maximum intake of cadmium, including all sources (food, air and water) reflecting to body weight is 1.0-1.2 $\mu g\ kg^{-1}$ (FAO/WHO, 1972). Heavy metals, as stressors decreases plant production. Their toxifying effect and accumulation in plants depend on many factors, such as the characteristics of the soil, the concentration of the contaminant, the time period of the contamination, the complex-forming materials of the rhizosphere, the plant species (Fodor, 2003). Cadmium can easily be uptaken by plants, especially on acidic soils, and is transported rapidly inside the plant. Besides plants can often accumulate it in huge amount without any visible symptoms. In my study two such plants (maize and sunflower) were involved that have different nutrient uptake mechanism, and directly or indirectly play an important role in human nutrition. The chosen plants also have an important industrial role. In 2012 the acreage of maize was 1.280.000 hectares, while of sunflower was 621.000 hectares (KSH data).

In my present work I have studied the effect of heavy metals, and within especially of the cadmium, to plant physiological processes and parameters of different maize and sunflower hybrids. The effect of cadmium in different living organisms has already been widely studied, but its total mechanism of action has not known yet. During my examinations I tried to give an overall picture on how cadmium effects to nutrient uptake of plants, to photosynthesis, and what defence mechanism the examined plant species react with. I looked for the answer on whether exist such sustainable solution, that can compensate the negative effect of cadmium

on plants, therefore can increase the utilisation of the contaminated areas as well. As such alternative solution I involved a bacteria-based biofertilizer into my trials.

To reach our goals we examined the followings:

- effect of Cd to the intensity of initial root growth
- effect of Cd to the composition and the intensity of secretion of root acid
- effect of Cd to photosynthesis, within this to the relative and absolute chlorophyll content, and to the pigment composition
- the study of the pH of the apoplastic solution, due to the different treatments, in case of plants grown on nutrient solution
- we examined in rhizoboxes how the content of soluble cadmium in soils changes as time passes, and what its relationship is with the availability of its uptake by plants, and with its accumulation in given plant organs, in soil samples contaminated by cadmium (*barren land of Gyöngyösoroszi mine*)
- involving different plant species and their hybrids into the trials – if we accept the suggestion that metal tolerancy and metal uptake are functionally linked --, then the data of uptake can become comparable in case of different species, giving inner data on the tolerancy operations of each of the genotypes.

This approach gives the possibility to compare in two different plant species the effect of heavy metals stress causing plant physiological changes, or to show the differences amongst hybrids of the same plant species. The information received such way can be well utilized in the presentation of the mechanism of stress tolerance, and even in plant breeding, as well as in the utilization of agricultural lands contaminated by heavy metals.

2. METHODS OF THE STUDY

2.1. Growing conditions

The plants were grown in the laboratory of the Department of Agricultural Botany and Plant Physiology in the Institute of Plant Sciences, in the Center for Agricultural and Applied Economic Sciences of the University of Debrecen. We used maize (*Zea mays L.*) and sunflower (*Helianthus annus L.*) hybrids as trial plants, their short review can be found in *Annex 11*. The seeds were germinated between wet filter paper layers, then were grown under

hydroponic conditions on nutrion solution (*Lévai és Kovács,* 2001). The composition of the nutrient solution was the following: 2,0 mM of $Ca(NO_3)_2$, 0,7 mM of K_2SO_4, 0,5 mM of $MgSO_4$, 0,1 mM of KH_2PO_4, 0,1 mM of KCl, 1 µM of $MnSO_4$, 1 µM of $ZnSO_4$, 0,25 µM of $CuSO_4$, and 0,01 µM of $(NH_4)_6Mo_7O_{24}$. In the nutrient solution for monocots the concentration of boron was 1 µM of H_3BO_3, while for dicots was 10 µM of H_3BO_3. Plants received iron in the form of 10^{-4} M of FeEDTA. Cadmium treatment happened in form of $CdSO_4$, the applied concentrations were occasionally changed according to the goal of the trial. During the trials the environmental conditions were regulated: the light intensity was 350 µmol m^{-2} s^{-1}, the periodicity of the temperature was 25/20 °C (daytime/nighttime), the relative humidity (RH) was 65-75%, the illumination/dark period was 16 h/8 h.

In case of certain trials the nutrient solutions contained live bacteria as well, what were given to the nutrient solution in the form of a commercial biofertilizer in the quantity of 1 ml dm^{-3}. The applied biofertilizer was a viscous liquid that contained two bacteria tribes; the free-living nitrogen fixing *Azotobacter chroococcum* (1-2 x 10^9 db cm^{-3}) and the phosphorus mobilizing *Bacillus megatherium var. phosphoricum* (1-2 x 10^8 db cm^{-3}), whose utilization is accepted in organic farming as well. Its biocontrol permission number is FM 9961/1992. According to the patents it has three important areas of application:

- enrichment of the nitrogen content of the soil and improvement of phosphorus uptake,
- intensification of the mineralization of stubble residues having high fiber content and
- stimulation of soil life

2.2. Determination of dry matter content

We determined the dry matter content of plant by termo gravimetric method. The trials were set in 6-8 replications, the shoot and root parts were put separately into a drying oven preheated to 85°C for 48 hours. After drying to constant weight we measured back the dry material weight with four decimal accuracy. For the measurements OHAUS Explorer (Switzerland) type analitycal balance was used.

2.3. Determination of the relative chlophyll content (SPAD-index)

We made the determination of the relative chlorophyll content (SPAD-index) by the SPAD-502 (Soil Plant Analysis System) (Minolta, Japan) chlorophyll meter. The equipment gives information on the total chlorophyll content, what is calculated from the intensity of the red (650 nm) and infrared (940 mm) lights passing through the leaf. We made our

measurements in ten-fold replicates in case of each leaf, evenly between the leaf base and the leaf apex. The advantage of the method is its rapidity, it is not harmful to the plants, therefore during the development of the plant the changes of this parameter can be continuously traced.

2.4. Determination of the absolute concentration of the photosynthetic pigments

For the determination of photosynthetic pigments we made a pigment extract from the samples after homogenisation with using acetone of 80% (*Wellburn,* 1994). We determined the concentrations of chlorophyll–*a*, chlorophyll–*b* and of total carotenoids spectrophotometrically (Metertek SP-830 UV/VIS, Japan). In the extract the determination of the concentrations (mg g^{-1}) was done on the basis of absorbancies registrated on 663, 646 és 470 nms. For eliminating the turbidity our results were corrected with the absorbancy measured on 750 nm. Chlorophyll–*a*, chlorophyll–*b* and total carotenoid contents were related to per unit dry weight. Namely I applied the method of *Moran and Porath* (1980) (In: *Vidican and Cachita-Cosma,* 2010), whereby I dissolved 50 mg fresh leaf sample in 5 ml of DMF (N,N-Dimethyl-formamide) during 72 hours at 4ºC. Then I measured photometrically the amount of chlorophyll–*a* (664 nm), of chlorophyll–*b* (647 nm), and of the carotenoids at 480 nm wavelength. For the determination of the amounts I applied the following formulas:

Chlorophyll– ***a*** (mg/gSP) = (11,65 A_{664} – 2,69 A_{647}) *v/sp

Chlorophyll– ***b*** (mg/gSP) = (20,8 A_{647} – 3,14 A_{664}) *v/sp

Carotenoids (mg/gSP) = (1000 A_{480} – 1,28 chlorophyll ***a*** – 56,7 chlorophyll ***b***) / 245 * v/sp

wkere: A480 – is the readable value at 480nm filter;

A647 – is the readable value at 647nm filter;

A664 – is the readable value at 664nm filter;

v – the applied solution in ml;

SP – mg fresh plant material used for the extraction.

2.5. Measurement of photosynthetic activity by the use of the method of chlorophyll-fluorescence induction

For the determination of the activity of photosynthesis – as an indirect method – we applied the method of chlorophyll fluorescence induction (*Schreiber et al.,* 1994). In the dark-adapted leaves the *in vivo* chlorophyll fluorescence, the parameters of the rapid phase of chlorophyll fluorescence induction were determined with a PAM-2001 type fluorometer

(WALZ Gmbh, Germany) végeztük. Before the measurements the plant sample was adapted to dark for 20 minutes.

During the measurement at first the dark-adapted sample was lighted by weak measuring light, and the level of basic fluorescence (F_o) was measured, then after the application of the saturating light pulse (6000 µmol m^{-2} s^{-1}), the equipment detected the maximum fluorescence (F_m). In the dark after about 20 seconds F_m falls back to the level of F_o *(Figure 1.)*. The difference between F_m and F_o is the variable fluorescence (F_v) (*Figure 1.*). We used the F_v/F_m rate to characterize the PSII maximum (potential) photochemic efficiency.

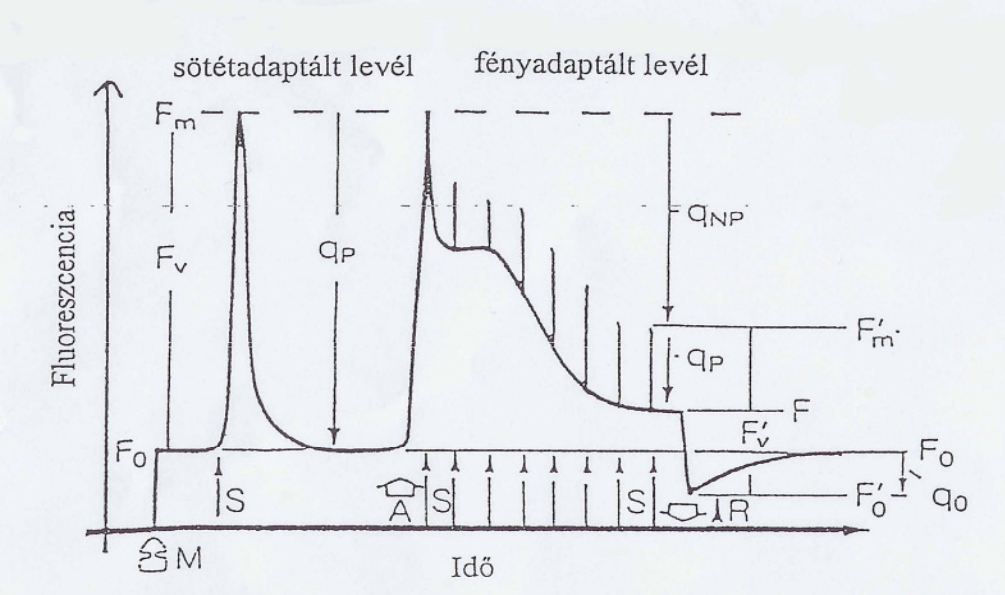

Figure 1.: Induction curve of the chlorophyll fluorescence

2.6. Examination of the acid secretion of the root

For the detection of root acids secreted by the plant was used agar sheets, the indicator was bromocresol purple. The preparation of the agar sheet was made according to the followings. The agar medium contained 100 ml of distilled water, 1,25 g of Agar-agar, which was boiled for approx.15 minutes on water-bath, then 1 ml of bromocresol purple indicator was added. (The indicator solution contained 1,25% of bromocresol purple (BCP-5',5"-dibromo-o-krezolszulfoftalein), whose pH value was set to 6.0 by using 1 n of NaOH and 1 n of H_2SO_4). The such made medium had the pH value of 6.0; its colour became purple. I made a colour range by changing the pH values (with the range of 0.5), whose help the range of colours of each pH value became identifiable with. The agar-agar medium poured into 3 mm sheets, and these sheets were used to identify the acid extraction of roots (*Marshner et al.,* 1982). This is a relatively subjective method for the visualization of the chosen root acid, or for the estimation of the pH of the chosen acid.

2.7. Determination of the element content of the samples

Treatment of the plant samples

The element content of the roots and shoots of the examined plants were determined separately. The iones bound to the surface of the roots were removed by rinse with 0,1n of HCl, while the remnants of hydrochloric acid with distilled water. Before the evaluations the roots and shoots were dried to constant weight at 85 °C in drying oven. Preparation of the plant samples for element determination was done as follows: from the properly prepared sample (dried to constant weight, chopped), during the pre-destruction, I gave 10 ml of HNO_3 to 1 g material, what I kept at 60 °C for 30 minutes. After their cooling down the samples were completed with 3 ml of H_2O_2, and I continued the destruction for further 90 minutes at 120 °C. The destructed samples, after their cooling down, were completed with deionized water up to 50 ml, then were filtered with MN 640 W filter paper, and the element content of the samples were determined by OPTIMA 3300DV ICP-OA (Perkin-Elmer) spectrophotometer. The extreme values of the deviations from the mean values were signed in the tables.

Treatment of soil samples

From the properly prepared (dried, ground) samples I weighted in 1 g, which was destructed during the pre-destruction with 5 cm^3 of distilled of cc. HNO_3, at 60 ^oC for 30 minutes. Then during the main-destruction with adding of 5 cm^3 of H_2O_2 of 30%, I destructed it for 270 minutes at 120 ^oC temperature. After the cooling down of the destructed material, I filled up the test tubes to 50 cm^3 with deionized water, then I filtered the samples with Filtrak 388 filter paper. Then we measured the total element content of the samples with ICP-MS equipment.

2.8. Rhizobox trials

I put 250 g of soil into each rhizobox, homogenously mixed with distilled water until corresponds to 50% of field water capacity, and I moistened them with $CdSO_4$-solutions corrresponding to the treatments. In case of the cadmium treatments I used $CdSO_4$-solutions concerning to 10, 30, 50, 100, 150 µmol. 100 mg kg^{-1} of cadmium. Before placing the soil into the rhizoboxes, I put filter papers moistened before with deionized water onto the bottom of each rhizobox. Thus we ensured the homogenous moisture dispersion in the soil. Every day I measured the weight of each rhizobox and the length of the roots of each plant, and I complemented the missing water amount (transpiration, evaporation). I evaluated the maize and sunflower plants sown into the rhizoboxes five days after the sowing.

2.9. Parameters of soils used during the trials

We used two different types of soil for the rhizobox trials. The first soil was a calcareous chernozem originated from the Látókép Pilot Plant of the Center for Agricultural and Applied Economic Sciences of the University of Debrecen. During the trials it took part as control, or was contained with cadmium in the concentration determined by me. The other soil originated from the barren land of the Gyöngyösoroszi mine, from the banks of the Toka-creek. This soil was contaminated for years due to the mining activities. Its parameters were the followings:

	Látókép	**Gyöngyösoroszi**		**Gyöngyösoroszi**	
		1st soil profile		***2nd soil profile***	
Depth (m)	0-0.3	0-0.4	0.4-0,8	0-0.4	0.4-0.8
pH (KCl)	5.71	7.20	4.97	6.27	3.76
pH (H_2O)	6.58				
Arany type of plasticity index	43				
Water-soluble total salt	0.015				
Ca (mg kg^{-1})		5740	40175	4750	27875
Humus (%)	4.75	4.775	1.085	4.605	1.065
KCL-soluble NO_3-N+NO_2-N:(mg kg^{-1})	8.04	39.63	16.15	32.91	23.99
AL-soluble P_2O_5 (mg kg^{-1})	199	46.5	0.75	34.84	0.77
AL-soluble K_2O (mg kg^{-1})	451	113.00	83.55	34.84	27.83
KCl-oldható Mg (mg kg^{-1})	332	8.59	0.925	8.59	4.47
KCl-EDTA soluble Fe (µg kg^{-1})		61.0	11.56	43.45	77.6
KCl-EDTA soluble Zn (µg kg^{-1})	7.9	93.4	3.1	88.1	22.63
KCl-EDTA soluble Mn (µg kg^{-1})	262	78.8	2.63	65.9	39.66
$Pb_{220.353}$ (mg kg^{-1})		1952,5	839	1913,5	616
$Cd_{228.802}$ (mg kg^{-1})		4,485	11,7	2,705	12,3

2.10. Statistical evaluations

The statistical evaluation of the results was made by the programs of Microsoft Excel 2007, Sigmaplot 8.0 (2001) and SPSS 14.0. During the evaluation the normality of the dispersion of the data was checked by the Kolmogorov-Smirnov test. The simultaneous comparison of the mean values was done by the Duncan-test. The authenticity of the significancy results received in case of the treatments of on-similar variencies was checked by the t-probe.

3. MAIN STATEMENTS OF THE DISSERTATION

Evolution of some physiological parameters of maize and sunflower seedlings due to the effect of cadmium

Effect of Cd treatments in the initial phase of root growth

During the incubation period maize was more sensitive than sunflower. The relapse of root growth showed significant decrease during all three treatment periods (1, 2, 3 h) (*Table 1.*). In case of sunflower significant difference was detected only between the 1 and 3-hour incubation times. In case of both species the root growth inhibitant effect of cadmium was the most pronounced during the 3-hour incubation time. In case of the same incubation times, we were not able to detect any statistical differences amongst the hybrids regarding to the root growth intensity occurred during 24 hours.

Table 1.: *Root growth of maize and sunflower hybrids (cm) depending on the treatment time, 24 hours after treatment (n=8, ** p <0,01; *** p <0,001; n.s.= there is no significant difference)*

	Treatment time			
Maize hybrids	**1 hour**	**2 hours**	**3 hours**	***F-value***
PR37D25	1.22 a	0.84 b	0.79 b	***8.30*** ***
DKC4490	1.17 a	1.13 a	1.03 a	***0.61 n.s.***
P9400	1.27 a	1.15 a	0.88 b	***5.87*** **
Sunflower hybrids	**1hour**	**2 hours**	**3 hours**	***F-value***
NK Brio	0.22 ab	0.19 b	0.13 cb	***2.50*** *
NK Ferti	0.21 ab	0.11 cb	0.12 b	***3.00*** *
NK Neoma	0.19 a	0.19 a	0.12 a	***1.36 n.s.***

In case of maize in 6, 24 and 30 hours after the treatment there were significant differences in the root growth in all three measurement times (*Table 2.*).

Table 2.: *Root growth of maize and sunflower hybrids (cm) depending on the time passed after treatment (n=8, *** p <0,001)*

	Time after treatment			
Maize hybrids	**6 hours**	**24 hours**	**30 hours**	***F-value***
PR37D25	0.41 c	1.02 b	1.39 a	***38.96*** ***
DKC4490	0.34 c	1.25 b	1.69 a	***67.80*** ***
P9400	0.41 c	1.19 b	1.68 a	***77.55*** ***
Sunflower hybrids	**6 hours**	**24 hours**	**30 hours**	***F-value***
NK Brio	0.06 b	0.20 a	0.26 a	***9.58*** ***
NK Neoma	0.11 b	0.19 b	0.37 a	***35.87*** ***
NK Feri	0.05 b	0.24 a	0.24 a	***11.45*** ***

In case of sunflower there was significant difference between the 6 and the 24-hour measurements, while between the 24 and the 30-hour measurements we didn't find significant difference in root growth. Root growth of sunflower stopped earlier by the effect of cadmium treatment than of maize. The different hybrids reacted the same way to the time passed after the treatment. In case of maize hybrids we could detected significant differences in their root growth per time unit 24 and 30 hours after the treatment. In case of sunflower in 6 and 30 hours after the cadmium treatment there was significant difference amongst the root growth of the given hybrids.

By the effect of the increasing cadmium concentrations (0, 5, 10, 30, 50, 100, 150 µM) root growth of both species has significantly decreased (*Table 3.*). Root growth of sunflower is significantly hindered by even low cadmium concentration (5 µM) (the growth of the root decreased with about ~63 % in 24 hours after the treatments). In case of maize such decrease in root growth (~60 %) was caused by only a cadmium concentration over 30 µM. The higher cadmium tolerance of maize was also signed by the fact that it could show some root growth even under a very high cadmium concentration. In case of sunflower over a cadmium concentration of 50 µM the root growth was totally hindered. Amongst the maize hybrids involved into the trial significant difference could be detected only in case of a cadmium concentration over 50 µM, while in case of sunflower at cadmium concentrations below 50 µM (over this concentration the root growth was totally hindered).

Table 3.: *Differences amongst the sensitivities of the root growth of maize and sunflower hybrids (cm) depending on the increasing cadmium concentrations, 24 hours after the treatment (n=8, *** p <0.001)*

Cd ($CdSO_4$) **concentration** (µM)	**PR37D25**	**DKC4490**	**P9400**	**NK Brio**	**NK Neoma**	**NK Ferti**
0	1.41 a	1.75 a	1.74 a	0.51 a	0.48 a	0.29 a
5	1.38 a	1.49 ab	1.42 b	0.20 b	0.14 b	0.16 b
10	1.21 a	1.39 b	1.20 bc	0.05 c	0.09 bc	0.10 bc
30	0.61 b	0.71 c	0.68 de	0.01 c	0.02 bc	0.04 c
50	0.56 b	0.56 c	0.93 cd	0.00 c	0.00 c	0.00 c
100	0.23 c	0.14 d	0.37 ef	0.00 c	0.00 c	0.00 c
150	0.03 c	0.09 d	0.11 f	0.00 c	0.00 c	0.00 c
F-value	***25.80 ******	***32.25 ******	***30.85 ******	***47.78 ******	***28.68 ******	***10.19 ******

Dry material production depending on the Cd treatments

Dry material of both the shoot and root of maize (*Zea mays L. cv* MV343) decreased by the effect of cadmium treatments, comparing to the control (*Table 4.*). As a result of the highest applied, the 150 µM of $CdSO_4$ treatment comparing to the control, the dry material of both of the shoot and the root was decreased approximately to half.

Table 4.: *Dry weight (g) of the shoot and root of a 10-day old maize seedling grown in rhizobox, depending on the cadmium concentration (n=3)*

	Maize (MV343)	
	Dry weight of the shoot	**Dry weight of the root**
Control	0.0633±0.01	0.1177±0.04
10 µM Cd	0.0607±0.03	0.1152±0.06
30 µM Cd	0.0593±0.01	0.0995±0.02
50 µM Cd	0.0485±0.01	0.0757±0.02
100 µM Cd	0.0358±0.02	0.0713±0.05
150 µM Cd	0.0290±0.01	0.0641±0.01

Dry weight of the shoot and roots of sunflower (*Helianthus annus L. cv* Nova) showed a very different result in comparison to the maize. In case of the dry weight of the shoot there was no significant difference as an effect of cadmium treatments (*Table 5.*). Even the dry weight of the root decreased by the effect of the cadmium treatments, but not as significantly as of maize. The results could be influenced by the different nutrient uptake mechanisms of the given species.

Table 5.: *Dry weight (g) of the shoot and root of a 10-day old sunflower seedling grown in rhizobox, depending on the cadmium (n=3)*

	Sunflower (Nova)	
	Dry weight of the shoot	**Dry weight of the root**
Control	0.0319±0.01	0.0358±0.01
10 µM Cd	0.0357±0.00	0.0325±0.01
30 µM Cd	0.0355±0.01	0.0237±0.01
50 µM Cd	0.0342±0.01	0.0238±0.01
100 µM Cd	0.0337±0.01	0.0211±0.01
150 µM Cd	0.0377±0.00	0.0255±0.01

Comparing the dry weight of shoots and roots of maize plants grown on different soil types (the non-contaminated, chernozem soil originated from organic farming from Látókép, which was my permanent control during the trials, and the barren, sandy soil of the Gyöngyösoroszi mine, which was contaminated by heavy metals for years) I realized that the hybrids grown on

the contaminated soil originated from Gyöngyösoroszi showed better dry material production than on the non-contaminated, control soil originated from Látókép (*Table 6.*). This must probably be justified by the differences between the soil structures. The soil originated from Gyöngyösoroszi is a sandy type, therefore roots were able to grow against less resistance. This could provide bigger root surface and therefore bigger surface for nutrion uptake, what effected advantageously the dry weight of the shoot. Besides the heavy metals accumulated for years in the soil of Gyöngyösoroszi transformed in different chemical reaction into such forms what are less uptakeable for the plants.

Table 6.: *Dry weight of shoots and roots of different maize hybrids (g) on diffeeent soils (**1:** soil from Látókép, **2:** soil from Gyöngyösoroszi) (n=3)*

	Siló King		**Mv343**		**De285**		**P9400**	
	root	**shoot**	**root**	**shoot**	**root**	**shoot**	**root**	**shoot**
1	0.1245±0.04	0.0154±0.00	0.1846±0.01	0.0214±0.01	0.1199±0.04	0.0188±0.01	0.1034±0.00	0.0183±0.00
2	0.1559±0.00	0.0201±0.01	0.2750±0.06	0.1053±0.12	0.1559±0.06	0.0254±0.01	0.1810±0.05	0.0223±0.00

Amounts of root acids secreted by the effect of cadmium

By the effect of the increasing cadmium concentration, after 24 hours from the treatment, the amount of the secreted root acids was decreased significantly, therefore pH of the rhizosphere increased. Amongst the given hybrids significant differences were detected.

Changing of parameters of young, several-week old plants by the effect of cadmium treatments

The effects of Cd treatments to the root growth of maize and sunflower

In case of the examination of root length the sunflower hybrids reacted with about the same sensitivity against the applied cadmium concentrations, while amongst the maize hybrids I got much hectical results. Comparing to the controls of the maize hybrids, the Mv500 made the biggest average root lenght (65 cm), but this hybrid was the most sensitive against the cadmium treatments. I measured the shortest root length (44 cm) at the Mv343 hybrid. At sunfower examining the root growth of the control, a hybrid (NKBrio) emerged out from the others with its significant (more than 60 cm) root length. The average lengths of the non-treated roots of the other examined hybrids were between 40 and 53 cm. In case of sunflower the 10 mg dm^{-3} Cd treatments meant 50% decrease in the averade root length. In case of both of the maize and the sunflower the amount and size of side roots and the root hairs were significantly decreased by the effect of cadmium treatments.

Changing of the relative chlorophyll content by the effect of Cd treatments

In case of maize the cadmium treatments in almost all cases resulted significant decrease in relative chlorophyll content comparing to the control (*Table 7.*). Amongst the given hybrids there were significant differences depending on the cadmium treatments; only in case of the cadmium treatment of 10 mg dm^{-3} the SPAD-values measured in the second leaf didn't show significant difference amongst the hybrids.

Table 7.: *Effect of cadmium treatments to the relative chlorophyll content of the maize hybrids (SPAD-value) (n=5, Significant difference comparing to the control: *p <0,05; **p <0,01; ***p <0,001; difference amongst the given hybrids was marked with letters in the superscript)*

	2nd leaf			
	Mv343	**Mv277**	**Mv500**	**De285**
Control	$39.3^{a} \pm 0.97$	$42.8^{a} \pm 3.18$	$37.1^{b} \pm 2.56$	$34.6^{b} \pm 2.33$
10 mg L^{-1} Cd	21.4 ± 3.13*	24.1 ± 2.79***	24.7 ± 3.32***	22.1 ± 3.10**
20 mg L^{-1} Cd	$14.9^{a} \pm 12.3$*	$15.4^{a} \pm 5.31$***	$21.2^{b} \pm 1.26$***	$14.8^{a} \pm 6.05$***
	3rd leaf			
Control	$43.4^{a} \pm 2.56$	$44.7^{a} \pm 2.11$	$40.6^{b} \pm 1.86$	$36.1^{c} \pm 1.18$
10 mg L^{-1} Cd	$31.9^{ab} \pm 1.46$***	$34.4^{a} \pm 2.12$***	$32.0^{ab} \pm 1.91$***	$29.1^{b} \pm 3.62$**
20 mg L^{-1} Cd	$27.7^{b} \pm 5.04$***	$30.4^{a} \pm 1.06$***	$28.2^{ab} \pm 1.46$***	$26.0^{b} \pm 3.38$***

The relative chlorophyll content of sunflower hybrids reacted with different sensitivity to the cadmium treatments (*Table 8.*). The chlorophyll measured in the second leaves decreased less by the effect of increasing cadmium concentrations, as of the relative chlorophyll content measured in the third leaves. Taking into account the value measured in both leaves, the NK Brio turned to be the most sensitive. Comparing the control results, even this hybrid had the highest relative chlorophyll content.

Table 8.: *Effect of cadmium treatments to the relative chlorophyll content of the sunflower hybrids (SPAD-value) (n=5, Significant difference comparing to the control: *p <0,05; **p <0,01; ***p <0,001; difference amongst the given hybrids was marked with letters in the superscript)*

	2nd leaf			
	NK Brio	**NK Neoma**	**NK Alego**	**Nova**
Control	$52.3^{a} \pm 1.6$	$44.2^{b} \pm 1.1$	$43.0^{b} \pm 1.49$	$42.7^{b} \pm 2.32$
1 mg dm^{-3} Cd	$44.7^{a} \pm 2.5$	$45.8^{a} \pm 1.9$	$40.7^{b} \pm 1.61$	$40.6^{b} \pm 1.28$
5 mg dm^{-3} Cd	42.6 ± 4.8*	41.1 ± 3.3	42.5 ± 0.79	41.8 ± 3.08
10 mg dm^{-3} Cd	$40.1^{a} \pm 3.3$*	$36.8^{b} \pm 0.8$*	$41.2^{a} \pm 1.19$	$39.6^{a} \pm 2.10$
	3rd leaf			
Control	$49.0^{a} \pm 1.69$	$47.1^{ab} \pm 0.90$	$41.2^{c} \pm 1.24$	$43.9^{bc} \pm 2.09$
1 mg dm^{-3} Cd	$45.7^{a} \pm 1.64$*	$45.0^{a} \pm 1.04$	$40.1^{b} \pm 0.36$	$42.7^{b} \pm 2.28$
5 mg dm^{-3} Cd	38.8 ± 1.50**	39.5 ± 0.26***	38.2 ± 1.17*	38.6 ± 3.13*
10 mg dm^{-3} Cd	$32.5^{a} \pm 3.20$***	$26.5^{b} \pm 2.11$***	$38.1^{cd} \pm 1.28$*	$35.9^{ad} \pm 2.41$*

Changing of the photosynthetic pigment content depending on the Cd treatments

The absolut chlorophyll content of the maize hybrids, in all studied groups of pigments (klorofill–*a*, –*b* and carotenoids) significantly (60-75%) decreased comparing to the control (*Figure 2.*). The three hybrids from Martonvásár (Mv277, Mv343, Mv500) performed almost the same results, while De285 was behind them a bit relating the amount of the total photosynthetic pigments. I determined in case of all four maize hybrids, that from amongst the three examined pigment groups the klorofill–*a* and –*b* reacted the most sensitively.

The carotenoids were less sensitive to the differences in cadmium concentrations. In case of all four maize hybrids there was significant difference amongst the control and the cadmium treatments. For the concentrations of the cadmium treatments the Mv500 hybrid reacted rhe most sensitively.

In case of sunflower the photosynthetic pigments reacted less sensitively to the cadmium treatments (*Figure 3.*). In all sunflower hybrids there were such pigment groups that didn't show significant decrease even at 10 mg dm^{-3} Cd concentration comparing to the control, this was true mainly for the results measured in the older, second leaves.

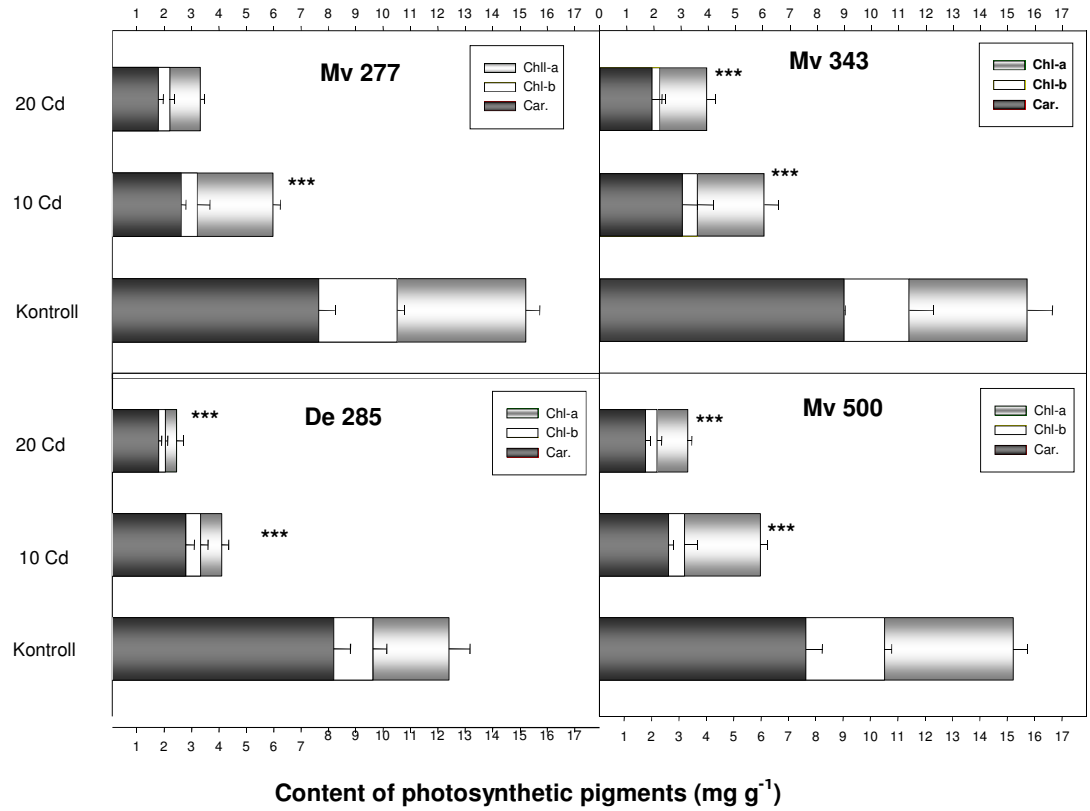

Figure 2.: *Changing of the absolute chlorophyll content (mg g^{-1}) by the result of different cadmium treatments in the third leaf of 26-day old maize plants (n=3 ± s.e.) cadmium treatments (1: control, 2: 10 mg dm^{-3} $CdSO_4$, 3: 20 mg dm^{-3} $CdSO_4$); p <0.001****

Considering the maize and sunflower results, we can determine that the synthesis of the photosynthetic pigments was less sensitive to the cadmium treatments in case of sunflower, than in case of maize.

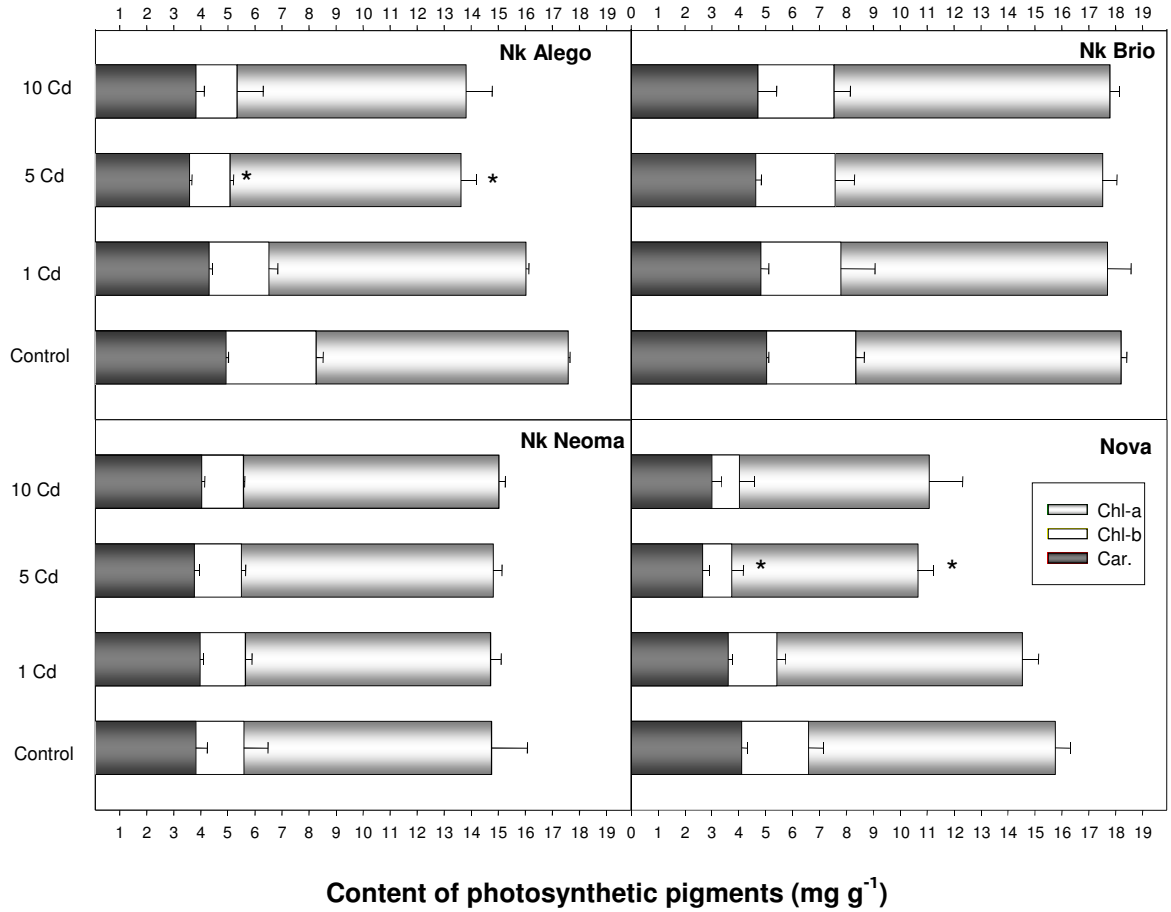

Figure 3.: *Changing of the absolute chlorophyll content (mg g^{-1}) by the result of different cadmium treatments in the third leaf of 26-day old sunflower plants (n=3 ± s.e.) cadmium treatments (1: control, 2: 10 mg dm^{-3} $CdSO_4$, 3: 20 mg dm^{-3} $CdSO_4$); p <0.001****

Changing of the apoplastic pH depending on the cadmium concentration

Determination of the apoplastic pH was made from guttation drops. Guttation is the exudation of drops of xylem sap (containing water and some soluble salts, carbohydrates etc.) on the tips or edges of leaves. During the examination of the apoplastic pH of leaves (*Table 9.*), I determined that the pH of the control plants in case of the all three maize hybrids (Mv343, Mv500, De285) fall into the basic range (pH 8.6 – 9.1). By the effect of the increasing cadmium concentrations the pH values decreased. In case of the 10 mg dm^{-3} Cd concentration the pH was in the 8.1 – 9.0 pH range, while in case of the 20 mg dm^{-3} Cd treatment the value of the pH changed between 7.2 and 8.1.

Table 9.: *Changing of the pH of guttation drops by the effect of cadmium ($CdSO_4$) treatments with different concentrations in maize hybrids (n=5-7)*

Hybrid	Treatment	$pH_{guttation\ drop}$	ΔpH
	Control	9.1 ± 0.71	
MV 343	10 mg dm^{-3} Cd	9.0 ± 0.44	-0.1
	20 mg dm^{-3} Cd	7.9 ± 1.41	-1.2
	Control	8.6 ± 0.22	
MV 500	10 mg dm^{-3} Cd	8.3 ± 0.00	-0.3
	20 mg dm^{-3} Cd	8.1 ± 1.26	-0.5
	Control	8.7 ± 0.74	
De 285	10 mg dm^{-3} Cd	8.1 ± 0.66	-0.6
	20 mg $dm^{-3\ Cd}$	7.2 ± 0.40	-1.5

Effects of cadmium treatments supplemented with biofertilizers to several physiological parameters of maize and sunflower

Effects of cadmium treatments supplemented with biofertilizers to the fresh weights of maize and sunflower

The cadmium treatment significantly decreased the fresh weight of maize comparing to the control. In case of sunflower the decrease of the fresh weight by the effect of cadmium treatment was bigger than in case of maize. The sunflower seemed to be more sensitive than maize respecting to the cadmium concentration, since in case of the 20 mg dm^{-3} cadmium concentration we were not able to even grow up the plants. By the effect of biofertilizer treatment we found that in case of both plants we could measure about 25-30% more fresh weight comparing to the control. In such cases when cadmium treatments were supplemented with biofertilizer, at 1 mg dm^{-3} Cd concentration the volume of fresh weight significantly increased in case of both maize and sunflower, while at 5 és 10 mg dm^{-3} Cd concentrations there were no detectable difference.

Effects of cadmium treatments supplemented with biofertilizers to the dry weights of maize and sunflower

Dry material content of maize was significanly less at 5, 10, 20 mg dm^{-3} Cd treatments comparing to the dry material content of the control plants (*Figure 4.*). The dry material content of the sunflower shoot was significantly less by the effect of even the 1 mg dm^{-3} Cd treatment comparing to the control. The biofertilizer increased the dry weight of the shoot and root of both plants. The cadmium treatment decreased the dry material weight of the shoot and root of the sunflower more than of the maize. The biofertilizer treatment increased the dry material weight of both of the shoot and the root. In case of the maize this increase was bigger

in case of the root (30%) than of the shoot (20%). Examining the dry material weight of the root of the sunflower we can state that at 1 mg dm^{-3}-es Cd treatment the auxiliary biofertilizer treatment gave significantly bigger value, but we were not able to detect the advantegous effect - found in case of the maize - at the other cadmium concentrations. (*Figure 5.*).

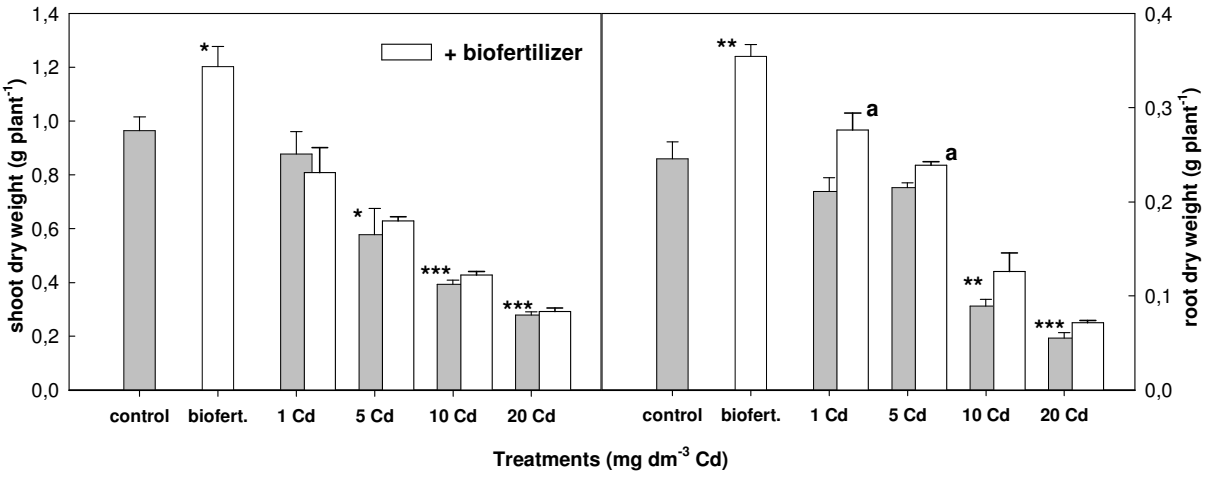

Figure 4.: *Changing of the dry material content (g/plant) of maize by the effect of different concentration cadmium (Cd) treatment, and in case of the use of biofertilizer (n=6-8 ±s.e.) (the effect of Cd treatment comparing to the control: p <0.05*, p <0.01**, p <0.001***; effect of biofertilizer treatment p <0.05[a])*

Changing of the dry material content of the shoot and root of sunflower by the effect of different concentrations of cadmium and biofertilizer treatments can be seen in Figure 31.

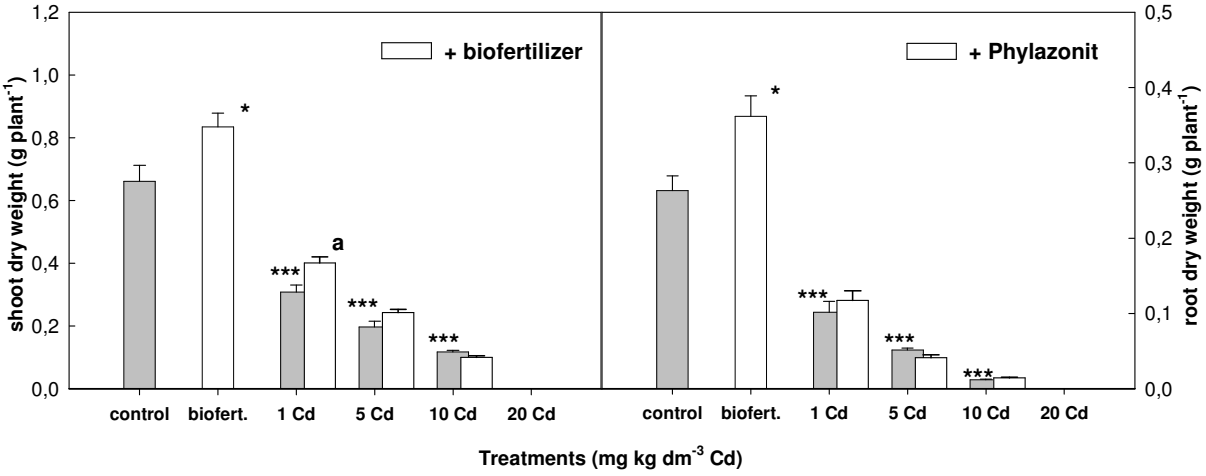

Figure 5.: *Changing of the dry material content (g/plant) of sunflower by the effect of different concentration cadmium (Cd) treatment, and in case of the use of biofertilizer (n=6-8 ±s.e.) (the effect of Cd treatment comparing to the control: p <0.05*, p <0.001***; effect of biofertilizer treatment p <0.05[a])*

Effects of cadmium treatments supplemented with biofertilizers to the relative chlorophyll content of maize and sunflower

By the effect of cadmium treatment the relative chlorophyll content of the leaves decreased significantly. While in case of the maize by the effect of the applied cadmium treatments the relative chlorophyll content decreased depending on the concentrations (*Figure 6.*), whilst in case of the sunflower even at higher concentrations there were no such changes (*Figure 7.*). The sunflower plants treated with higher cadmium concentrations remained shorter, but there were no change in their relative chlorophyll content. Probably the growth decreased in a smaller amount than the chlorophyll content. In case of the sunflower the biofertilizer treatment applied at 1 mg dm^{-3} cadmium concentration in all measurement days inreased significantly the value of relative chlorophyll content, but it was not effective against the other cadmium concentrations.

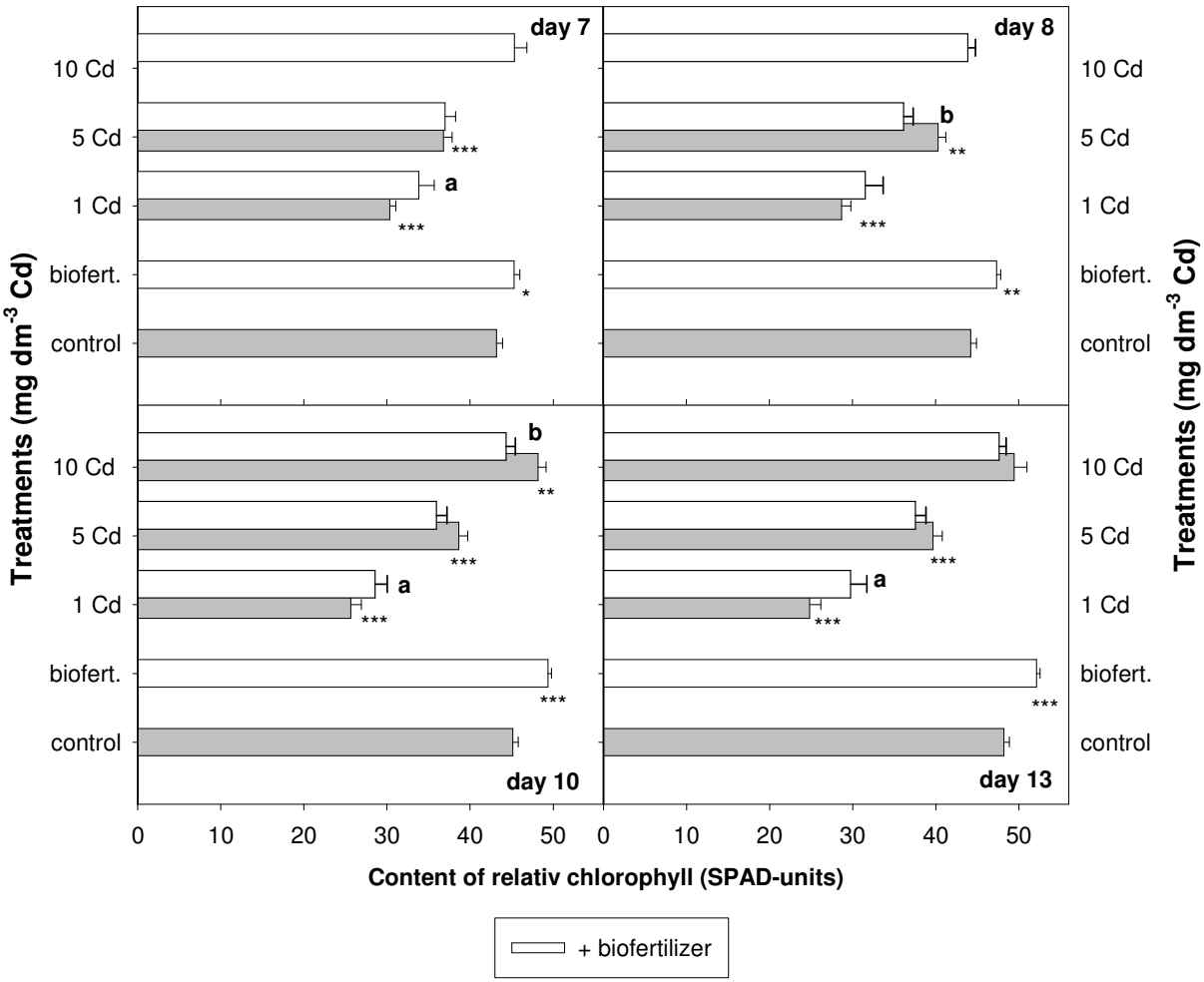

Figure 6.: *Changing of the value of the relative chlorophyll content (SPAD - units) of sunflower shoot by the effect of treatments at different cadmium (Cd) concentrations and of biofertilizer in case of plants treated for 7, 8, 10 and 13 days. (n=80-100 ±s.e.) (effect of the Cd treatment, comparing to the control: p <0.05*, p <0.01**, p <0.001****
effect of biofertilizer treatment p <0.05[a], p <0.01[b])

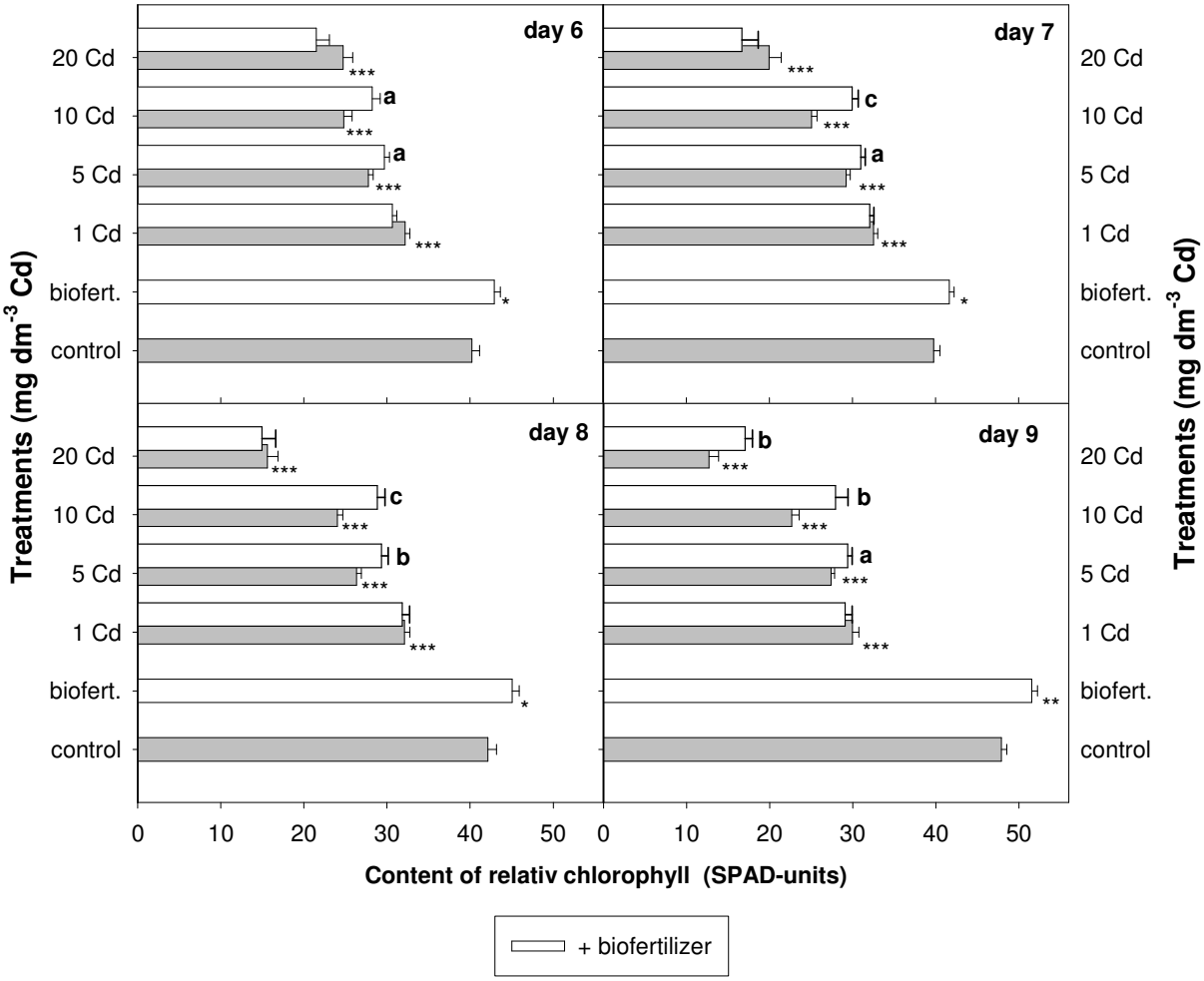

Figure 7.: *Changing of the value of the relative chlorophyll content (SPAD-units) of maize by the effect of treatments at different cadmium (Cd) concentrations and of biofertilizer in case of plants treated for 6, 7, 8, and 9 days. (n=80-100 ±s.e.) (effect of the Cd treatment, comparing to the control: p <0.05*, p <0.01**, p <0.001****
effect of biofertilizer treatment p <0.05[a], p <0.01[b])

Effects of cadmium treatments supplemented with biofertilizers to the photosynthetic pigment content of maize and sunflower

According to our results the cadmium treatments decreased the amount of chlorophylls and carotenoids in the shoot of the maize, but the carotinoids were less sensitive. The alone applied biofertilizer increased the amount of photosynthetic pigments, while applied with cadmium treatment, it decreased the negative effect of cadmium. We found similar changes in case of sunflower as well, the cadmium treatments decreased the amount of chlorophylls, but the 10 mg dm^{-3} Cd treatment gave a value close of the control, as we saw at SPAD-indexes as well. So long as we detected the decrease of the photosynthetic pigments in the shoot of the maize by the effect of cadmium treatment, while in case of the sunflower, the high

concentration cadmium treatment increased the amount of photosynthetic pigments related to dry material content.

Effects of cadmium treatments supplemented with biofertilizers to the photochemical effectiveness of maize and sunflower

The value of the potential photochemical efficiency is characterized, that in case of both examined plant groups the F_v/F_m value of the control approaches the optimal estimated value, and by the effect of biofertilizer treatment it remained the same high (*Figure 8.*). By the effect of different concentration cadmium treatments the F_v/F_m, decreased, in case of sunflower more than of the maize. In case of the maize the 10 mg dm^{-3} cadmium treatment supplemented with biofertilizer compensates the effect of cadmium, i.e. we also measured higher F_v/F_m values with biofertilizer supplement, than without. In case of sunflower even no detectable difference can be found between the effect influencing the F_v/F_m values of the 10 mg dm^{-3} cadmium treatment and the biofertilizer supplementation.

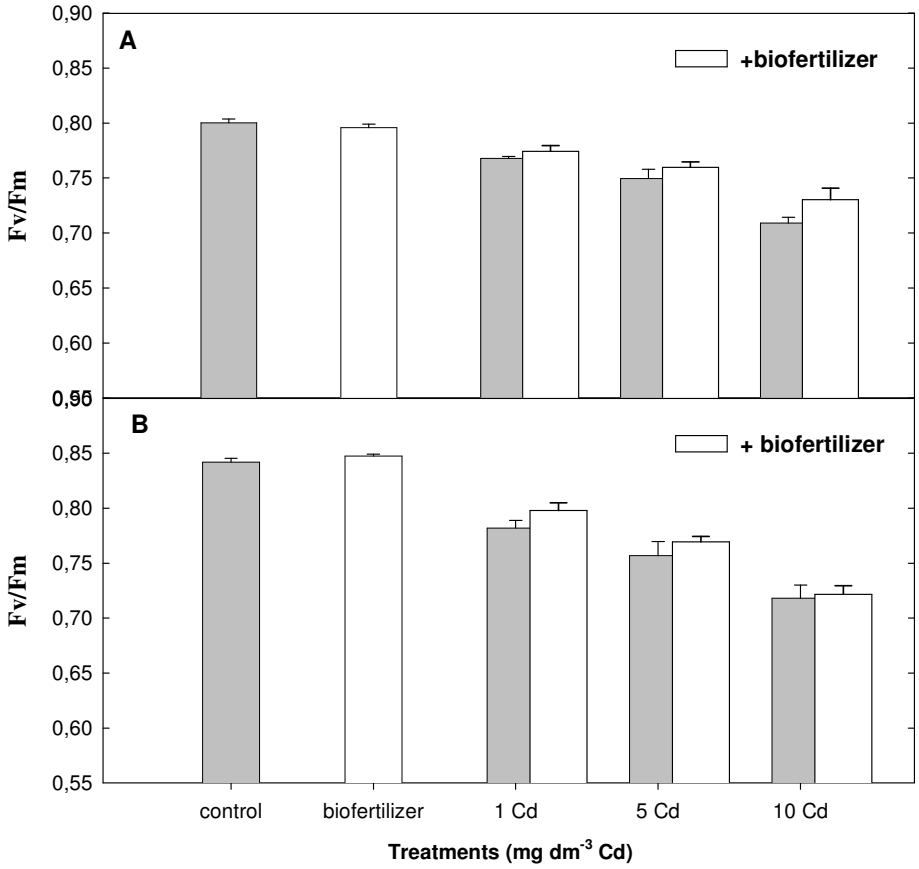

Figure 8.: *Changing of the value of potential photochemical efficiency (F_v/F_m) by the effect of different concentration cadmium(Cd) and biofertilizer treatments in case of maize (A) and sunflower (B) n=6 ±s.e.*

Effects of cadmium treatments supplemented with biofertilizers to the cadmium content of maize and sunflower

By the effect of cadmium treatments, the cadmium content in the shoot and root of both plants increased comparing to the control (*Tables 10-11.*). The cadmium content of the root is multiple than of the shoot, i.e. the uptaken cadmium remains in the root, and only a relatively small part of it is transported into the shoot. By the effect of biofertilizer treatment the detected amount of cadmium declined both in the root and shoot of maize.

Table 10. *Cadmium content of the shoot and root of maize treated with cadmium and biofertilizer (n=3±s.e.) (significancy examination between the cadmium treatment and the biofertilizer supplementation, *p <0,05, **p <0,01)*

	Shoot (µg/plant)	**Root** (µg/plant)
Control	1.31 ±0.10	11.50 ±0.72
1 mg dm^{-3} Cd	134.67 ±9.06	597.00 ±23.52
5 mg dm^{-3} Cd	215.33 ±18.09	950.33 ±24.39
10 mg dm^{-3} Cd	345.50 ±20.50	1602.33 ±64.68
20 mg dm^{-3} Cd	514.00 ±5.51	2548.33 ±118.44
1 mg dm^{-3} Cd+biofertilizer	159.33 ±22.26	653.33 ±39.22
5 mg dm^{-3} Cd+biofertilizer	209.67 ±12.41	880.67 ±80.88*
10 mg dm^{-3} Cd+biofertilizer	249.67 ±6.38**	1179.00 ±49.86**
20 mg dm^{-3} Cd+biofertilizer	563.33 ±28.39	3520.00 ±236.43

Sunflower accumulated more cadmium in its shoot and root, than maize. This explains the results of the above trials, according to which the sunflower showed bigger sensitivity against cadmium, than the maize. It is assumed that the defence mechanisms of maize and sunflower are different, but this will be a subject of my further research.

Table 11. *Cadmium content of the shoot and root of sunflower treated with cadmium and biofertilizer (n=3±s.e.) (significancy examination between the cadmium treatment and the biofertilizer supplementation, *p <0,05, **p <0,01)*

	Shoot (µg/plant)	**Root** (µg/plant)
Control	4.68 ±0.07	12.67 ±1.35
1 mg dm^{-3} Cd	272.67 ±16.75	604.00 ±84.36
5 mg dm^{-3} Cd	797.67 ±13.86	2047.33 ±61.65
10 mg dm^{-3} Cd	922.33 ±76.91	13001.33 ±1220.34
1 mg dm^{-3} Cd+biofertilizer	216.00 ±19.86	512.00 ±155.47
5 mg dm^{-3} Cd+biofertilizer	800.67 ±52.87	2329.33 ±352.77
10 mg dm^{-3} Cd+biofertilizer	504.00 ±57.04**	6745.67 ±676.96**

4. NEW AND NOVEL SCIENTIFIC RESULTS OF THE DISSERTATION

- In case of the seedlings of the two examined species (maize and sunflower) **I determined that during the comparative study of the root growth, at the $CdSO_4$ concentrations above 50 µM the relative root growth didn't change significantly. Besides some plant species, for example sunflower in the result of my study, would react more sensitively to the high cadmium concentrations.** At 50 µM cadmium concentration root growth was totally hindered.

- **The acid secretion of the roots ofseveral-day old seedlings was closely related to the growth of the roots.** In prportion of the inhibition of the root growth the acid secretion of the roots also decreased. In case of sunflower acid secretion was more intensive in the root collar zone, while in case of maize this was stronger in the zones of the root collar and the apex.

- Seeing the results of the maize and the sunflower, it can be stated that **the synthesis of photosynthetic pigments is more sensitive to the cadmium treatments in case of sunflower, than in case of maize**. I.e. it doesn't hinder the dry material production of the sunflower through the decrease of the photosynthetic pigments, but it hinders photosynthesis through an other process.

- In practice, the aims of biofertilizer treatments are to mobilize the nutrients, and to promote the nutrient uptake of the plants. Therefore it is assumed that cadmium uptake would increase as well. But ont he basis of **our measurments the cadmium uptake didn't increase significantly by the effect of additional biofertilizer treatments, even in some cases the accumulated amount of cadmium decreased in both plant species.**

5. PRACTICAL USE OF THE RESULTS

- **The useful microorganisms of the biofertilizer compensate for the negative effects of the cadmium**, they promote the nutrient uptake of the plants, **even in contamined soil, while they don't strenghten significantly the uptake of heavy metals, presumably they would play a role in hinderance of the uptake of heavy metals.**

- **I determined the change of apoplastic pH had occured by the effect of cadmium treatments by the measurement of the pH-value of guttation drops**, instead of the spinning, which was preferred more in the general practice. **This is much cheaper and faster measurment method for the determination of apoplastic pH, and the test plants without truncation remain available for further investigations. By the effect of the increasing cadmium content the value of apoplastic pH decreased comparing to the control.**

6. PUBLICATIONS OF THE DISSERTATION

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