THESIS OF DOCTORAL (PhD) DISSERTATION

PLANT PHYSIOLOGICAL EXAMINATION OF INDUSTRIAL WASTES

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1. INTRODUCTION

Protecting our environment is one of the most important tasks nowadays. The Earth is injured and is not able to bear the rising environmental loud without damages. It also came to the surface that there are no such phenomena which can be locked, excluded or separated. Everything is related to everything on Earth. It is particularly true of the changes of the environment. The continuity of deforestation of the Brazilian rainforests is seemingly a case of one country. The economic growth of the most developed and the most quickly developing countries is not its own case of the certain country either, seems the high production level or fast development result so polluting emission which conclusions can be felt by everyone.

The effect of the increased CO_2 and SO_2 emission is well-known. Apart from the continuous global warming acid rains could result the death of vegetation. However, acidification of our soils is not only the result of acid rains. By the deforestation of several-year-old plants such as wood high quality nutrient disappears which exist in the trees but actually comes form the soil of the forest. The buffer capacity and pH of the soil decrease dramatically resulting the increasing of heavy metal uptake. Although Al is not considered as a heavy metal its physiological effects are the same. As a result of the increasing Al-uptake membranes of the cell are damaged, the regulation of metabolism finishes and the plant dies. The main reason for the death of European forests can be seen in the increased Al-uptake by a lot of scientists. Other heavy metal uptake increases similarly with the decrease of pH. Today Hungarian population consumes the biggest amount of fresh vegetables and fruits in a hardly controlled form because we have really small amount of data about the products of the markets which come from the small gardens. If we have some, those refer to pesticide content. The heavy metal load of population is a latent risk concerning the situation.

The scientific technical development of 1950 brought fundamental changes in life of mankind. Revealing the energy sources and introducing them to the production, furthermore the automatization of the production and the wide range use of polymer materials multiplied the productivity. In the 21st century the industrial production increased by 50-times and the four-fifth of it has reason from 1950. The increase of the industrial production in the 1990s is almost the same as the whole European industrial activity at the end of 1938-1940s. The natural and environmental protection is the result of the effects of accelerated industrial and economic development after the Second World War. However, unfavourable changes which appear in the natural environment may endanger the maintenance of life. The air pollution is a catastrophe in many places. The power plants, factories, chemical plants, cars emit high quantity of CO_2, SO_2, NO_X and CO, chemical materials, dust and heavy metals in the atmosphere, consequently they change the content of the air.

The fossil materials cause the biggest change in the air, during their burning between 1860-1980 185 billion tons of carbon came to the atmosphere. Nowadays, the amount of carbon emission is approximately worth 5 billion tons. The CO_2 content of the atmosphere was 260-270 ppm in 1910 which rose to 360 ppm in 1985. According to some researchers this amount could reach 550-600 ppm if we do not change our manufacturing behaviour. The emitted CO_2 during burning together with our gases can boost the gas content of the atmosphere around the Earth, which is similar to the glass-bell hinders and to the movement of reflected heat-ray from the Earth to space. The contribution of manmade air polluting materials to the evolution of the global green-house effect was the following in 1986; CO_2 50%, CH_4 19%, PCB 17%, troposphere ozone 8%, N_2O 4%, stratospheric stem 2%.

Approximately based on the current tendencies and compared to the middle of previous century the CO_2 content of the atmosphere will probably have been doubled by 2030-2050s, causing 1,5-4,5 ºC temperature rise (Murray, 1995). Significant part of SO_2, NO_X, CO_2, CO and CH_4 appearing in the atmosphere get back and acid sedimentation, moreover it highly contributed to the acidification of our soils.

2. RAISING THE SUBJECT

The agricultural crops reduce the green-house effect because they bind CO_2 which contributes to the causing of green-house effect by about 50%. The production of fertilizers is not only expensive but it is a procedure which needs enormous energy. The reduction of fertilizer's portion has become essential in the crop production by now. One of the reasons for that is during other industrial activities such as by-products are produced in high quality in which the necessary nutrients for plants can be found in a big amount. These nutrients have high organic content. This is a fundamental essential condition for the microorganisms appearing in the soil without which the sustainable crop production cannot be achieved.

Besides the high fertilizer prices the use of produced wastes is economically reasonable. Finally, the other reason for the reduction of fertilizer use is that the inappropriate use of the fertilizers may cause environmental pollution.

During my work I would like to find solution with the use of wastes and by-products for the reduction of CO_2 and other green-house gases which appear in the atmosphere. During the different industrial and production procedures and probably during everyday use some by-

products and wastes are generated which have high micro- and macro element content and they do not endanger the environment. They should not be handled as wastes but rather as nutrient amendments.
In my experiments I examined the effect of applied wastes and by-products (flue gas, sewage sludge-compost, extruded poppy-heads, sewage sludge, grinding sludge and lime sludge) on the germination, their element content and their physiological effect on plants. Apart from the enumerated materials I examined soot, acid, cellulose sludge oil and oily emulsion but because of the limited space I cannot introduce these results in my thesis.

3. MATERIALS AND METHODS

3. 1. Chemical analysis of the samples

After the drying of plant samples on 85 ºC and their milling 1 g was measured on analytical scale.
To measure the elements concentration of plants ten ml HNO_3 (65 % v/v) was added to 1 g of sample for overnight incubation at room temperature. Then, the samples were pre-digested for 30 min at 60 ºC. Finally, 3 ml H_2O_2 (30m/m %) was added for 90 min, boiling at 120 ºC. the solutions were made up to 50 ml, homogenized and filtered through Filtrak 388 filter paper.
To measure the elements concentration of examined by-products 15 ml HNO_3 (65 % v/v) was added to 1 g of sample for overnight incubation at room temperature. Then, the samples were pre-digested for 30 min at 60 ºC. Finally, 5 ml H_2O_2 (30m/m %) was added for 270 min, boiling at 120 ºC. The solutions were made up to 50 ml, homogenized and filtered through Filtrak 388 filter paper.
For the production of solutions analytical quality HNO3 (65%) solution (produced by Merck) was used. Standard solutions made by Merck and BDH, furthermore analytically clean chemicals were applied to the production of basic solution.

To the analytical defining an OPTIMA 3300 DV type ICP-OES was used which has the following parameters:

Type:	OPTIMA 3300 DV
Producer:	Perkin-Elmer Ltd.
Optical system:	Echelle system by Ar gas

Wave-length scale:	160-782 nm
RF generator:	40 MHz
Detector:	SCD
Observation of plasma:	axial
Type of atomizing:	concentric (Meinhard Type A)
Type of peristaltic pump-pipe:	black-black
Definition of optical system:	normal
Ability for dissolving:	0.007 nm

3. 2. Determination of relative chlorophyll content

The relative chlorophyll content was measured on the second and third leaves. The relative chlorophyll content was measured with SPAD-502 (MINOLTA, Japan) Chlorophyll Meter. The results are shown as SPAD-units in the tables.

3. 3. Determination of chlorophyll-*a*, *b* and carotenoids by spectrophotometer

50 mg fresh weight of leaves were collected from second and third leaves while extraction of chlorophyll using N,N-dimethylformamide (DMF) was blended with 5 ml and then cooled at 4^{o}C for 72 hours. The extraction content of the pigment was determined using spectrophotometer. The data after the spectrophotometrycal determination was mathematically processed using formula proposed by Moran and Porath (1980).

3. 4. Measurement of dry matter

The plant samples were dried at 85 ^{o} C for weight permanence and were measured with analytical scale (OHAUS) after cooling back them to room temperature.

3. 5. Toxicity test

Mustard (*Sinapsis alba* L.) seeds were used in the toxicity test. The examined by-products and the filtrates of the by-products were put to the Petri-dishes on filter paper and they were watered. 100 seeds were put into each Petri-dishes. Six treatments and 1 control were conducted. The number of repetition was three.

3. 6. Examination with plants grown on nutrient solution

The experimental plants were maize (*Zea mays* cvs. Norma) and sunflower (*Helianthus annus* cvs. Arena). The seeds were soaked in 10 mM $CaSO_4$ for 4 hours after sterilization and then germinated on moistened filter paper at 22 ^{o}C. The seedlings were transferred to continuously aerated nutrient solution of the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 1µM H_3BO_3, 1µM $MnSO_4$, 10 µM $ZnSO_4$, 0.25 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$. Iron was added to the nutrient solution as Fe(III)-EDTA at a concentration of 100 µM.

For different treatments, 92, 91, 97, 86 and 100 ml dm^{-3} of compost, extruded poppy-heads, sewage sludge, grinding sludge and lime sludge were added into the nutrient solution, respectively. The filtrates were made from 10 g of each materials and 100 ml distilled water was being shaked for 2 hours, and after that it was vacuum filtrated.

The native materials were used in the quantity of 2 g dm^{-3}

The seedlings were grown under controlled environmental conditions (light/dark regime 10/14 h at 24/20 ^{o}C, relative humidity of 65–70 % and a photosynthetic photon flux of 300 µmol m^{-2} s^{-1}). The plants were grown in the growth chamber of Agricultural Botany and Crop Physiology Department at Institute of Plant Sciences.

3. 7. Light electroscopic examination of plant samples

For the light microscopy examination the samples were taken from the second leaves. The maize was 11 days old and the sunflower was 15 days old. The middle and edge part of leaf blade were hand cut and fixed in 70 % alcohol, formalin and acetic acid in 90:5:5 mixture, for 1 day. After that the samples were washed with alcohol (20-40 %). The samples were dehydrated in graded ethanol (25 %, 50 %, 70 %, 80 %, 90 %, 96 % and absolute alcohol). After this procedure, the samples were soaked in alcohol:benzole=1:1 for 1 hour and taken in clear benzole for 5 min. Paraffin was added to the benzole and the samples were taken in thermostat for a half day at 30-40 ºC. For the fixing egg white:glycerol=1:1 mixture was used. Benzole was used for the deparaffination. For the dyeing 0.2 % toluidin ?? blue was used.

Finally, ultra thin sections of leaf tissue were examined and photographed with a Zeiss Axioskop 2 Plus light microscope, Sony Hyper HAD camera and DigitPlan program were used.
The segments were made at the Department of Plant Anatomy, Eötvös Lóránd University. The pictures were taken at the Department of Agricultural Botany and Crop Physiology, University of Debrecen.
For the chlorophyll calculation 6 segments were used, 10 cells were counted per segment.

3. 8. Electron microscopic examination of plant samples

For the electron microscopy examination the samples were taken from the second leaves. The maize was 11 days old and the sunflower was 15 days old. The middle and edge part of leaf blade were hand cut and fixed in 2.5 % glutaraldehyde solution buffered in 0.1 M phosphate-buffer, pH 2, for 3 hours at room temperature. After that the samples were washed with phosphate-buffer (pH 2) for 2 hours. The samples were postfixed for 2 hours in 1% OsO_4, and after that being washed with 0.1 M phosphate-buffer for 30 min. The samples were dehydrated in graded ethanol (25 %, 50 %, 70 %, 80 %, 90 %, 96 % and absolute alcohol). After that the samples were infiltrated with propilene-oxid and synthetic resin for 1 hour. The samples were in the clear synthetic resin for 3 days at 60 ºC. ultra thin (70 nm) sections were cut on a Reichert-Jung Ultracut E ultramicrotome. Finally, ultra thin sections of leaf tissue were examined and photographed with a Hitachi 7100 transmission electron microscope, Mega View III. camera and analysis Pro 3.2 program (Soft Imaging System GmbH) were used. The pictures were made at the Department of Plant Anatomy, Eötvös Lóránd University.

3. 9. Statistical evaluation of the results

For the statistical evaluation Microsoft Excel 2003 and Sigma Plot 8.0 version were used.

4. RESULTS

4. 1. Effects of examined side products on germination of white mustard

White mustard (*Sinapis alba* L.) is one of our most sensible plants, therefore it is commonly used for toxicity tests. The effects of different side products on germination of mustard seeds are shown in Table 1.

Table 1. Effect of filtrates of examined by-products (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the germination of mustard. n=3± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

		Treatments		
Measuring day	Control	Flue gas	Compost	Extruded poppy-heads
		Germination %		
2.	25.67±8.74	1.67±1.53**	25.00±16,64	42.67±2.52*
3.	56.00±6.66	1.67±0.00**	57.67±8.74	64.34±0.58
4.	68.33±7.02	1.67±0.00*	68,67±6.43	72.01±3.21
5.	79.00±8.62	1.67±0.00	73,34±3.06	76.68±3.06
		Germination %		
Measuring days	Sewage sludge	Grinding sludge	Lime sludge	
		Germination %		
2.	63.67±1.53**	4.67±2.08*	3.67±1.15*	
3.	84.34±2.52	9.67±1.00**	3.67±0.00**	
4.	86.67±1.53	13.00±2.08	3.67±0.00*	
5.	89.00±2.52	17.00±3.46	5.00±0.58	

The highest germination rate was observed at sewage sludge. The germination percentage was over the control on the second day of the measurements, when extruded poppy-head, and sewage sludge were examined. Beside the germination rate the intensity of germination was also examined. It was higher by 166% at extruded poppy-head, and by 258% at sewage sludge in comparison to the control. The higher germination intensity means that the germination takes shorter time, and as a consequence the plants will be unified that makes the planning of field works better.

The germination was retarded when flue-gas and lime-sludge were examined.

To get detailed information the effects of water-diluted part of side products were examined on the germination. The results are shown in Table 2.

The germination rates were better in general when the dilutions were used. The germination rates were increasing when the dilution of flue-gas, compost, grinding sludge, lime sludge were applied, while it was decreasing when sewage sludge was examined.

Table 2. Effect of native examined by-products (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the germination of mustard. n=3± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

	Treatments			
Measuring days	Control	Flue gas	Compost	Extruded poppy-heads
	Germination %			
2.	19.00±16.64	4.00±5.20	20.67±11.02	8.00±9.54
3.	31.33±9.24	8.33±1.53***	38.67±3.61	13.67±3.06
4.	52.33±2.00	12.33±2.00	61.67±7.55	32.00±6.66
5.	62.66±4.16	20.00±0.58	68.34±4.93	43.67±2.31
6.	70.99±6.43	27.67±0.58	75.01±4.93	55.34±2.31

	Treatments		
Measuring days	Sewage sludge	Grinding sludge	Lime sludge
	Germination %		
2.	11.67±5.51	13.33±7.02	32.67±2.52
3.	19.67±0.00**	23.00±2.08	50.00±3.06
4.	30.34±2.31	31.67±7.51	66.67±5.03
5.	37.34±0.00	38.67±1.00	74.34±4.04
6.	52.34±8.66	50.00±2.89	79.67±4.16

4. 2. Examination of filtrates of by-products

4. 2. 1. The effects of examined by-products on the nutrient uptake of test plants grown on nutrient solution.

The effect of any nutrients can be considered directly, when the plants take it up, and the element gets into the living cell. Then the elements will be transferred to the different part of plant. The tolerant plants exclude the harmful elements from their metabolism, through the excretion of the elements to the vacuole. The sensitive plants are not able to transfer these elements from the roots, therefore these accumulate, causing the changing of membrane functions, and also affect on osmotic potential. The examinations were conducted with corn and sunflower plants while there are differences in the nutrient uptake mechanism between the mono and dicot plants.

The concentrations of examined elements in the shoots and roots of corn can be seen in Table 3-4. treated by the filtrates of by-products

Table 3. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the shoot of maize treated by different filtrates of by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	23,07± 13,80	10.604,33± 569,19	71,86± 11,49	76.739,67± 4.488,16	2.302,66± 170,05	10.249,00± 1.441,71	2.524,33± 319,78
Flue gas	27,96± 15,42	9.088,67± 749,71	70,10± 12,32	64.964,33± 6.808,21	1.858,00± 235,84	7.527,00± 275,77	2.689,00± 235,07
Compost	20,83± 8,00	9.683,67± 354,48	73,80± 9,70	65.551,33± 1.451,67	2.265,00± 125,21	12.292,00± 1.402,90	3.509,00± 307,04
Extr. poppy-h.	14,60± 4,00	7.930,33± 68,21	60,20± 14,68	77.889,66± 8.152,61	2.015,33± 271,92	11.331,33± 1.336,86	3.765,66± 784,10
Sewage sludge	13,29± 6,01	10.693,00± 617,04	63,60± 3,20	84.928,33± 4.030,81	2.444,00± 40,92	11.429,00± 353,20	4.089,33± 305,51
Grinding sludge	17,46± 11,07	7.757,66± 413,67	63,60± 18,16	51.221,00± 514,74	1.976,00± 47,67	8.481,00± 573,00	3.641,33± 337,12
Lime sludge	10,57± 1,70	9.172,00± 125,33	57,43± 3,50	78.496,67± 1.745,61	2.115,33± 102,12	9.587,00± 323,13	3.012,00± 173,69

Table 4. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the root of maize treated by different filtrates of by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	38,65± 10,96	11.967,33± 762,14	264,33± 80,00	63.865,33±2.344,80	5.224,66±310,41	10.313,33±2.345,33	7.604,66±429,33
Flue gas	255,33±158,41	12.991,67± 685,05	1.986,00± 9,89	46.702,67±1.149,23	4.147,66±368,07	4.202,00± 475,17	9.541,00±445,67
Compost	1.486,00±290,46	11.018,33± 548,33	1.051,33± 38,91	61.347,67± 647,33	5.544,66±341,33	12.678,33± 380,26	12.681,00±548,47
Extr. poppy-h.	126,00± 4,24	7.075,33±3.417,36	247,00± 29,69	43.223,00±1.408,00	3.269,66±678,32	11.489,50± 460,17	11.951,00±501,89
Sewage sludge	242,66± 35,81	11.294,66± 460,96	357,33± 17,21	65.470,33±4.691,69	5.760,33±187,88	8.885,00± 798,74	12.062,66±144,09
Grinding sludge	442,00±371,93	4.460,46± 279,33	1.313,50±310,42	19.626,67±1.743,67	1.598,66±105,37	9.379,00± 610,34	7.323,50±954,85
Lime sludge	717,00±135,33	11.880,33± 398,86	602,33± 80,62	61.838,33±1.901,64	4.991,33±195,17	9.349,00± 272,94	9.645,00±132,72

The concentration of examined elements were higher – except the aluminium – in the shoots of control plants in comparison to the flue gas dilution treated plants. Due to the flue-gas dilution treatment the concentration of aluminium was increased by 18% in comparison to the control.

We measured higher concentrations of K, Mg and P in the roots of control plants in comparison to the side product treated plants. Increasing concentration of Al and Fe were observed in comparison to the control.

There were no significant differences in element concentrations between the control and sewage sludge treated plants, although the concentrations of K, P, and S were higher in the shoots of treated plants.

Special difference was observed in the case of S, while its concentration extremely increases in the roots due to the treatment. On the basis of similar increase of concentration of Fe and S, we suppose the increase of ferredoxin bounded reactions.

The extrude poppy-head, and its dilution can be considered as nature-like products with high organic matter contents. This can be the reason, why I did not find differences in the uptake, transfer, and distribution of elements. The transport of S to the shoots was retarded both in control and treated plants, while intensive transport was observed in case of K. The reason for the differences in the intensity of transport mechanism can be based on the different charge of K, and S, while the membrane transport is simpler in case of positively charged K ions.

Increased concentrations of K, P, and S were observed in shoots of treated plants with sewage sludge, while significant increase of Al and Fe were measured in the roots. The transport of K to the shoots was more intensive in case of treated plants. The high concentration of Al in roots that is in line with high Fe contents is also remarkable in this case.

The concentrations of all examined elements –except S- were higher in the shoots of control plants when grinding sludge was used, while the concentration of S was higher by 44 % in the shoots of treated plants.

The concentrations of examined elements decreased in the roots – except the Al and Fe- , while the transport rate to the shoots was higher at the treated plants. It is well seen in case of K, because the K concentration was 3-fold in the shoots of treated plants, while it was only by 20 % higher in the shoots of control plants in comparison to its roots.

The parallel increase of Fe and Al concentrations in the roots of treated plants is well seen in this case as well.

The concentration of Al increased when lime sludge treatment was applied, although this increase was not extraordinary. The concentration of S was also higher in the shoots of treated plants, while the element concentrations in the roots were being formed particularly. The concentrations of almost all elements decrease due to the treatment, but in contradiction with this, the concentration of Fe and Al increased by many folds.

The in line increase of Al and Fe cannot be considered casual, while this increase was observed in almost all cases. Our knowledge is incomplete in the field of Fe-Al interactions. We can exclude that the two ions determine their oxidation rate, because the charge of Al is not changeable. We suppose that Al has significant effect on the membrane-structure, enzyme-activity, perhaps on the iron transporters.

My observation can be interesting because behind the damage of forest – caused by acid rains- the scientists presume the intensive uptake of Al. It can be very dangerous if this active Al uptake is in line with high Fe uptake, because due to the high reduction capacity of roots huge amounts of bivalent Fe will be produced, which develop free radicals. The further investigation of this observation can bring us closer to the reasons of damage of European forest caused by low soil pH.

The concentrations of elements in the shoots and roots of sunflower can be seen in Table 5-6. The concentrations of elements were higher –except the Al and Ca – in the shoots of sunflower. The concentration of Fe decreases by 22 % due to the flue gas dilution treatment in the shoot, while the concentration of Al, Fe, and K increases in the roots of sunflower. The differences between the element concentration of roots and shoots can be explained with the element accumulation in the roots, and with the retarded root to shoot transport.

The concentrations of all examined element -except the Ca- were higher when the plants were treated with compost-dilution. The concentration of Al was higher by more than twice.

The concentrations of Fe, Al, S, and Mg were significantly higher in roots in comparison to the control. The parallel increase of Fe and Al concentration raises up some questions, and needs further examinations on the field of iron transport mechanisms. The increase of S concentration may be the consequence of ferredoxin-type reactions, while the high Mg contents indicate the increase of ATP dependent phosphorilation. The energy demand of intensive nutrient uptake is provided by the kinase-type reactions.

The treatment with extruded poppy-head causes significant changes in roots. The concentration of Al was many-fold in comparison to the control, while there was no difference in the concentrations of iron. Strong retardation of root to shoot transport of Fe was observed when the plants were treated with the dilution of extruded poppy head. One fifth of root iron was transferred to shoots while this value was one fourth in case of control plants.

The xylem transport of iron depends on several conditions. The transported Fe is mainly double charged, and bound to citric acid. The transport has several preconditions in case of maize. The maize takes up the Fe-siderofore complex as tree charged cation. The iron, inside the living cells will be reduced to two charged ion, forms complex with citric acid, and will be transferred to the shoots in this form. The weakness of reduction capacity of roots or missing of sufficient amounts of citric acid easily prevents this process.

Decrease of concentrations of examined elements – except the Al – were observed when the sunflower was treated with dilution of sewage sludge. The most intensive decreases were in case of K and Ca. In contradiction with this, the concentrations of Al, Fe, Mg, and S increased due to the treatment. The high concentrations of Fe and Al show also synergism in this case.

The concentration of Al increased by twofold in the shoots when the plants were treated with grinding sludge. The increases were moderated in case of Fe, Mg and P concentrations. Double concentrations of Al and Fe were observed in the roots of sunflower due to the treatment. This increase seems to be moderate in comparison to that of observed at other side products, but the tendency is the same. We observed increasing concentrations of Ca, Fe, and K. The differences between the concentrations of different elements related to the accumulation in the roots, and to the retarded root to shoot transport.

The Al concentration was threefold higher in the shoots of sunflower in comparison to the control, when the plants were treated with diluted lime sludge. Significant increases were observed in case of Ca, and S. The concentrations of examined elements in the roots were usually over the control. This increase is remarkable in case of Fe and Al. The intensive transport of Ca to the shoots is uncommon, because of its bridge-forming ability, as well as the low, 0,1mM Ca concentration, as optimal Ca concentration of living cells. I suppose similar Mg-transport, although the physiological role of Mg differs from that of Ca.

Table 5. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the shoot of sunflower treated by different filtrates of by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	5,72± 0,34	28.133,33±2.183,33	177,33± 1,52	44.366,33±5.392,00	3.788,33±862,00	4.455,00±736,81	4.640,00± 458,38
Flue gas	12,87± 6,25	29.100,00± 981,33	91,63±14,29	43.015,00± 521,33	3.430,33±123,33	3.925,33±552,67	3.2687,00± 506,30
Compost	15,30± 4,10	26.401,33± 245,67	129,66±15,56	51.177,67±1.514,61	4.1194,00±241,33	5.152,66±269,41	5.811,66±242,86
Extr. poppy-h.	8,17± 0,53	24.786,00± 305,47	90,73± 6,83	44.169,50±1.635,54	3.693,00±140,29	4.595,66±256,05	4.405,50± 546,59
Sewage sludge	7,44± 1,90	23.959,67± 359,33	111,00± 8,71	38.632,00± 152,67	3.356,00±155,33	3.999,50± 77,07	4.387,00± 483,66
Grinding sludge	11,29± 2,14	27.613,00± 647,33	133,50±31,31	55.638,00± 541,67	3.990,33±135,33	4.596,33±324,15	4.523,66± 525,01
Lime sludge	16,53± 2,47	34.021,67± 777,00	164,00±26,28	40.078,67± 655,00	3.827,00±115,33	5.428,50±706,39	7.676,00± 28,28

Table 6. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the root of sunflower treated by different filtrates of by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	22,75± 1,91	3.902,66± 241,33	454,33± 51,08	58.219,67± 455,25	2.953,00± 89,57	7.148,33±778,99	5.708,00± 4,24
Flue gas	106,00± 1,41	3.745,33± 48,23	4.681,00±263,04	84.413,33±4.541,67	1.327,66±213,33	4.108,50±342,94	4.709,33±210,07
Compost	857,50±58,68	3.082,00± 154,33	1.074,50± 51,61	42.299,67±2.412,67	6.373,33±347,33	6.104,66± 84,76	7.396,50±641,35
Extr. poppy-h.	813,50±31,63	3.159,50± 185,96	463,50± 4,94	53.388,50± 71,41	3.158,50±306,17	7.779,50±654,07	6.383,00±597,88
Sewage sludge	326,50±20,51	3.389,33± 151,21	887,33± 16,86	43.415,00± 417,12	5.672,00±541,33	6.324,66±150,50	5.951,33±603,37
Grinding sludge	53,63± 8,61	4.532,33± 214,67	1.151,33± 64,78	65.281,00± 621,51	1.572,00± 54,13	6.522,66±594,39	4.447,66±339,53
Lime sludge	478,50±28,99	3.918,33± 313,00	863,00± 25,45	54.876,67±2.395,00	3.610,00± 81,21	8.286,00±411,53	7.288,00±171,56

4. 2. 2. The effects of filtrates of examined materials on the dry matter of plants grown on nutrient solutions.

The accumulation of dry matter was measured. The results are shown in Table 7.

Table 7.: Effect of filtrates of examined by-products (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the dry matter of maize (g plant^{-1}) n=9± S.E. Significant differences compared to the control: *p<0.05.

Treatments	Shoot	Root
Control	0.318± 0.11	0.101± 0.05
Flue gas	0.212± 0.08*	0.068± 0.03*
Compost	0.309± 0.13	0.086± 0.02
Extr. poppy-heads	0.323± 0.07	0.081± 0.00
Sewage sludge	0.411± 0.10*	0.121± 0.04*
Grinding sludge	0.216± 0.02	0.057± 0.03*
Lime sludge	0.362± 0.02	0.104± 0.01

Decrease of dry matter of roots and shots was observed when we made the treatments with flue gas. Behind the decrease I suppose the increased Al concentration. The shoot dry matter decreased by 34 %, while the decrease of root dry matter was 33 % in comparison to the control.

There was no significant decrease observed at corn when filtrates of compost was applied. The dry matter of shoots decreased by 3 %, while the roots dry matter by 17 %.

The reason of more intensive decrease in root dry matter can be the accumulation of Al.

Increase of shoots dry matter, and decreased root dry matter was measured when extruded poppy head was applied. The reason of decrease in root dry matter is the same, that was written earlier.

The effect of sewage sludge treatment was advantageous on dry matter accumulation of corn shoots and roots. The results are significant. The increase of shoots was more intensive than the increased dry matter accumulation of roots. The more intensive dry matter accumulation of shoots can be originated from its higher K contents.

The treatment with grinding sludge solution decreases the dry matter of shoots and roots of corn. The increase was more significant in case of roots. The reason of grow retardation can be very complex. I suppose, that a general disorder, and the toxic effect of different heavy metals can be the main reasons of retarded dry matter accumulation.

Table 8.: Effect of filtrates of examined by-products (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the dry matter of sunflower (g $plant^{-1}$) n=9± S.E. Significant differences compared to the control: *p<0.05; **p<0.01; ***p<0.001.

Treatments	Shoot	Root
Control	0.960± 0.16	0.210± 0.04
Flue gas	0.722± 0.05*	0.190± 0.03**
Compost	1.030± 0.16*	0.220± 0.04
Extr. poppy-heads	0.840± 0.05	0.160± 001**
Sewage sludge	1.030± 0.36	0.210± 0,02
Grinding sludge	0.601± 0.0***	0.124± 0,03***
Lime sludge	0.880± 0.02	0.180± 0.01**

The dry matter accumulation of sunflower shoots decreased approximately by 25%, while the decrease was 10 % in case of roots, when filtrate of flue-gas treatment was applied (table 8.). The decrease was significant in the roots, but the extremely high ion concentration of roots did not cause necrosis. The conclusion can be that the reason of retarded growth rather is the osmotic stress than the toxic effect of accumulated ions.

Increase of dry matter accumulation of sunflower shoots and roots was observed when diluted compost treatment was used. The effect of diluted compost was more advantageous in case of sunflower in comparison to the corn. The differences can be explained with the different nutrient uptake mechanisms of two plants. The other possible reason can be the more intensive growth of sunflower in the time of evaluation. The higher nutrient providing ability of diluted compost satisfies more easily the nutrient demand of sunflower therefore the growth can be more intensive. This idea is supported by changed morphology of roots.

Decreased dry matter accumulation was observed of sunflower shoots and roots when filtrate of extruded poppy-head was applied. I suppose that the treatment caused more slack tissue differentiation.

The sewage sludge treatment caused 7 % increase in dry matter of sunflower shoots in comparison to the control, while there was no difference in dry matter accumulation between the treated and the control root dry matter. It is surprising, that this equality in root dry matter accumulation developed at lower concentration of all examined elements in the treated plants.

Significant decrease was measured in dry matter accumulation of sunflower shoots and roots when the plants were treated with grinding sludge. Although the decrease of root dry matter accumulation is significant, the accumulated ions in high concentration did not cause the mortality of root, or root parts.

Due to the diluted lime sludge treatment decrease was observed in dry matter accumulation of sunflower roots and shoots. The decrease in root dry matter was more expressed.

4. 2. 3. The effects of examined by-products on SPAD value of plants grown on nutrient solution.

All treatment affected the dry matter accumulation. I suppose the changes of photosynthetic activity, and chlorophyll contents behind this effect.

The treatments had effects on chlorophyll content by my measurements (Table 9.).

Table 9.: Effect of different treatments (flue gas, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the relative chlorophyll content (SPAD-unit) of second and third leaves of maize. n=60± S.E. Significant differences compared to the control: *p<0.05; **p<0.01; ***p<0.001.

Second leaves			
Treatments	6th day	9th day	11th day
Control	38.14± 4.95	48.20± 2.85	49.31± 5.12
Flue gas	36.53± 7.74	40.42± 5.35*	44.71± 4.72*
Compost	41.26± 5.82	43.07± 4.68*	47.37± 3.60
Extr. poppy-heads	34.57± 5.83	41.51± 4.27*	41.07± 4.83*
Sewage sludge	34.07± 7.75	47.27± 4.08	47.92± 4.10
Grinding sludge	34.39± 8.74***	36.81± 5.47	36.52± 2.73***
Lime sludge	38.10± 4.78	44.05± 2.08	47.40± 2.86***
Third leaves			
Treatments	6th day	9th day	11th day
Control	30.06± 6.06	43.43± 2.49	45.87± 1.98
Flue gas	32.41± 6.99	37.46± 5.54**	42.55± 3.15**
Compost	32.11± 6.86	38.85± 3.50**	44.83± 3.27
Extr.poppy-heads	29.69± 7.04	41.34± 3.42	42.48± 4.49*
Sewage sludge	32.13± 5.19	41.77± 4.60	46.75± 4.30
Grinding sludge	26.63± 6.93	36.27± 1.83***	35.10± 3.57***
Lime sludge	33.69± 3.64	42.88± 3.39	47.71± 1.62***

Continuous decrease of SPAD values were observed in the second leaf of maize, when diluted flue gas treatment was applied. The decreases were significant on the 9th day (17 %), on the 11th day (10 %). The SPAD values increased on the 6th day, while decreased on the 9th and 11th days in the third leaf of corn. Behind the decrease of SPAD values we suppose the damage of biochemical processes of pigment synthesis. There was no difference in Fe contents between the treated and control plants, while the concentration of Mg decreases as a consequence of treatment.

The SPAD values decreased – except the 6th day - when diluted compost was applied. The differences are not important, but the results are significant in some cases. We found similarities between the dry matter accumulations and SPAD values. The dry matter accumulation slightly decreased, due to the moderated photosynthetic activity caused by lower chlorophyll contents.

Decrease of SPAD-units was observed in each day in the second and third leaves of corn when diluted extruded poppy head treatments were used. The decrease shows week significance in some cases. We suppose tight connection between the decrease in SPAD units and dry matter accumulation. The decrease of SPAD units can be the consequence of lower Fe and Mg concentration of shoots.

Decreased SPAD values were observed –except two cases- in the second and third leaves of corn when sewage sludge treatment was applied. We suppose that behind the decrease of SPAD units there is a more effective photosynthetic apparatus. We base this statement on the observation that the dry matter accumulation increased when this treatment was applied. We did not find differences between the Fe and Mg concentrations of treated and control plants leaves.

Decreased SPAD values were observed in both leaves when the plants were treated with diluted grinding sludge. The decrease can be the consequence of damage of several biochemical processes. The reason of lower SPAD units can be the lower Fe and Mg concentrations of treated plants. The most of the results were significant.

The SPAD units were lower on each measuring days in the second leaves of treated plants. The decrease measured on 11th day was significant. In contrast the SPAD units of younger leaves increased on 6th and 11th days in comparison to the control. Significant values in SPAD units were measured only on the last measuring day. Decrease in the second leaf, while increase in the leaf was observed. The differences were not so evident, to be able to change the dry matter accumulation of corn.

The SPAD values measured in sunflower leaves are shown in table 10.

The reason for decrease in SPAD units can be originated from the lower intensity of pigment synthesis. The reason can be the lower concentration of Fe and Mg.

The SPAD units were decreasing on all measuring days in comparison to the control, when diluted flue gas treatments were used. The decreases were significant on the 13th and 15th days.

Table 10.: The formation of the 10-13-15-day-old sunflower's relative chlorophyll content after the different treatments (Spad-value) n=60± S.E. Significant difference compared to the control: *p<0,05; **p<0,01;***p<0,001.

Relative chlorophyll content in the second leaves of sunflower (Spad Units)			
Treatments	10th day	13rd day	15th day
Control	47.57± 1.93	50.63± 1.34	50.33± 0.40
Flue gas	46.12± 0.86	46.48± 2.15***	44.47± 3.44***
Compost	46.78± 1.34	49.87± 0.64	50.02± 1.28
Ext.poppy-heads	45.50± 2.92	48.04± 0.88**	47.68± 0.97**
Sewage sludge	46.71± 3.09	48.36± 0.95**	48.88± 0.11
Grinding sludge	44.20± 1.11**	46.89± 0.78***	47.52± 0.45***
Lime sludge	44.45± 0.68**	45.54± 0.47***	46.35± 1.43***

Decrease also was observed on all measuring days in SPAD units when diluted compost was applied. These decreases were not significant. Supposedly there was no modification in photosynthetic apparatus, but its effectiveness became higher, since the dry matter accumulation was higher due to the treatments.

Slight decrease of SPAD values were measured on each measuring day when we applied diluted extruded poppy head treatments. We suppose connection between the decrease of SPAD units and decrease of dry matter accumulation.

The treatment with diluted sewage sludge caused decrease in SPAD units on each measuring day. This observation is in contradiction with the increased dry matter accumulation of shoots. The reason for lower SPAD units can be the lower Fe and Mg concentrations. The consequence can be the moderated pigment synthesis.

The diluted grinding sludge treatment made significant decrease in comparison to the control. The reason can be the lower intensity of pigment synthesis. However, the SPAD units were decreasing due to the treatments, but these are high. Only the decrease of SPAD units should not cause the decrease of dry matter accumulation that was measured.

The decrease in SPAD units observed at diluted lime sludge treatment could be the consequence of moderated synthesis of photosynthetic pigments. This observation is in harmony with the lower dry matter accumulation of shoots. The concentration of Fe and Mg did not decrease in comparison to the control, but supposedly the effectiveness of photosynthetic apparatus was moderated.

4. 3. Effects of examined by-products on physiological parameters of experimental plants

4. 3. 1. Effects of examined by-products on nutrient uptake of experimental plants

The concentrations of examined elements in the shoots and roots of maize can be seen in Table 11-12, when the plants were treated with the diluted form of examined side products.

The concentrations of examined elements decreased in the shoots of maize at flue-gas treatment, except the Fe, where slight increase was measured. The highest decrease was in case of P. The Al concentration was around 50 % in comparison to the control in the shoots. In general, the concentration of all examined element decrease in the shoots due to the treatments, while significant increase of Ca and Fe were observed in the roots. The Al concentration in the roots of treated plants was over the control value. The increase of Ca concentration raises several questions. The optimal Ca concentration in the citosol is 1 mM. To obtain this relatively low Ca concentration, the cells excrete Ca in energy demand processes. The total Ca concentration includes the apoplasmatic and symplasmatic Ca concentration, we can suppose the increase of ca concentration inside the cells. The root cells were not able to release these higher amounts of Ca, because there was not enough energy (ATP) to cover the energy demand of process.

The data of Tables 11-12 give interesting details according to the transport processes. The root to shoot transport of K was extremely intensive at the control, and at the treatment by sewage sludge compost. The observation was the same in case of P. The “together” movements of Fe and Al are well seen in this case as well, but it is not so expressed as it was in the other cases. The high concentration of P and K in the shoots let us make a conclusion of a more intensive carbohydrate metabolism.

The concentration of all examined elements decreased in the shoot of corn when extruded poppy head treatment was applied. This decrease happened in spite of the fact that the concentrations of measured elements were extremely high in the roots. This observation draws our attention to the importance of transport processes. Intensive root to shoot transport of K was observed at the control plants.

The increase of the concentration of Al and Fe is well seen in the roots, that raises further questions. Both ions are positively charged, therefore the competition can be supposed between them for the negatively charged places of apoplasma and plasmalemma. I observed special “together” movement instead of competition.

Table 11. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the shoot of maize treated by different by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	67,60± 2,54	8.006,50± 147,78	92,70± 3,12	80.074,00±5.593,21	2.003,50±132,23	13.987,00± 38,18	3.507,50± 77,07
Flue gas	30,50± 3,60	6.307,66± 280,01	95,73± 4,51	49.838,67±1.049,86	1.744,33± 61,13	3.590,60±243,60	2.478,30± 83,96
Compost	18,50± 8,34	6.479,00± 497,33	137,00± 9,89	67.856,00±1.431,09	1.755,00±356,38	11.769,50±881,60	3.218,50±615,00
Extr. poppy-h.	10,05± 0,30	74.656,00± 329,36	76,15± 6,05	37.820,50±1.450,33	1.218,00±340,82	7.500,50±237,17	2.198,00±150,12
Sewage sludge	13,80± 0,95	9.884,00± 393,25	152,50± 0,71	101.446,00±4.621,65	2.606,00± 60,81	15.770,50±651,24	4.854,00± 77,78
Grinding sludge	6,59± 0,73	11.245,65± 674,35	53,72± 1,98	22.205,08± 991,68	1.516,78±122,29	5.990,80±491,21	2.066,20± 48,27
Lime sludge	3,11± 0,41	5.339,76± 251,38	25,42± 1,63	10.527,71± 835,07	711,34± 18,02	2.812,90±253,59	975,20± 43,28

Table 12. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the root of maize treated by different by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	49,75± 3,04	7.773,50± 43,13	117,00± 7,07	51.065,50±1.898,58	4.714,50± 86,97	5.412,50± 85,18	10.469,50± 12,02
Flue gas	89,90± 7,23	83.937,00±6.485,33	1.017,00± 45,30	9.471,00± 143,67	1.108,00± 81,23	2.426,00± 46,20	5.324,00± 76,54
Compost	108,85±20,01	6.983,00± 237,72	240,00± 22,62	52.323,50±3.988,78	3.794,00±506,28	5.940,50± 53,88	10.512,50±182,99
Extr. poppy-h.	450,50±31,42	8.142,50± 195,86	594,50± 18,18	48.986,00±5.279,25	4.404,00±226,27	6.453,00± 65,91	10.790,00± 69,74
Sewage sludge	92,25± 1,06	5.311,50± 252,59	284,00± 4,24	41.211,50±2.502,95	2.947,50±314,66	4.020,00± 88,12	8.891,50± 74,28
Grinding sludge	39,25± 5,16	2.461,00± 41,01	58.508,50±678,11	57.169,50±3.851,61	865,50± 6,36	14.613,50±210,02	4.562,50± 53,03
Lime sludge	161,50± 0,71	2.444,00± 0,00	1.075,50± 53,03	65.881,00±2.818,52	962,30± 10,61	4.911,50± 75,66	5.254,00± 65,05

It is a fact that I cannot make differences between the apoplasmatic and symplasmatic Al, but let us suppose that most of Al is extracellular bound, while the accumulation of Fe is rather inside the cell. This observation needs further investigations because we should give answer for the question how can the high Fe uptake be possible in the presence of high Al concentration which –because its tree charges- modifies the structure of plasmalemma.

Only the Al concentration was decreased among the other examined elements when sewage sludge was applied. This observation is interesting, because the sewage sludge contains lots of Al, but the plant were not able to accumulate it. So the Al contents in the root, that is higher than it is in the control roots, cannot be considered high. The highest increase was observed in case of Fe in the shoot of maize. The concentration of most of examined decreased in the roots of maize, while concentration in the shoots were over the control value. This shows the intensive root to shoot transport.

The concentrations of examined elements decreased in the shoots of maize, except the Fe, Mg and S, where slight increases were observed, when we applied grinding sludge treatments. The decrease of Al concentration in shoots was about 8.5 fold due to the grinding sludge treatments. The concentrations of Fe were 432 fold higher in the roots of treated plants in comparison to the control. It can be explained with the extremely high Fe concentration of grinding sludge (683,000 mg kg^{-1}).

Decreasing concentrations of examined elements were observed - except of S - in the shoot of maize when lime sludge was used. The most significant decrease was observed in case of Al. In spite of this, the concentration of Al in the roots of treated plants was 4-fold higher in comparison to the control. As it has already been observed several times, the concentration of Fe increases in line with Al in this case as well.

The concentrations of examined elements in the roots and shoots of sunflower can be seen in table 13-14. treated with the by-products.

The concentration of most elements decreased in the soot of sunflower, while the Al and the S increase when the plants were treated with flue gas. The concentration of Ca in the roots of sunflower was 44-fold higher in comparison to the control. The high concentration of Ca seems to be the most questionable. The localization of this huge amounts of Ca is the question we have to answer in the future research work. We suppose, that most amounts of Ca is located in the apoplasma, because such high Ca-concentration inside the cell causes the death of living organism. It seems to be obvious, that the Ca ions are bounded to the negatively charged places of apoplasma. The Ca –because its bridge forming ability- has a strong effect on the structure of biological membranes, makes the membrane more contracted, therefore the permability will be weaker, therefore the uptake of other nutrients will low. I suppose that this can be the main reason of the lower measured concentrations of other examined elements in the treated plants.

The highest differences were measured in the concentration, transport and distribution of K, and Ca in the plants treated with extruded poppy head treatment. The root to shoot transport of Ca was very intensive in the control plants, and as a consequence we measured many fold higher Ca concentration in the shoot than in the roots.

Less intensive root to shoot transport of Ca was observed at the treated plant, therefore the Ca concentration remained below the control, although this relatively lower Ca concentration meant in general high concentration! In contradiction with this observation, the K concentration of sunflower roots treated with poppy head remained on low level in comparison to the control, meanwhile the K concentration in the shoots of treated and control plants were similar. The ion antagonism between the Ca and K is well known, that was observed in the roots. The K concentration in the control roots was high, while the concentration of Ca was low. This can not be observed in the shoot, there was no differences in the concentrations of these elements.

The decrease of all examined elements was observed in both parts of plants. The element uptake of roots was low except the Ca, and the intensity of root to shoot transport was high only in the case of P and Mg.

In contradiction with our experience with corn, the concentration of Al decreased in the roots and shoots of sunflower due to the sewage sludge treatment. To use the sewage sludge in agriculture, there is one important point of view, namely its heavy metal content. The Al is not considered heavy metal, although its physiological similar to that. The sewage sludge contain lots of Al, but there was no observed uptake and accumulation by the sunflower roots.

Decrease was observed in the concentration of all examined elements in the shoot of sunflower when the plants were treated with grinding sludge. Increase was measured in comparison to the control in the concentration of Ca, Fe, P, and S in the roots. As it was mentioned at the corn, extremely high Fe concentration was measured in this case as well (58.508 $mgkg^{-1}$). The Fe reduction on the surface of roots was well seen in this case too. Supposedly the Fe was strongly bounded in the apolastic space. The huge amounts of apoplastic bounded Fe was observed at the roots of both experimental plants, having different Fe uptake mechanism. Therefore we suppose, that rather exists a special, but similar defensive mechanism at both plants. The question is in this case: how can accumulate so much negatively charged place in apoplasma, that become able to bond this huge amount of Fe? The other question, we can base our further work on is: is there any defence mechanism against Fe toxicity, which is induced by high Fe concentration, or can we prove, that the synthesis of pectin will be increased as a consequence of high Fe concentration? There is difference in nutrient uptake mechanism between the mono-, and dicot plants. In contradiction with this statement the observation at maize regarding the interaction of Al and Fe is true in case of sunflower. I suppose, that this interaction can be the reason of low concentrations of K, Mg, and P in the roots. Retarded root to shoot nutrient transport was observed at several elements as well.

Table 13. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the shoot of sunflower treated by different by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	12,70±1,55	33.816,50±2.843,77	137,00± 4,00	55.531,50±685,18	4.203,00±35,35	4.455,00±736,81	4.640,00± 458,38
Flue gas	23,85± 2,33	25.690,33± 910,75	97,70±10,32	55.066,50±1.709,07	4.198,50±197,28	4.006,00±50,63	4.772,50± 729,02
Compost	14,55± 0,21	27.672,00± 60,81	135,00± 4,58	55.066,50±1.709,07	4.198,50±197,28	15.522,50±481,53	5.441,00±3.169,71
Extr. poppy-h.	17,15± 0,35	28.942,00± 210,71	139,50± 0,71	57.452,50± 3,53	4.020,50±139,30	15.844,50±672,45	5.392,00± 83,43
Sewage sludge	13,80± 0,95	98.840,00± 393,25	152,50± 0,71	101.446,00±4.621,65	3.693,00±140,92	11.429,00±353,20	4.089,33± 305,51
Grinding sludge	11,29± 2,14	27.613,00± 647,33	133,50±31,31	55.638,00± 541,67	3990,33±135,33	4.596,33±324,15	4.523,66± 525,01
Lime sludge	16,53± 2,47	34.021,67± 777,00	164,00± 26,28	40.078,67± 655,00	3.827,00±115,33	5.428,50±706,39	7.676,00± 28,28

Table 14. The content of examined elements (Al, Ca, Fe, K, Mg, K, S) (mg kg^{-1}) in the root of sunflower treated by different by-products (flue-gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) n=3± S.E.

Treatments	Al	Ca	Fe	K	Mg	P	S
Control	60,15± 0,21	2.420,50± 142,12	174,00± 2,82	66.463,50± 344,36	1.103,50± 4,95	7.733,00± 14,14	4.530,00± 62,23
Flue gas	56,90± 0,45	107.354,00±7.420,47	331,00± 15,20	9.871,00± 741,67	627,00± 24,13	1.428,00± 45,23	2.880,00± 45,87
Compost	49,64± 1,26	54.482,53±4.771,56	226,00±28,46	28.312,15± 679,72	701,89± 15,91	3.443,00±249,68	3.019,60±197,42
Extr. poppy-h.	29,50± 7,84	64.627,04±4.346,51	187,20± 4,83	27.195,93±1.578,56	403,91± 21,41	3.146,40±419,56	1.608,50± 95,58
Sewage sludge	92,25± 1,06	5.311,50± 252,59	284,00± 4,24	41.211,50±2.502,98	2.947,50±314,66	4.020,00± 88,12	8.891,50± 74,28
Grinding sludge	53,63± 8,61	4.532,33± 214,67	1.151,33±64,78	65.281,00± 621,41	1.572,00± 54,13	6.522,66±594,39	4.447,66±339,53
Lime sludge	478,50±28,99	3.918,33± 313,00	863,00±25,45	54.876,67±2.395,00	3.610,00± 81,21	8.286,00±411,53	7.288,00±171,56

4. 3. 2. Effect of examined materials on the dry matter of plants grown on nutrient solutions

The dry matter of maize is shown in Table 15. The dry matter of shoots and roots of maize significantly decreased at the flus gas treatment. The formation of lateral roots was cramped which can be explained with the high pH of flue gas and with the insufficient nutrient supply.

The dry matter of shoot significantly increased, while the dry matter of root remained at the control value.

The dry matter of shoot and root of maize decreased at the extruded poppy-heads treatment. The decrease is not significant. The decrease of dry matter of root can be explained by the high concentration of Al of root. The other possible explanation is the unfavourable Fe^{2+}/Fe^{3+} ratio in the young leaves. If the iron is oxidizing in the tissue, it will precipitate in insoluble ferric-phosphate and do not have role in the metabolism.

The dry matter of shoots and roots of maize is increased at the sewage sludge treatment. The increase was significant in the shoot.

Table 15.: Effect of by-products (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the dry matter of maize (g plant^{1}) n=9± S.E. Significant differences compared to the control: *$p<0.05$;***$p<0.001$.

Treatments	Shoot	Root
Control	0.199± 0.02	0.064± 0.01
Flue gas	0.128± 0.02***	0.023± 0.00***
Compost	0.254± 0.03***	0.064± 0.01
Ext. poppy-heads	0.180± 0.04	0.052± 0.01
Sewage sludge	0.262± 0.06*	0.067± 0.01
Grinding sludge	0.282± 0.03***	0.066± 0.01
Lime sludge	0.285± 0.03***	0.072± 0.01

The dry matter of shoots and roots increased at the grinding sludge treatment. The results are contradictory because the dry matter decreased when filtrate was examined. There are different effects of filtrate of grinding sludge and grinding sludge needs more investigation. But I assume that the solubility of grinding sludge in the nutrient solution is not the same as at the making filtrate because the excreted organic acids can change the solubility of grinding sludge.

The dry matter of shoot and root of maize increased at the lime sludge treatment. The increase was significant in the shoot. The results are similar to the results of filtrate.

The result of dry matter of sunflower is shown in Table 16.

Table 16.: A különféle kezelések hatása (füstgáz, komposzt, extrudált mákgubó, szennyvíziszap, köszörűiszap, mésziszap) a napraforgó száraz tömegére (g növény^{-1}) n=9± S.E. Szignifikáns különbség a kontrollhoz viszonyítva: *p<0,05; **p<0,01;***p<0,001.

Treatments	Shoot	Root
Control	0.332± 0.07	0.061± 0.02
Flue gas	0.168± 0.03***	0.047± 0.06
Compost	0.433± 0.07*	0.091± 0.02*
Exr. poppy-heads	0.373± 0.07	0.074± 0.02
Sewage sludge	0.431± 0.07*	0.089± 0.01**
Grinding sluge	0.308± 0.03	0.071± 0.01
Lime sludge	0.447± 0.08*	0.116± 0.03***

The dry matter of shoot and root of sunflower was decreased. The decrease was higher at the shoots than at the roots. One of the causes of the decrease may be the pH increasing effect of flue gas in the nutrient solution (11.40±0.56). Other cause may be the high Ca-content of root.

The dry matter of shoot and root increased significantly at the compost treatment. The dry matter of shoot increased by 30%, and the dry matter of root increased by 49% compared to the control.

The dry matter of shoot and root of sunflower increased at the extruded poppy-heads. The increase of root expressed more.

The dry matter of shoot and root of sunflower also increased at the sewage sludge treatment more than at the maize. The increase is significant.

The dry matter of shoot of sunflower decreased at the grinding sludge treatment and the dry matter of root increased.

The dry matter of shoot and root of sunflower increased significantly at the lime sludge treatment compared to the control. The increase was more intensive at the root than at the shoot.

4. 3. 3. Effect of examined materials on the relative chlorophyll content (SPAD-units) of plants grown on nutrient solutions

There is a connection between the dry matter accumulation and chlorophyll content as I mentioned previously. So, I measured the relative chlorophyll content in the second and third leaves on the 6th, 9th and 11th days of experiments (Table 17.).

The SPAD-unit is decreased in the second leaves of maize on all measuring days at the flue gas treatment. The relative chlorophyll content decreased by 2 SPAD-units on the 6th day, by 4 SPAD-units on the 9th day and by 8 SPAD-units on the 11th day. The decrease was significant on the 9th and 11th days. The SPAD-unit decreased on the 6th and on the 11th days in the third leaves, while this value increased by 1 SPAD-units on the 11th day.

The SPAD-unit increased in the second and third leaves of maize on the all measuring days. The differences between the treated and non-treated (control) plant can be found in the visuals.

Table 17.: The relative chlorophyll content (SPAD-unit) in second and third leaves of maize on the 6th, 9th and 11st days of the experiment. n=60± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

Second leaves			
Treatments	6th day	9th day	11th day
Control	36.77± 3.35	37.27± 3.92	38.38± 2.31
Flue gas	34.70± 3.82	33.34± 4.43*	30.36± 2.73***
Compost	39.42± 3.25	41.95± 3.83**	41.62± 2.70**
Extr. poppy-heads	39.35± 3.56	42.30± 4.61**	39.49± 3.67
Sewage sludge	38.69± 2.11	43.67± 3.51***	43.79± 1.64***
Grinding sludge	43.10± 3.35***	48.68± 2.85***	46.35± 2.09***
Lime slusge	38.02± 1.39	42.81± 3.12***	43.92± 1.58***
Third leaves			
Treatments	6th day	9th day	11th day
Control	36.77± 3.35	37.27± 3.92	38.38± 2.31
Flue gas	34.70± 3.82	33.34± 4.43*	30.36± 2.73***
Compost	39.42± 3.25	41.95± 3.83**	41.62± 2.70**
Extr. poppy-heads	39.35± 3.56	42.30± 4.61**	39.49± 3.67
Sewage sludge	38.69± 2.11	43.67± 3.51***	43.79± 1.64***
Grinding sludge	43.10± 3.35***	48.68± 2.85***	46.35± 2.09**
Lime sludge	38.02± 1.39	42.81± 3.12***	43.92± 1.58***

The SPAD-unit is increased on all measuring days in the second leaves of maize at the extruded poppy-heads. The increase was significant on the 11th day in the third leaves. I suppose that the synthesis of photosynthetic pigments is a slower process than the growth of leaf. Thus, the total pigment concentration has not finished at the total leaf size developing. The synthesis of photosynthetic pigments needs energy and materials. So, I suppose that one of the highest quantities of the produced energy during the leaf-differentiation turns to the structure formation and after the finishing the synthesis of photosynthetic pigments.

The SPAD-unit increased in the second and third leave of maize on all measuring days at the sewage sludge treatment compared to the control. The increase was significant on the 9th and on the 11th days.

The SPAD-unit is significantly increased in the second and third leaves of maize on all measuring days at the grinding sludge treatment.

The SPAD-unit also increased at the plants which were treated by lime sludge. The increase was significant on the 9th and 11th days of the experiment.

The relative chlorophyll content of sunflower is shown in the Table 18.

Table 18.: The relative chlorophyll content (SPAD-unit) in the second leaves of sunflower on the 10th, 13rd and 15th days of measuring. n=60± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

Treatments	10th day	13rd day	15th day
Control	36.32± 3.14	39.88± 2.53	43.95± 2.32
Flues gas	49.13± 3.86***	47.39± 4.04***	46.81± 3.19*
Compost	37.44± 2.07	39.17± 2.43	41.56± 1.29*
Ext. poppy-heads	37.71± 1.73	39.25± 2.65	41.57± 3.08*
Sewage sludge	36.82± 2.87	40.37± 3.15	41.50± 3.18*
Grinding sludge	44.82± 2.41***	46.86± 2.74***	49.36± 2.73***
Lime sludge	40.93± 3.49**	44.71± 1.87***	44.58± 2.23

The SPAD-unit increased significantly in the second leaves of sunflower on all measuring days. This increase was 13 Spad-unit on the 10th day, 7.5 Spad-unit on the 13th day and 3 Spad-unit on the 15th day.

The SPAD-unit did not change significantly at the compost treatment compared to the control.

The SPAD-unit decreased significantly on the 15th at the extruded poppy-heads treatment. On the other hand the dry matter of shoot of sunflower increased so it is a contradiction. It draws attention to changes in the photosynthetic cells. The dry matter of treated plants increased while the SPAD-unit decreased. So, it draws attention to complex effect that the treatment has different effect on dry matter accumulation and on chlorophyll synthesis.

The SPAD-unit did not increase significantly on the 11th and 13th days and decreased on the 15th day when plants were treated by sewage sludge. There is a continuous growth in the control and in the treated plants, but the increase of SPAD-unit was more intensive in the control leaves.

The SPAD-unit increased significantly at the grinding sludge treatment. This result comes into conflict with the decrease of dry matter of shoot. The content of Fe and Mg decreased in the shoot of sunflower. This contradiction needs more examinations. Presumably the photosynthetic system is also damaged.

The increased SPAD-unit raises new questions because the ion content of shoot decreased at the lime sludge treatment. As a result, the content of Fe and Mg also decreased. The essential nutrients of the chlorophyll synthesis decreased while the SPAD-unit increased, so it suggests that the decreased Fe and Mg content is enough for the chlorophyll synthesis. This amount of Fe and Mg was physiological active and basically localized in the cell and easily accessible for the chlorophyll synthesis.

4. 4. Effect of the by-products tested on the chlorophyll-*a*, *b* and carotenoids content of the experimental plants

Since taking relative chlorophyll values gives only relative readings the volumes of photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b*, carotenoids) were also taken (Table 19).

Chlorophyll content readings are similar to SPAD-value readings of flue gas treatments. On the basis of the detailed measurement it can be stated that the treatment brings about changes in chlorophyll-*b* concentrations, primarily. Chlorophyll-*a* contents decreased in the older second leaves while they increased in the subsequent mature ones under the influence of the treatment. The increase in the chlorophyll-*a*/chlorophyll-*b* ratios calls our attention to the defective operation of the photosynthetic equipment.

The increases in SPAD-values under the influence of the compost treatment are supported by the values showing absolute concentrations of photosynthetic pigments.

The absolute volumes of photosynthetic pigments decreased in the case of leaves of any age when extruded poppy-heads treatment was applied. In the third, the younger leaf, this decrease is significant, which supports the earlier explanation, namely that the time curves of leaf growth and syntheses of photosynthetic pigments do not coincide. The decrease in the pigment levels in older leaves in relation to the control may be a cause of the more moderate organic matter accumulation in the plants treated.

Table 19. Chlorophyll -*a* and *b* carotenoid contents (mg g^{-1}) and changes in tthe chlorophyll *a* / chlorophyll –b ratio in the 2nd and 3rd leaves of maize under the effects of different treatments (flue gas, compost, extruded poppy-heads, sewage-sludge, grinding sludge, lime sludge) n=3± S.E. Significant differences compared to the control: *p<0.05.

Second leaves				
Treatments	Chlorophyll-*a*	Chlorophyll-*b*	Chlorophyll-*a*/*b*	Carotenoids
Control	12.44± 0.61	3.51± 0.41	3.54	8.53± 0.89
Flue gas	11.53± 0.60	2.92± 0.57	3.94	7.60± 0.02
Compost	13.18± 1.55	3.89± 0.75	3.38	8.54± 0.86
Extr. poppy-heads	11.22± 0.52	2.97± 0.59	3.77	6.95± 0.76
Sewage sludge	13.63± 1.75	3.54± 0.74	3.85	8.16± 0.75
Grinding slugde	16.75± 0.98**	5.57± 0.37*	3.00	10.86± 0.37
Lime sludge	15.13± 0.57**	5.01± 0.38*	3.02	10.23± 0.45
Third leaves				
Treatments	Chlorophyll-*a*	Chlorophyll-*b*	Chorophyll-*a*/*b*	Carotenoids
Control	8.79± 0.38	3.48± 0.09	2.50	5.92± 0.69
Flue gas	9.95± 0.22*	2.67± 0.25	3.73	6.92± 0.31
Compost	13.45± 0.44**	3.54± 0.98	3.79	8.63± 0.02*
Extr. poppy-heads	7.55± 0.43*	1.79± 0.59	4.21	5.24± 0.14
Sewage sludge	14.04± 0.55*	4.15± 0.69	3.38	9.12± 0.71*
Grinding sludge	16.64± 0.61***	6.17± 0.63*	2.69	11.77± 0.65***
Lime sludge	15.12± 0.71***	5.64± 0.72*	2.68	10.17± 0.67***

Similarly to SPAD-values, chlorophyll-*a*, chlorophyll-*b* and carotenoids contents also increased in older leaves of corn when a sewage-sludge treatment was applied. A more significant increase was observed in the third leaf than in the second one. The dry matter accumulation in shoots in excess of that in the control and the increases in the relative and absolute chlorophyll contents of the plants that were treated reveal positive causal correlations.

The observation of the photosynthetic pigment confirms the SPAD readings since concentrations of photosynthetic pigments increased significantly when a grinder-sludge treatment was applied. In all the cases concentrations of photosynthetic pigments increased when lime sludge treatment was applied. Although at different levels, but the increases were significant. The higher number of photosynthetic pigments manifested itself in higher SPAD-values and increased organic matter accumulations.

Table 20. Changes in chlorophill-*a*, *b*, carotenoid contents (mg g^{-1}) and chlorophyll-*a* / chlorophyll-*b* ratio found in the second and third leaves of sunflower under the effects of different (flue gas, compost, extruded poppy-heads, sewage-sludge, grinding sludge, lime sludge) treatments n=3± S.E. Significant differences compared to the control: *p<0.05.

Second leaves				
Treatments	Chlorophyll-*a*	Chlorophyll-*b*	Chlorophyll-*a*/*b*	Carotenoids
Control	11.77± 0.98	3.57± 0.09	3.29	7.13± 0.22
Flue gas	11.25± 0.46	3.51± 0.37	3.20	7.47± 0.14
Compost	10.32± 0.18	3.01± 0.12*	3.42	6.61± 0.20
Extr. poppy-heads	8.90± 0.78*	3.01± 0.76	2.95	5.36± 0.31*
Sewage sludge	9.66± 0.74	2.97± 0.34	3.25	6.00± 0.61
Gringing sludge	12.46± 0.68	4.23± 0.08	2.94	9.24± 0.61*
Lime sludge	11.38± 0.17	3.52± 0.18	3.23	7.67± 0.26
Third leaves				
Treatments	Chlorophyll-*a*	Chlorophyll-*b*	Chlorophyll-*a*/*b*	Carotenoids
Control	12,29± 0,41	3.69± 0.05	3.33	8.85± 0.05
Flue gas	12,19± 0,72	4.23± 0.35	2.88	8.99± 0.75
Compost	10,98± 0,26	3.20± 0.31	3.43	7.62± 0.25
Extr. poppy-heads	11,89± 0,81	3.96± 0.35	3.00	8.24± 0.64
Sewage sludge	11,27± 0,13*	3.56± 0.08	3.16	7.98± 0.14***
Grinding sludge	10,57± 0,32*	3.03± 0.19**	3.48	7.68± 0.19**
Lime sludge	11,62± 0,51	3.46± 0.17	3.35	8.41± 0.86

4. 5. The effect of examined materials on the leaf area of the experimental plants

The effects of different treatments on the leaf area also were examined. The results are shown in table 21-22.

Table 21.: Effect of examined materials (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the second and third leaves area (mm^2) of maize. n=3± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

Treatments	2nd leaves	3rd leaves
Control	44.51± 2.92	25.24± 0.67
Flue gas	16.22± 2.71***	35.72± 2.77*
Compost	21.50± 7.24**	39.77± 9.24
Ext.poppy-heads	21.18± 0.30***	47.68± 7.19*
Sewage sludge	20.66± 3.05***	42.09± 9.45
Grinding sludge	21.39± 2.34***	41.94± 3.59**
Lime sludge	22.72± 0.85***	43.28± 1.32***

The leaf area decreases significantly at the second leaf of maize when the plants were treated with flue gas in comparison to the control. At the same time increase was observed at the area of third leaf. The results are contradictory. There were significant differences between the dry matter of control and treated leaves, while the dry matter of third leaf was below the control. We suppose that the contradiction can be explained with the different thickness of leaves, and with the damage of mesophyll cells.

The area of second leaf, which was developed earlier, decreased, while increase was observed in the area of third leaf after compost treatment. In line with this the dry matter of treated shoots increased significantly. I suppose that the reason of this contradiction can be the different thickness of leaves. The other possible reason can be the supra-optimal nutrient supply that may retard the elongation of cells, meanwhile there is no change in the volume of cells.

The area of second leaf of maize decreased significantly, while the area of third leaf increases significantly when extruded poppy head treatment was applied. The consequence is the same what we already did: the reason can be the different leaf thickness.

The area of third leaf increases when sewage sludge treatment was applied, the area was larger by 67 % in comparison to the control. The area of second leaf decreases. This decrease was significant. The observation is in agreement with the dry matter accumulation. The advantageous effect of treatment starts in line with appearance of third leaf, because the more intensive mobilization of nutrients happens in this developmental phase. The increase of leaf area was observed by Thomas et al. (2006) when sewage sludge was applied.

The results are contradictory at the treatment with grinding sludge, and lime sludge. I measured decrease of area of second and increase of area of third leaf.

The treatment with flue gas inhibited the leaf growth (table 22.). The inhibition is significant at leaves of both ages. The measurements are in harmony with results of dry matter accumulation and with the chlorophyll contents. The retardation effect was higher than it was expected. The only reason cannot be the different thickness of leaves.

Table 22.: Effect of examined materials (flue gas, compost, extruded poppy-heads, sewage sludge, grinding sludge, lime sludge) on the second and third leaves area (mm^2) of sunflower. n=3± S.E. Significant differences compared to the control: *p<0.05; **p<0.01;***p<0.001.

Treatments	2^{nd} leaves	3^{rd} leaves
Control	134.33± 32.64	170.93± 12.74
Flue gas	46.45± 3.73*	21.75± 2.94***
Compost	105.97± 18.78	157.26± 6.10*
Ext. poppy-heads	118.11± 6.69	158.15± 7.89
Sewage sludge	116.07± 18.25	170.84± 17.03
Grinding sludge	82.08± 7.76	93.07± 15.89**
Lime sludge	102.86± 19.02	127.30± 15.92*

Different decreases of the leaf area were observed of the second (22 %) and the third (8%) leaf in comparison to the control. The effect of treatment was the same for the younger and older leaf. The decrease of leaf areas happened in line with the increase of dry matter accumulation. We suppose the slight increase of cell volumes behind this observation.

The leaf area decreases when poppy head treatment was applied. I also suppose the increase of leaf thickness, but it cannot be accepted as a single reason.

Decrease of leaf area of second and third leaves of sunflower was observed due to sewage sludge treatment. The decrease was significant in case of older, second leaf, while the area of third leaf was around the control value.

Significant decreases of leaf area were observed after grinding sludge treatment. The decrease was more intensive in case of 3^{rd} leaf.

Also decrease of leaf area was observed when lime sludge treatment was applied, while the dry matter of shoot increased. The volume of shoots was not measured, therefore only presumable, that the reason can be the more developed mesophyll tissue.

4. 5. Effect of compost, extruded poppy-heads, flue gas and grinding sludge treatments on the ultra-structural changes of maize and sunflower

The different treatments have effect on lot parameters which is connected to photosynthesis. The photosynthesis' cell is chloroplasts. That is why I examined the effect of different by-products on the number of chloroplasts per cell.

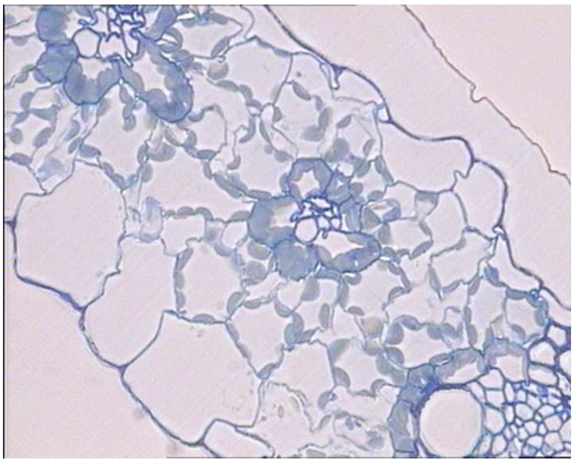
The chloroplasts of control maize are in equal arrangement in the light microscope picture (Pic.1.). Their size is average. The number of chloroplasts per cell is 5.5± 0.02.

The size of chloroplasts is increased when maize was treated with sewage sludge-compost (Pic.2.) where there are chloroplasts. The average number of chloroplasts is 5 which is explained by the unequal distribution of chloroplasts.

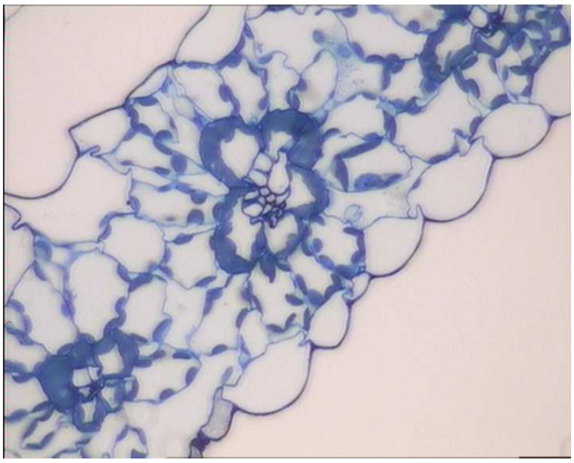
The cells are damaged (Pic.3.) and deformed at the flue gas treatment. The number of size of chloroplasts decreased compared to all treatments and controls. The average number of chloroplasts is 3.5. The decreased photosynthetic pigment contents can be explained by the decreased number of chloroplasts.

The distribution of chloroplasts of maize treated with grinding sludge is equal (Pic. 4.). The average chloroplasts number is 6, which is the explanation for the increased relative chlorophyll and photosynthetic pigment content.

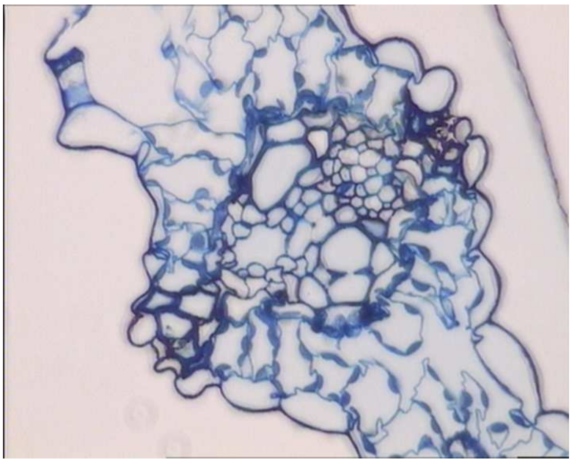
The distribution of chloroplasts of maize treated with extruded poppy-heads is equal (Pic. 5.), and their number and size did not change compared to the control.



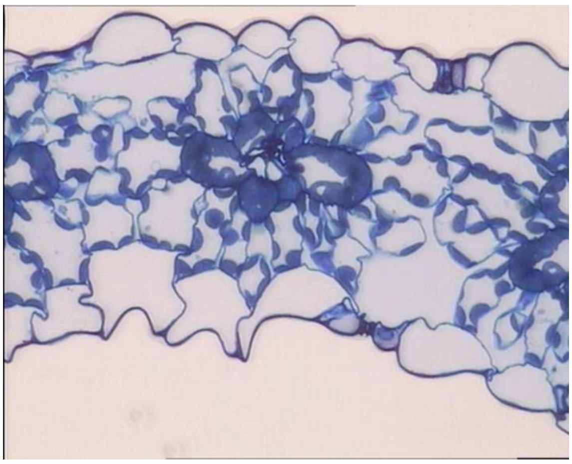
Picture 1. Chloroplasts in the second leaves of control maize (Photo: B. Toth, 2012)



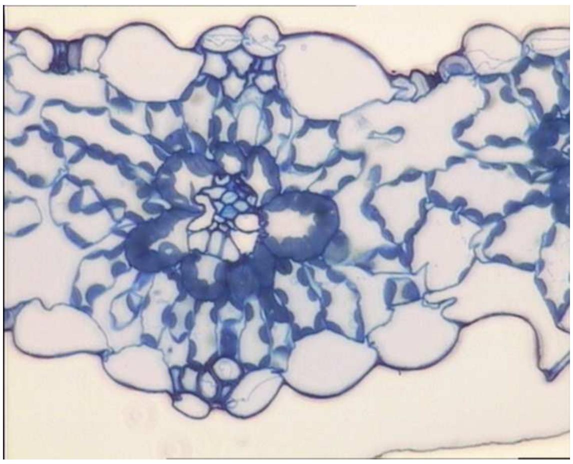
Pic. 2. Chloroplasts in the second leaves of maize treated with sewage sludge-compost (Photo: B. Toth, 2012)



Pic. 3. Chloroplasts in the second leaves of maize treated with flue gas (Photo: B. Toth, 2012)



Pic. 4. Chloroplasts in the second leaves of maize treated with grinding sludge (Photo: B. Toth, 2012)



Pic. 5. Chloroplasts in the second leaves of maize treated with extruded poppy-heads (Photo: B.Toth, 2012)

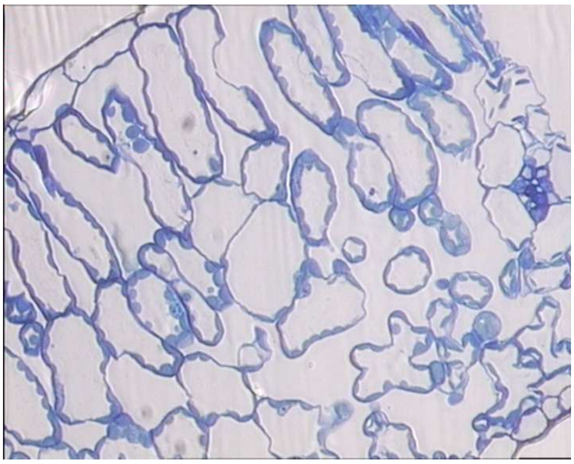
The distribution of chloroplasts of control sunflower is equal (Pic. 6.). Their size is average, but there are some bigger chloroplasts in 1-2 cells. The average number of chloroplasts is 12.

The size of chloroplasts decreased compared to the control when sunflower was treated with sewage sludge-compost (Pic.7.). The average number of chloroplasts is 10 which reflects the decreased relative and photosynthetic pigment content.

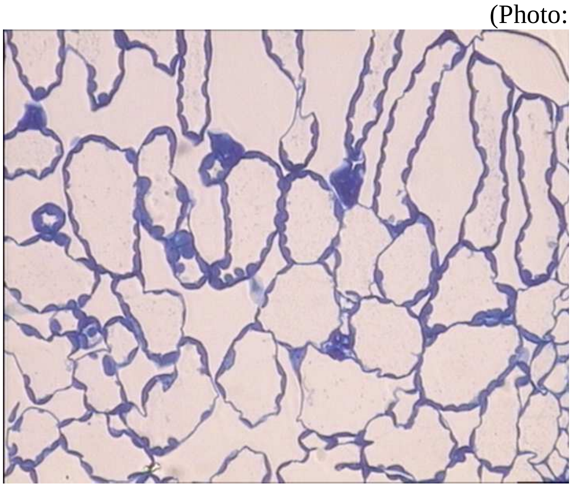
The cells are damaged (Pic.8.) and deformed at the flue gas treatment. The number of size of chloroplasts decreased compared to all treatments and controls. The average number of chloroplasts did not change compared to the control.

The distribution of chloroplasts of sunflower treated with grinding sludge is equal (Pic.9.). The average chloroplasts number is 13, which increased compared to the control.

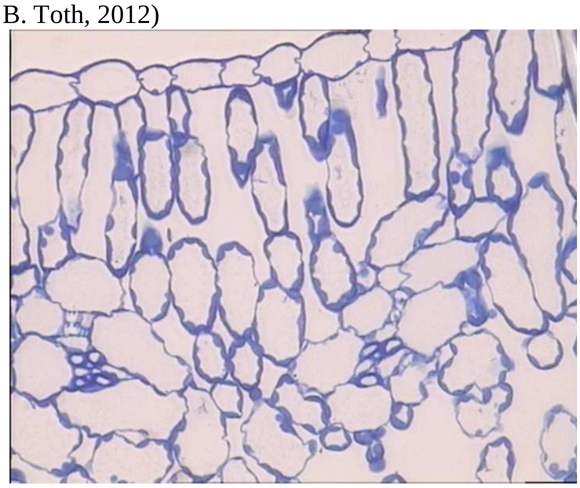
The size of chloroplasts is smaller than at the control when sunflower was treated by extruded poppy-heads (Pic. 10.). The smaller chloroplasts contain less pigment and the increased chloroplasts (13) did not compensate the differences.



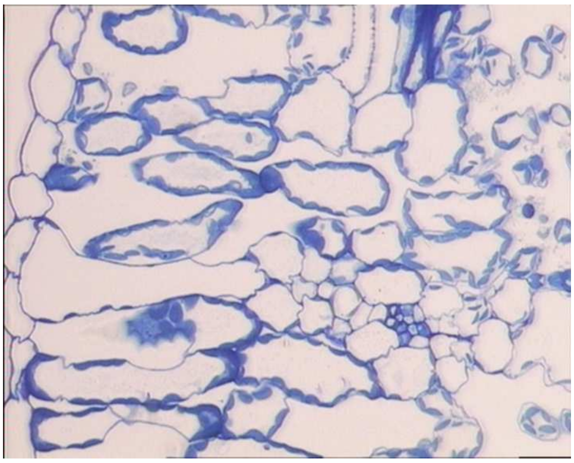
Pic. 6. Chloroplasts in the second leaves of control sunflower
(Photo: B. Toth, 2012)



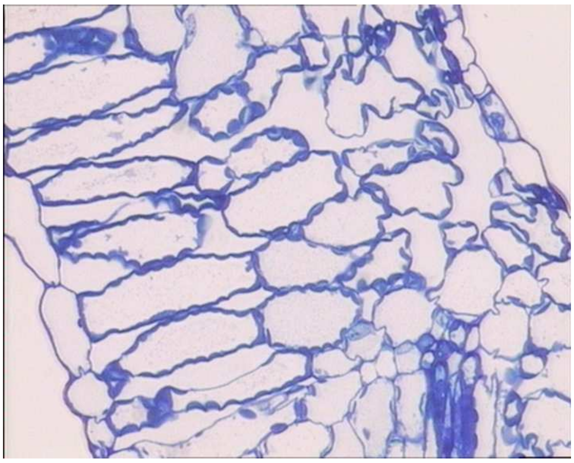
Pic. 7. Chloroplasts in the second leaves of sunflower Treated with sewage sludge-compost
(Photo: B. Toth, 2012)



Pic. 8. Chloroplasts in the second leaves of sunflower treated with flue gas (Photo: B. Toth, 2012)



Pic. 9. Chloroplasts in the second leaves of sunflower treated with grinding sludge (Photo: B. Toth, 2012)



Pic. 10. Chloroplasts in the second leaves of sunflower treated with extruded poppy-heads (Photo: B. Toth, 2012)

Those by-products were selected to the microscope analysis which has interesting results. The two experimental plants (maize and sunflower) have different reactions to the treatments. That is why I had to select those by-products which have the same effect on the plants. I took the SPAD-unit and dry matter into consideration.

The electron microscope picture of control maize is related to plants grown under the optimal circumstances. Deformation is not observed on this. The thylakoid membrane is well developed, while the stroma is homogenous (Pic.11.).

The size of chloroplasts is big at the sewage sludge compost which is similar to the control sunflower (Pic.12-13.).

There are some dilatations in the thylakoid and starch accumulation can be seen in Pic. 14 when maize was treated with flue gas. I assume that the decomposition and transport of photosynthetic starch is damaged. Thus, the starch is temporarily accumulated. The endoplasmic reticulum became multi-layer and grown wider.

More endoplasmic reticulum can be observed when maize was treated with grinding sludge (Pic. 15.) and extruded poppy-heads (Pic. 16.).

The electron microscope picture of control sunflower is related to the plants grown under optimal circumstances (Pic. 17.).

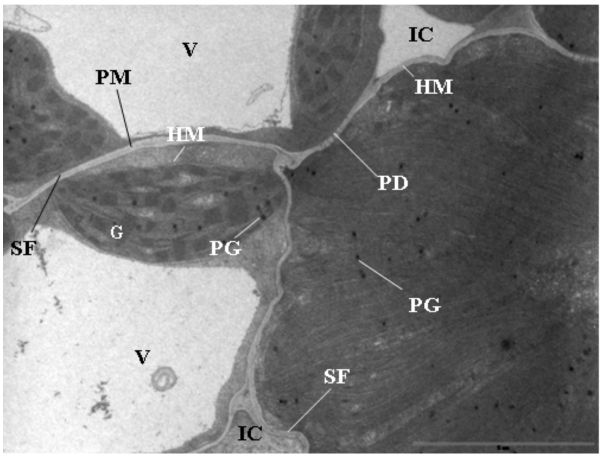
The mitochondrium is puffy and the formation is ring. The size of peroxisomal is bigger and the structure is looser that at the control. The structure of chloroplasts is the same as the control and the number of endoplasmic reticulum is more (Pic. 18.):

The dilutation of thylakoid can be observed in the Pic. 19. The chloroplasts are round which may indicate stress circumstances. The grana of chloroplasts are not in one level. The plastoglobulus is black and small. The chloroplasts are damaged (Pic. 19.).

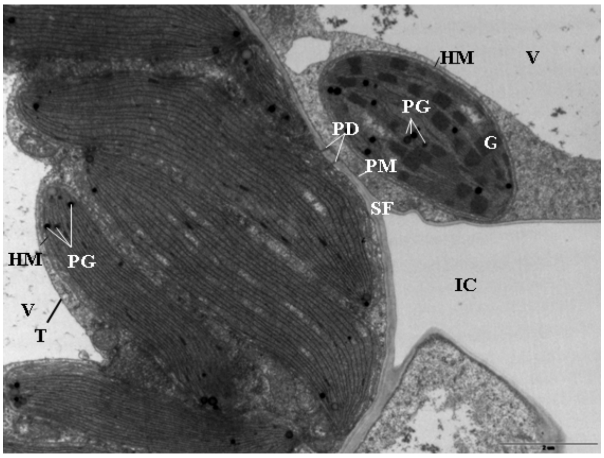
The displacement of thylakoid system can be observed at the grinding sludge treatment (Pic.20.). The number of grana in thylakoid is less than in the control.

There was no difference when sunflower was treated with extruded poppy-heads compared to the control (Pic. 21.).

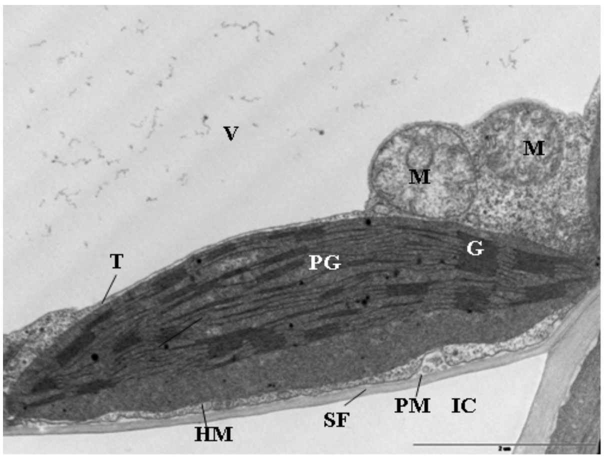
The grinding sludge had the most effects on the chloroplasts structural changes of sunflower. The conditions of chloroplast are the worst at the grinding sludge treatment. The next is the flue gas treatment where the largest dilatation can be observed in the thylakoid. There were some small changes at the compost treatment compared to the control. The extruded poppy-heads treatment did not have effect on structural changes of chloroplasts.



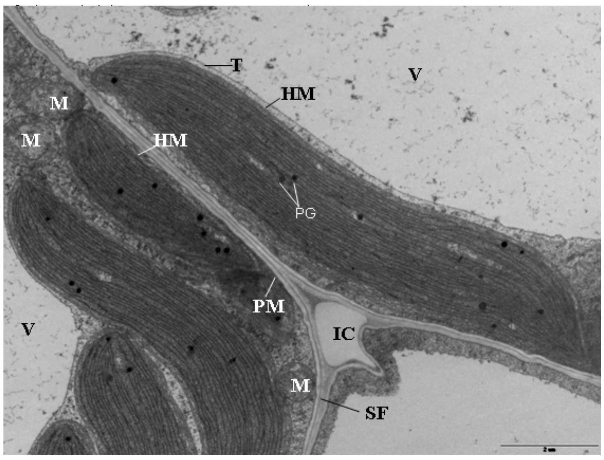
Pic. 11. Electron microscope picture of mesophyll and bundle sheath cell of 11 days old control maize



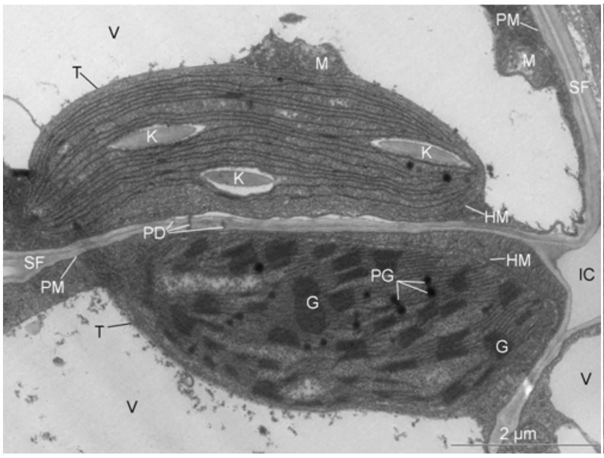
Pic. 12. Electron microscope picture of mesophyll and bundle sheath cell of 11 days old maize treated with extruded poppy-heads



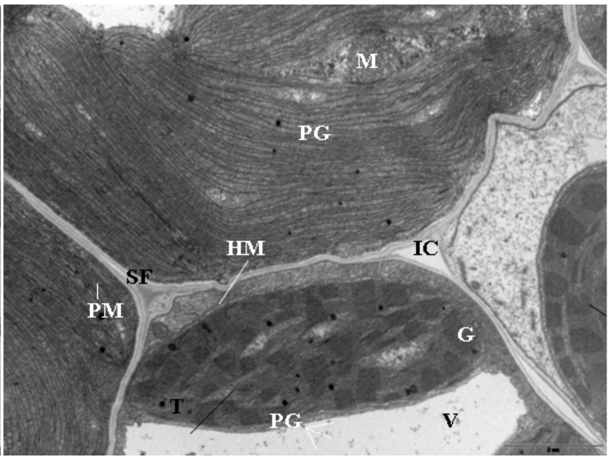
Pic. 13. Electron microscope picture of chloroplasts of mesophyll of 11 days old maize treated with compost



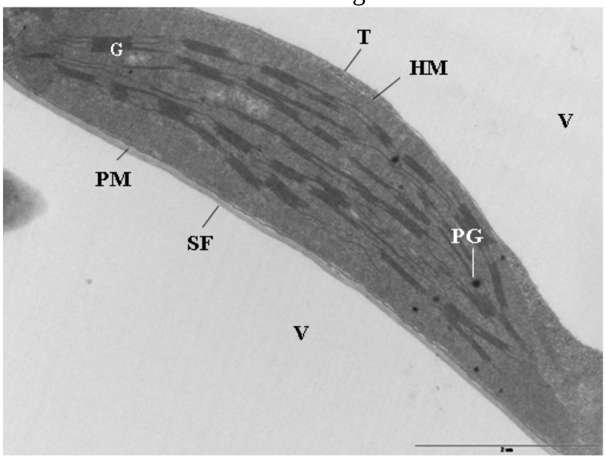
Pic. 14. Electron microscope picture of chloroplasts of bundle sheath cell of 11 days old maize treated with compost



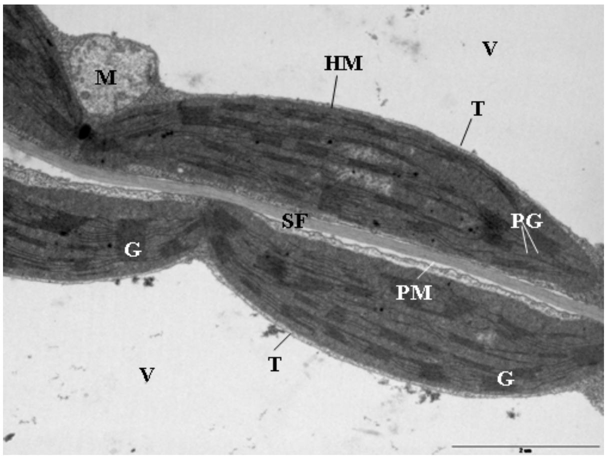
Piv. 15. Electron microscope picture of mesophyll and bundle sheath cell of 11 days old maize treated with flue gas



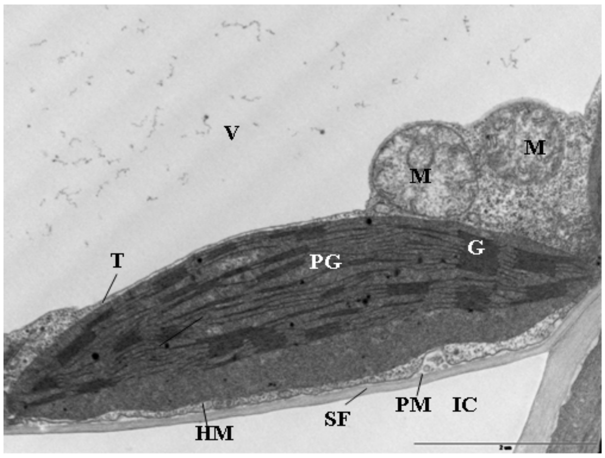
Pic. 16. Electron microscope picture of mesophyll and bundle sheath cell of 11 days old maize treated with flue gas



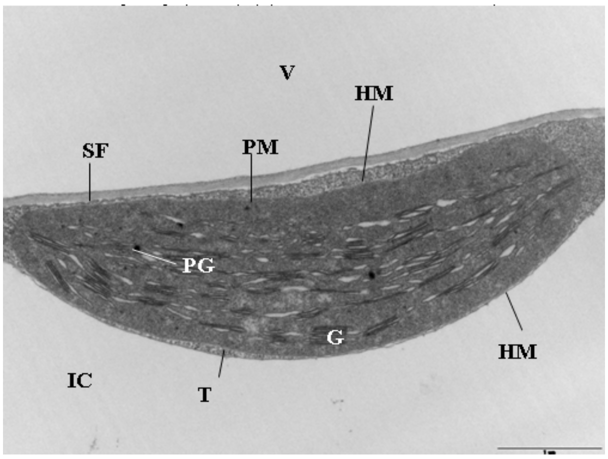
Pic. 17. Electron microscopy picture of mesophyll cell's chloroplasts of 15 days old control sunflower



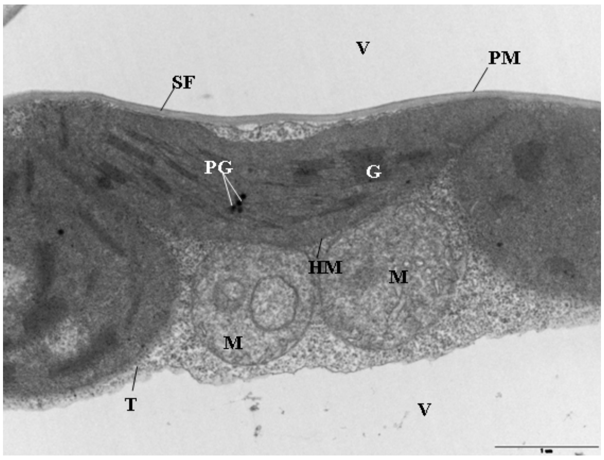
Pic. 18. Electron microscopy picture of mesophyll cell's chloroplast of 15 days old sunflower treated with extruded poppy-heads



Pic.19. Electron microscopy picture of mesophyll cell's chloroplasts of 15 days old sunflower treated with compost



Pic.20. Electron microscopy picture of mesophyll cell' chloroplasts of 15 days old sunflower treated with flue gas



Pic.21. Electron microscopy picture of mesophyll cell' chloroplasts of 15 days old sunflower treated with grinding sludge

NEW AND NEW-TYPE SCIENTIFIC RESULTS

1. The CO_2 emission is a global ecological problem nowadays. The agriculture keeps one of the greatest emitting sectors, because of the huge amounts of chemicals being used in producing processes. All solutions we can reduce are the use of fossil energy with exceptional importance. These solutions have both ecological and economic impacts on our daily lives.
The general plant physiological examinations of selected industrial side products were conducted in my work according to the above mentioned statements.

2. The dry matter accumulation of crop plant depends on two basic physiological processes, namely it is given by the differences of photosynthesis and respiration. The higher the difference for the benefit of photosynthesis is, the higher the expected yields are. Therefore, all anthropogenic or environmental effects have exceptional importance which is able to influence the intensity of photosynthesis. **My work demonstrated the fact that the examined by-products realize their effects through the changes of photosynthetic apparatus, but the connection with other metabolic processes cannot be excluded.**

3. The activity of photosynthesis depends on exogenous and endogenous factors. The processes of photosynthesis are going on in the chloroplasts. Therefore the number of chloroplasts in the mesophyll cells has exceptional importance. **My experiments show, that the treatments with flue gas, sewage-sludge compost, grinding sludge and extruded poppy heads make changes in the number of chloroplasts in the mesophyll cells.**

4. The light reactions of photosynthesis are coupled to the fine ultra-structure of chloroplasts, the light reaction that the life on the Earth is based on. This sensible membrane structure provides the ATP and reduction force (as NADPH) that are necessary to the CO_2 fixation. All changes of membrane structure affect on photochemical reactions, and in line with this on amounts of fixed CO_2. The ecological importance comes from the well known fact: the green plants fix the CO_2, therefore the greenhouse effect can be reduced, and this is a global aim. **It is proved, that the treatment with flue gas caused changes in the ultra-structure, and membrane structure of chloroplasts, enlargements were developed inside the thylakoid membranes that has influence on the effectiveness of light reactions.**

5. The iron is one of the most investigated ions nowadays. This is a crop production limiting factor on several parts of the world. The speciality of iron “problem” is, that the double charged iron easily uptakable, but it is toxic in larger amounts, while the triple charged form is not soluble, therefore the uptake needs lots of energy. The Al is considered toxic metal, although the tee needs Al in huge amount for normal growth. Both ions are multi charged cations, so they form strong connections with the negatively charged places of proteins and membranes, and modify the structures. The competition would be acceptable between the two ions. **Synergism was proved between the Fe and Al in my experiments that force us for further investigations on the field of membrane transporters regarding the two ions.**

PRACTICAL RESULTS

The plant physiological effects of industrial by-products were investigated in my experiments. This research work belongs to the basic science, therefore the direct, and immediate use of my results need further experiments on arable land, and continuous monitoring.
On the basis of my research the following results are kept for use in the practice in shorter time.

I proved the possible use of industrial side products in the nutrition of crop plants.

By the continuous control of the practical use of examined side products we can reduce the CO_2 emission, which has ecological and economic effects as well.

With the use of examined by-products we can take huge amounts of microelements into the soils where the nutrient disorder is the main yield limiting factor. The use of by-products can be very effective in Zn and Fe deficiency.

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