PhD thesis

The effect of mineral fertilization on the mineral element and protein content of winter wheat (*Triticum aestivum* L.)

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1. INTRODUCTION

The researches of mineral elements which come to the front by spread of health-conscious diet are represented two lines in quantitative aspects. Firstly, the mineral element concentration decreases in human diet. One of the reasons for this decreasing is the high yield level as a result of the intensive agricultural production. The trend which shows that the mineral element content of cereals were reduced by producing higher and higher yields, using mineral fertilizers and varieties or hybrids which make higher volume production in a shorter time was observed by several scientists. Another reason of the reduction of mineral element content in nutrition is the change caused by food processing. Despite of the fact that nowadays we can increasingly see food products in the supermarkets which were enriched mineral component to make it healthier, the food processing of different agricultural products in many cases leads to the decreasing of mineral element content of the primary goods. On the other hand, these procedures are often necessary to make the digestibility and the further use of the product easier (e.g. milling process of cereals).

Analyzing the accumulation of chemical elements in the environment represents another research line. It is important because the different contaminants can get into the human body through the food chain, therefore these are potential hazardous to health. These contaminants have several sources and most of these sources are anthropogenic. These are the chemicals which are used in agriculture, transport and every other activities generate energy by burning fossil fuels, mining, different by-products which were made by industrial production, sewage and the refuse which generated by the households, etc. The problem is not caused by the presence of the chemical elements but brought about by their accumulation in human body. Although some contaminants only damage health when taken into human body in large doses, however, some chemical elements may be toxic in very small quantities. As it was previously shown, the concentration of mineral elements has great importance in researches thus the analytical methods used as quantitative measurements also have high relevance. Nowadays, the mineral element content can be precisely measured by modern analyzing systems (e.g. inductively coupled plasma optical emission spectrometer, ICP-OES).

2. AIMS OF THE RESEARCH

Wheat is an important source of mineral elements and nutrients for human consumption and farm animals and it is produced in one of the three largest quantities of cereals. Cereals have a major role in the daily ingestion so it is very important that grains must be able to provide the human body with macroelements, microelements and other nutrients such as proteins. A number of researchers are working on that our ingested food utilize the potential opportunity of the applied agricultural raw materials (e.g. cereals) and as much as possible be able to meet this criterion. Before all this the in-depth knowledge of the tissue composition of plants and their nutrient uptake with the influencing factors are required. The population growth is followed by the intensified food needs and people demand good quality and healthy food products. In addition, the importance of the environmental protection, the ecological management and sustainable agriculture increases. It is a hot issue to understand how these factors can influence the composition of crops. Our main aims were as follows:

- During our research we were searching for the answers to those questions whether it is possible to increase the microelement content of wheat grain by proper nitrogen and NPK fertilization strategies.
- We examined the effect of different NPK fertilization doses on the P, K, S, Mg, Ca, Fe, Mn, Zn, Cu and Sr content of kernel and flour.
- The influence of different growing sites and NPK treatments on the mineral element content of winter wheat grain and flour were also investigated.
- With the investigaion of the mineral element concentration of crops, their protein content and composition were also studied, as protein is an essential nutrient for animals and human beings, and the protein concentration of wheat grain determines the baking quality of wheat flours.
- In this study we also investigated the effect of different production sites and different NPK treatments on the protein content of crops and the influence of mineral fertilizers on the amino acid composition and net protein ratio (NPR) of wheat grain.

3.MATERIALS AND METHODS

3.1. Descripion of the experiment

The Hungarian National Long-term Fertilization Trials were set up in 1966 for studying the effect of different NPK levels. The experiments marked as 18 and 19 have a split-split-plot design with 40 treatments in 4 replications. Samples of winter wheat variety Mv Magvas were collected from the years 2001 and 2002 and grains of variety Mv Csárdás were harvested in 2004 and 2005. Tested samples were derived from plots which were treated with different doses of mineral fertilizers. At first nitro-chalk, 26% N later ammonium-nitrate were applied as N fertilizer during the trials. P and K were ejected in the form superphosphate, 18% P_2O_5 and potassium chloride, 60% K_2O. Samples of peas variety Janusz were collected from the control plots (plots without fertilization) and derived from those plots which were treated with 125 kg/ha N, 180 kg/ha P and 150 kg/ha K.

Table 1. The NPK doses of the selected mineral fertilization

Treatments	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
1	0	0	0
9	150	0	100
11	150	100	100
15	200	150	100
17	250	100	100
21	250	200	200
28	150	50	100
30	150	100	0
34	200	0	100
36	150	100	200
40	200	0	0

Most of the examined samples were collected from the field station of Nagyhörcsök, located in Fejér County, in the Transdanubian region of Hungary. The experimental station is 140-150 meters above the sea level. It has arid climate. The soil is calcareous chernozem formed on loess. It has slightly alkaline pH, the humus layer is thick. The ploughed layer has loam mechanical composition and the soil has crumb structure with excellent soil moisture management. The cultivated layer has the following characteristics: pH (KCl):

7.3, $CaCO_3$: 4.27%, humus: 3.45%, S-Value: 26.8 meg/100 g, whereof Ca^{2+}: 92.6%; Mg^{2+}: 5.4%; Na^+: 0.1%; K^+: 1.9%. AL-extractable P_2O_5- and K_2O content: 60-80 and 180-200, KCl-Mg-: 150-180, Mn-, Cu- and Zn content which were digested with KCl+EDTA solution: 80-150, 2-3 and 1-2 $mg \cdot kg^{-1}$. In this study samples which were collected from Iregszemcse, Karcag, Kompolt and Putnok field stations were also investigated. These fields had different soil types and climatic conditions.

According to the reports of the Hungarian National Weather Service, the national average rainfall was 610 mm in the year 2001 which was analogous with the 30 year averages and 570 mm rainfall was measured in the year 2002. 2003 was a drought year when rainfall was 470 mm. In the year 2004 rainfall exceeded 12% in amount the 30 year averages and it was 686 mm and 748 mm rain was measured in 2005.

3.2. Methods of laboratory analysis

3.2.1. Mineral element content

The element content of samples was measured using OPTIMA 3300 type of inductively coupled plasma optical emission spectrometer (ICP-OES) manufactured by Perkin-Elmer Ltd. following digestion with HNO_3-H_2O_2 solution.

3.2.2. Protein content

The protein content was determined using Tecator 1007 digestion system and Tecator 1026 distiller according to Khjeldal’s method (MSZ 6830-4:1981)[1].

3.2.3. Net Protein Ratio

The net protein ratio of crops was investigated by animal feeding experiment during 10 days. The experimental animals were 21-day male rats of phylum Wistar. The animals got standard rat food with 16% protein content during 3 days. They were divided into

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[1] Hungarian Patent, MSZ 6830-4:1981 Takarmányok táplálóértékének megállapítása. Nitrogéntartalom meghatározása makro-Kjeldahl-módszerrel a nyersfehérje-tartalom számításához

groups of five animals based on body-weight on the 0th day of the experiment. The average of the groups did not differ from each other. The animals were kept in group metabolic cages and food and water intake was ad libitum. The rat food quantity was measured in each group from the feeder to investigate the consumed N-amount and body weight was individually measured every day. The protein content of diet was 6% during the 1st feeding experiment. There were two control groups. A protein-free control group (PFCG, 4%) which was on diet with 4% protein content and a positive control group (EGGPRO, 6%). 100% of the protein which was consumed in these groups was came from egg. Food was completed with mineral elements and vitamins only in the control groups.

During the 2nd feeding experiment, rat food had 10% protein content. In the test groups 50% of the protein intake has been replaced from cereal protein to white of egg. (The protein content of diet which contained peas was also 10%.) The NPR value was calculated according to the study of Pellet and Young (1980)[2].

3.2.4. Amino acid composition

The amino acid composition of the wheat samples was analyzed by ion exchange chromatography method following the annex X. of the regulation 44/2003. FVM (IV.26)[3].

3.3. Methods of statistical analysis

All data were examined by descriptive statistical analysis. The significant effect of NPK treatments on the mineral element and protein content of wheat grain and flour were analyzed using independent samples T-test. The significant effect of the years and production sites on the element and protein content were determined by ANOVA method, but after detection of significant differences ($p<0.05$) data were subjected to Duncan's test to allow separation of means. Data analysis was performed using SPSS for Windows 13.0 Software package.

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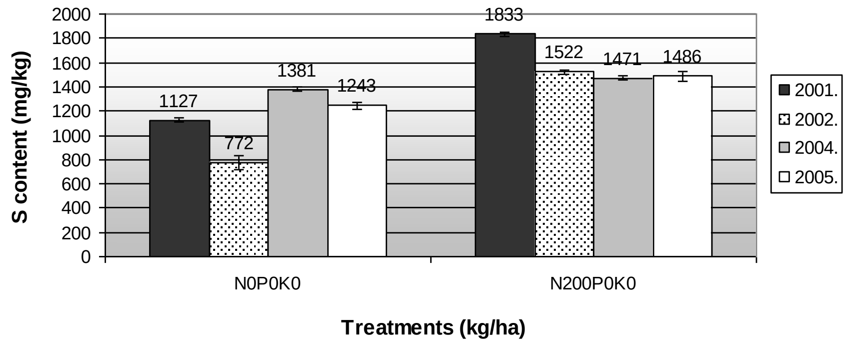
[2] Pellet, P.L., Young, V.R. (1980): Nutritional evaluation of protein foods: Methodology for net protein utilization. Tokyo: The United Nations University. 103. p.

[3] 44/2003. FVM rendelet a Magyar Takarmánykódex kötelező előírásairól. 10. sz. melléklet

4.RESULTS AND DISCUSSION

4.1. The effect of different NPK treatments on the mineral element and protein content of wheat grains

The aim of this study was to examine whether the application of mineral fertilizers can increase the macro-, microelement and protein content of crops. We investigated the effect of N treatments on the K, P, S, Mg, Ca, Fe, Mn, Zn, Cu, Sr and protein concentration of the whole wheat grain. The influence of treatment 1 ($N_0P_0K_0$) and treatment 40 ($N_{200}P_0K_0$) on the mineral element content was shown on the following diagrams. Grain samples were collected in Nagyhörcsök in the years 2001, 2002, 2004 and 2005.

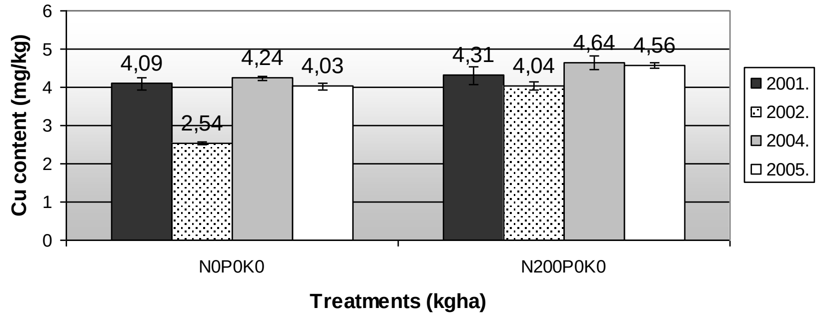


Year	2001	2002	2004	2005
p-value N_0-N_{200}	0.000	0.000	0.012	0.001

Figure 1. The effect of N treatments on the S content of winter wheat grains

The N fertilization had no influence on the potassium and phosphorus content of samples but N doses caused statistically significant positive changes in the S concentration of grains ($p<0.05$) in the years 2004 and 2001, 2002 and 2005 ($p<0.01$) (Figure 1.).

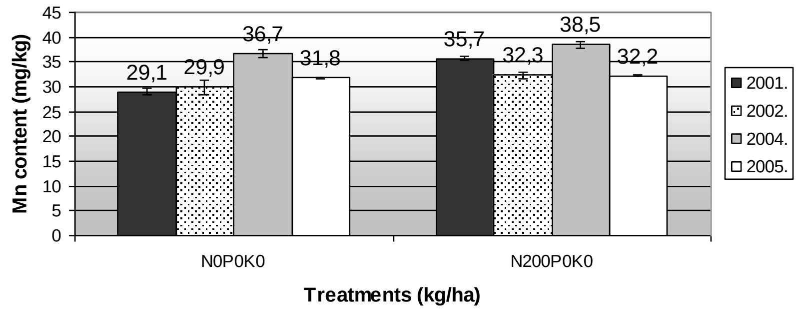
The N treatments had the same effect on the Ca concentration of wheat ($p<0.05$) in each year which could be the influence of the chalk content of the nitro-chalk fertilizer applied in the early period of the experiment. The Mg content also increased in the years 2001 and 2002 ($p<0.05$). The Fe content raised in parallel increasing N doses in the year 2001 ($p<0.05$) but the effect of N fertilization on the Fe concentration of grains was not proven statistically in 2002, 2004 and 2005.



Year	2001	2002	2004	2005
p-value N_0-N_{200}	0.451	0.000	0.070	0.005

Figure 2. The effect of N treatments on the Cu content of winter wheat grains

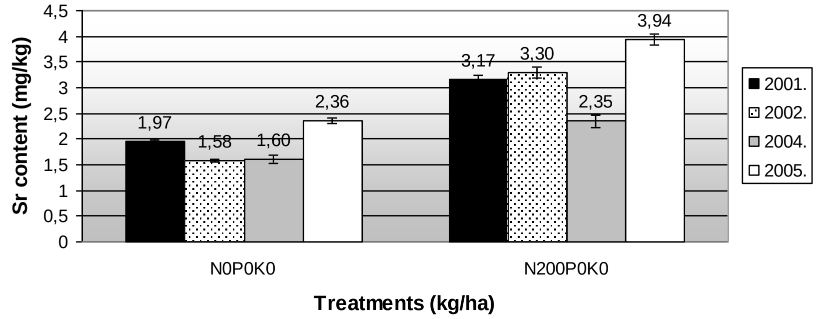
The N treatments had positive significant influence on the copper concentration in every year (*p<0.05*), and the Mn uptake of the grains was also stimulated by N fertilizer (Figure 2. and 3.) which was a strong positive correlation in the years 2001 (*p<0.01*) and 2002 (*p<0.01*).



Year	2001	2002	2004	2005
p-value N_0-N_{200}	0.000	0.000	0.128	0.129

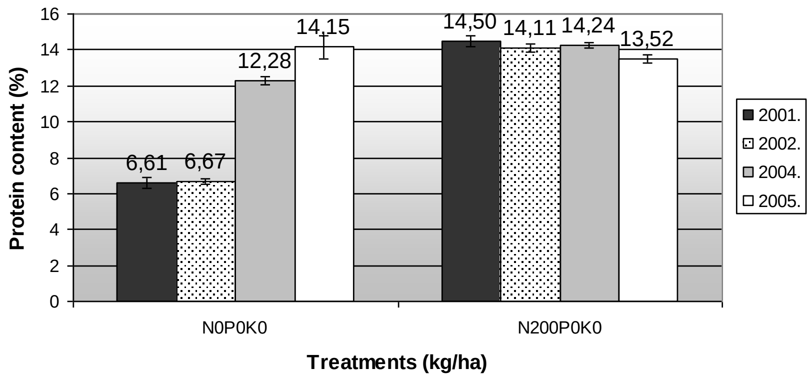
Figure 3. The effect of N treatments on the Mn content of winter wheat grains

Zn concentration of wheat decreased in parallel increasing N levels in the years 2001, 2004 and 2005 (*p<0.05*). The examination of the Sr content of samples shown, that N treatments contributed the Sr accumulation of the wheat (*p<0.01*) in every year (Figure 4.).



Year	2001	2002	2004	2005
p-value N_0-N_{200}	0.000	0.000	0.002	0.000

Figure 4. The effect of N treatments on the Sr content of winter wheat grains



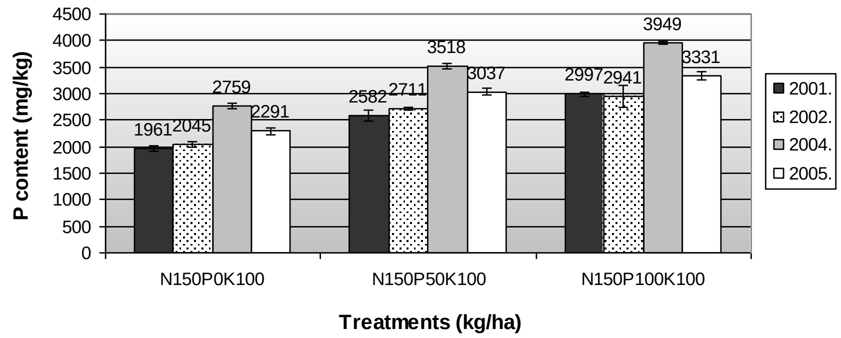
Year	2001	2002	2004	2005
p-value N_0-N_{200}	0.000	0.000	0.001	0.396

Figure 5. The effect of N treatments on the protein content of winter wheat grains

The N fertilizer enhanced the protein content of the whole grain only exception was in the year 2005. It was a statistically significant positive effect ($p<0.01$) (Figure 5.). After that we examined the influence of the N fertilization on mineral element concentration of samples we investigated the influence of P treatments.

The application of superphosphate had positive statistically significant effect on the potassium quantity of seeds in every year ($p<0.01$).

P concentration of plant tissues were higher in those samples which were taken in higher P treatments ($p<0.01$) (Figure 6.).

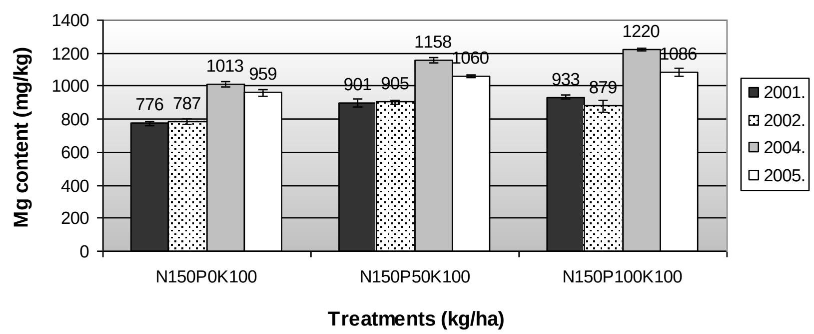


Year	p-value P_0-P_{50}	p-value P_{50}-P_{100}	p-value P_0-P_{100}
2001	0.002	0.009	0.000
2002	0.000	0.311	0.005
2004	0.000	0.001	0.000
2005	0.000	0.018	0.000

Figure 6. The effect of P treatments on the P content of winter wheat grains

Despite of the fact that previously several researchers described the positive correlation between superphosphate and S accumulation there was no connection between P doses and S content of grains.

The P treatments had no influence on the Ca concentration of samples but there were positive correlation between P fertilization and the Mg intake in every year which was proved by statistical analysis (*$p<0.01$*).

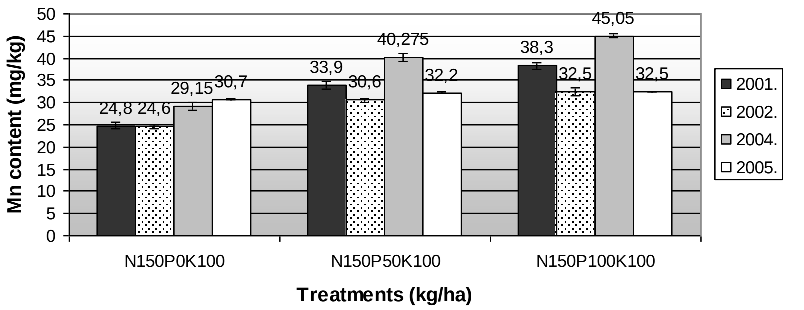


Year	p-value P_0-P_{50}	p-value P_{50}-P_{100}	p-value P_0-P_{100}
2001	0.004	0.280	0.000
2002	0.001	0.489	0.050
2004	0.000	0.010	0.000
2005	0.002	0.346	0.011

Figure 7. The effect of P treatments on the Mg content of winter wheat grains

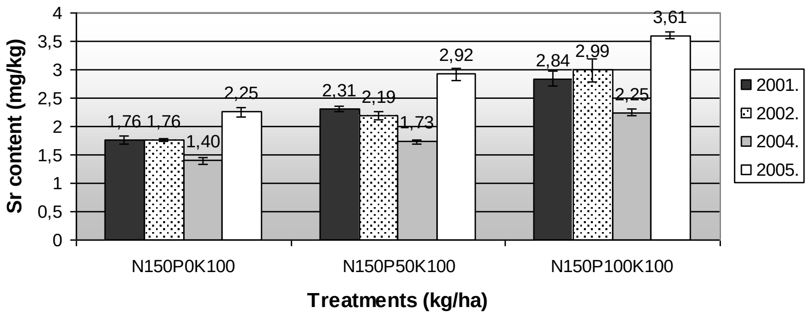
Only exception was in the year 2002 when the increasing of P doses was not followed by the Mg accumulation in wheat grains (Figure 7.).

The effect of superphosphate application on the iron concentration of samples was disagreeing in the different years. P treatments enhanced the Fe quantity in grains in the years 2004 (*p<0.01*) but there was no correlation between P doses and Fe content in the years 2001, 2002 and 2005.



Year	p-value P_0-P_{50}	p-value P_{50}-P_{100}	p-value P_0-P_{100}
2001	0.000	0.010	0.000
2002	0.000	0.084	0.000
2004	0.000	0.004	0.000
2005	0.002	0.158	0.000

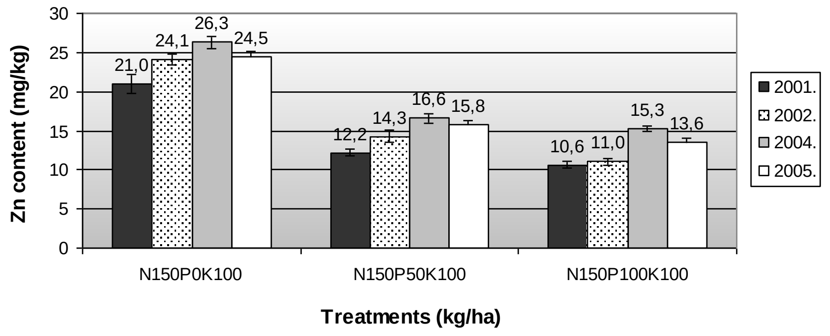
Figure 8. The effect of P treatments on the Mn content of winter wheat grains



Year	p-value P_0-P_{50}	p-value P_{50}-P_{100}	p-value P_0-P_{100}
2001	0.001	0.007	0.000
2002	0.002	0.010	0.001
2004	0.003	0.000	0.000
2005	0.007	0.002	0.000

Figure 9. The effect of P treatments on the Sr content of winter wheat grains

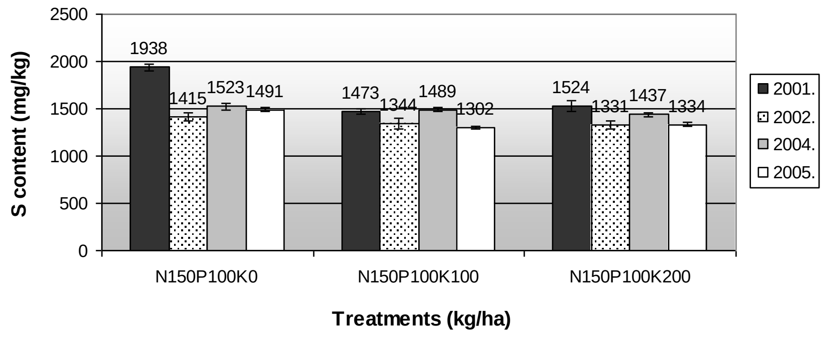
P fertilization had positive significant effect on the manganese content of seeds in every year ($p<0.01$) (Figure 8.) and Sr was also accumulated in samples following superphosphate doses ($p<0.01$) (Figure 9.).



Year	p-value P_0-P_{50}	p-value P_{50}-P_{100}	p-value P_0-P_{100}
2001	0.000	0.040	0.000
2002	0.000	0.013	0.000
2004	0.000	0.098	0.000
2005	0.000	0.024	0.000

Figure 10. The effect of P treatments on the Zn content of winter wheat grains

The P fertilization however reduced the Zn content in every year ($p<0.01$) (Figure 10.), like Cu concentration in 2001, 2002 and 2005 ($p<0,05$).



Year	p-value K_0-K_{100}	p-value K_{100}-K_{200}	p-value K_0-K_{200}
2001	0.000	0.430	0.001
2002	0.386	0.863	0.197
2004	0.481	0.184	0.120
2005	0.000	0.294	0.004

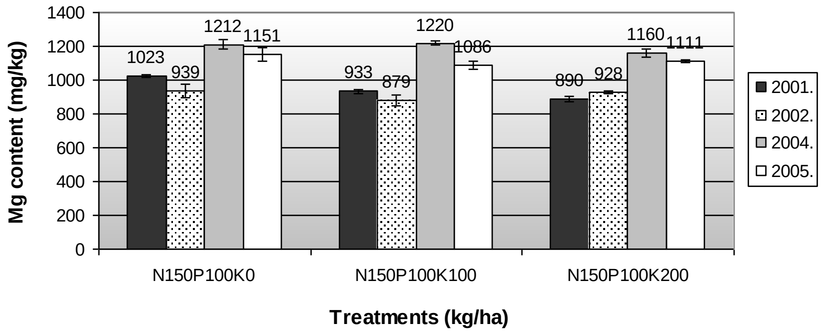
Figure 11. The effect of K treatments on the S content of winter wheat grains

However there was no negative correlation between the superphosphate treatments and Cu accumulation in 2004 (Figure 11.).

The protein content of samples shown downward trend in 2001 and 2002 but somewhat enhanced in 2004 and there was no correlation between protein concentration and superphosphate doses in the year 2005.

During our research the influence of K treatment on the mineral element content of the whole grain was also investigated. K fertilization had no influence on phosphorus, iron, zinc, copper and manganese accumulation of wheat and it had no significant effect on the K concentration but the amount of potassium was higher in those samples which were treated with high K doses.

The S content (in Figure 11.) shown negative correlation to K treatments in the years 2001 and 2005 ($p<0.01$) but it wasn't a statistical effect in the years 2002 and 2004.

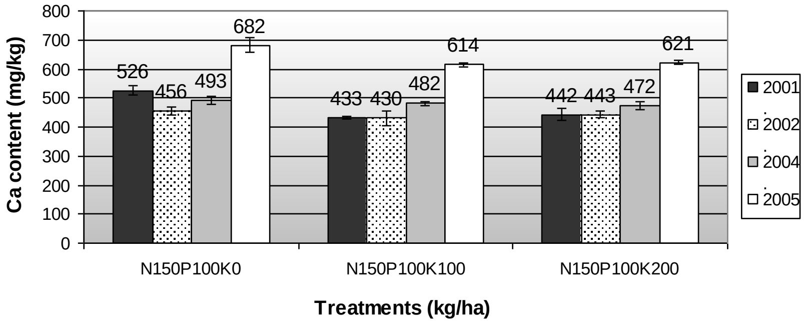


Year	p-value K_0-K_{100}	p-value K_{100}-K_{200}	p-value K_0-K_{200}
2001	0.001	0.079	0.000
2002	0.291	0.203	0.796
2004	0.807	0.045	0.038
2005	0.237	0.368	0.397

Figure 12. The effect of K treatments on the Mg content of winter wheat grains

The K fertilization significantly reduced the Mg accumulation through the cation antagonism in the years 2001 and 2004 ($p<0.05$) (Figure 12.) and the Ca concentration of samples shown negative trend in 2001 and 2005 ($p<0.05$) (Figure 13.).

The K fertilization hindered the accumulation of strontium in seeds. The negative effect of K treatments was proved by ANOVA in the year 2001, 2004 and 2005 ($p<0.05$).



Year	p-value K_0-K_{100}	p-value K_{100}-K_{200}	p-value K_0-K_{200}
2001	0.002	0.665	0.018
2002	0.422	0.666	0.528
2004	0.516	0.531	0.329
2005	0.032	0.457	0.048

13. The effect of K treatments on the Ca content of winter wheat grains

Finally we investigated the correlation between K fertilization and protein content of grains and we found that the K treatments had no significant effect on protein production of wheat.

4.2. The effect of different NPK treatments on the mineral element and protein content of flour

In this study, the effect of NPK fertilization on the mineral element and protein content of wheat flour was also investigated. During the statistical analysis it was proved that N treatments caused significant difference in the amount of Ca, Cu and Sr ($P<0.05$) and there were strong significant correlation between N fertilizer and S, Mn and protein content of the flours. N treatments had no significant influence on the K, P, Mg and Fe concentration but reduced the Zn content of the flour. The superphosphat had strong positive significant effect ($P<0.01$) on the P, Mn, and Sr content but had strong negative significant influence ($P<0.01$) on the amount of Zn and Cu.

There were no correlation between P treatments and the K, S, Mg, Ca, Fe and protein concentration.

The K fertilization had no significant effect on the K, P, Mg, Fe, Mn, Zn, Cu and protein content of wheat flour but reduced the accumulation of S, Ca and Sr ($P<0.05$) content of samples through the cation antagonism (Table 2., 3., 4.).

Table 2. The effect of N treatments on the mineral element and protein content of wheat flour

Treatment(kg/ha)	K(mg/kg)	P(mg/kg)	S(mg/kg)	Mg(mg/kg)	Ca(mg/kg)	Fe(mg/kg)	Mn(mg/kg)	Zn(mg/kg)	Cu(mg/kg)	Sr(mg/kg)	Protein(%)
$N_0P_0K_0$	1160±18	1162±8	904±19	216±4	189±4	9.55±0.86	7.08±0.15	5.69±0.19	1.80±0.03	0.545±0.014	8.50±0.08
$N_{200}P_0K_0$	1211±23	1152±27	1180±44	211±9	243±12	9.02±0.36	6.99±0.65	3.99±0.06	1.91±0.08	0.845±0.071	11.98±0.28
$N_{150}P_{100}K_{100}$	1350±43	1188±18	1151±36	221±6	240±15	10.1±0.4	7.76±0.09	3,56±0.15	1.59±0.01	1.020±0.061	9.68±0.36
$N_{250}P_{100}K_{100}$	1352±4	1208±33	1362±77	231±10	295±11	13.6±3.1	9.24±0.18	3,83±0.13	1.79±0.04	0.936±0.050	12.42±0.12
p-value N_0-N_{200}	0.129	0.078	0.001	0.626	0.007	0.588	0.896	0.000	0.266	0.006	0.000
p-value N_{150}-N_{250}	0.964	0.633	0.068	0.412	0.046	0.321	0.002	0.237	0.011	0.347	0.002

Table 3. The effect of P treatments on the mineral element and protein content of wheat flour

Treatment(kg/ha)	K(mg/kg)	P(mg/kg)	S(mg/kg)	Mg(mg/kg)	Ca(mg/kg)	Fe(mg/kg)	Mn(mg/kg)	Zn(mg/kg)	Cu(mg/kg)	Sr(mg/kg)	Protein(%)
$N_{150}P_0K_{100}$	1345±14	863±12	1114±38	218±7	239±10	8.79±1.13	4.64±0.20	6.28±0.50	1.99±0.06	0.467±0.028	10.97±0.42
$N_{150}P_{50}K_{100}$	1379±12	1130±20	1122±37	227±5	240±15	10.0±0.1	7.76±0.098	4.46±0.10	1.95±0.05	0.793±0.033	10.29±0.19
$N_{150}P_{100}K_{100}$	1350±43	1188±18	1151±36	221±6	240±15	10.1±0.4	8.09±0.53	3.56±0.15	1.59±0.01	1.02±0.06	9.68±0.36
p-value P_0-P_{50}	0.119	0.000	0.887	0.318	0.935	0.322	0.001	0.012	0.653	0.000	0.187
p-value P_{50}-P_{100}	0.743	0.096	0.523	0.446	0.989	0.743	0.627	0.017	0.002	0.003	0.169
p-value P_0-P_{100}	0.894	0.000	0.607	0.774	0.921	0.376	0.000	0.006	0.002	0.006	0.076

Table 4. The effect of K treatments on the mineral element and protein content of wheat flour

Treatment(kg/ha)	K(mg/kg)	P(mg/kg)	S(mg/kg)	Mg(mg/kg)	Ca(mg/kg)	Fe(mg/kg)	Mn(mg/kg)	Zn(mg/kg)	Cu(mg/kg)	Sr(mg/kg)	Protein(%)
$N_{150}P_{100}K_0$	1354±22	1125±44	1176±22	221±11	246±4	9.59±0.35	7.72±0.34	3.35±0.12	1.88±0.04	1.07±0.09	10.57±0.06
$N_{150}P_{100}K_{100}$	1350±43	1188±18	1151±36	221±6	240±15	10.1±0.4	7.76±0.09	3.56±0.15	1.74±0.07	1.02±0.06	9.68±0.36
$N_{150}P_{100}K_{200}$	1258±12	1108±38	1079±21	209±6	220±7	10.3±0.4	8.23±0.50	3.57±0.26	1.59±0.01	0.796±0.014	9.69±0.03
p-value K_0-K_{100}	0.945	0.257	0.588	0.956	0.743	0.347	0.909	0.343	0.053	0.643	0.070
p-value K_{100}-K_{200}	0.062	0.154	0.126	0.254	0.239	0.745	0.463	0.090	0.129	0.009	0.982
p-value K_0-K_{200}	0.080	0.778	0.027	0.353	0.039	0.275	0.466	0.155	0.183	0.015	0.080

4.3. The effect of different production sites on the mineral element and protein content of wheat grain

During our investigation it was proved that different growing places of the Hungarian National Long-term Fertilization Trials such as Iregszemcse, Karcag, Kompolt, Nagyhörcsök and Putnok caused significant ($p<0.01$) difference in the macro- and microelement ($p<0.01$) and protein content ($p<0.05$) of winter wheat samples (Table 5. and 6.). Wheat samples were collected in the year 2004 when the weather conditions were humid and wet.

Table 5. Macroelement content of wheat grain samples from different production sites

Control samples			Treated samples		
Production site	Mean	$LSD_{5\%}$	Production site	Mean	$LSD_{5\%}$
K content (mg/kg)					
Karcag	3170	a	Karcag	3176	A
Iregszemcse	3300	a, b	Iregszemcse	3411	B
Kompolt	3544	b	Kompolt	3549	C
Nagyhörcsök	3608	b	Nagyhörcsök	3654	D
Putnok	4137	c	Putnok	4147	E
P content (mg/kg)					
Karcag	2887	a	Karcag	3166	A
Kompolt	3043	b	Kompolt	3492	B
Nagyhörcsök	3275	b	Iregszemcse	3591	B, C
Iregszemcse	3587	c	Nagyhörcsök	3643	C
Putnok	4187	d	Putnok	4314	D
S content (mg/kg)					
Kompolt	1318	a	Kompolt	1482	A
Nagyhörcsök	1381	a	Nagyhörcsök	1497	A
Karcag	1469	b	Karcag	1725	B
Iregszemcse	1787	c	Iregszemcse	1946	C
Putnok	1832	c	Putnok	2033	D
Mg content (mg/kg)					
Karcag	959	a	Karcag	926	A
Kompolt	1110	b, c	Kompolt	1066	B
Iregszemcse	1164	c	Nagyhörcsök	1164	C
Nagyhörcsök	1189	c	Iregszemcse	1165	C
Putnok	1422	d	Putnok	1299	D
Ca content (mg/kg)					
Karcag	308	a	Karcag	298	A
Kompolt	372	b	Kompolt	404	B
Nagyhörcsök	482	c	Nagyhörcsök	491	C
Putnok	529	c	Putnok	596	D
Iregszemcse	571	c	Iregszemcse	628	E

The left side of the tables show "control samples" and you can see "treated samples" on the right side. "Control samples" were derived from the untreated control plots and "treated samples" were collected every other plots which were treated different doses of N, P and K fertilizer. Means (mg/kg) express the means of four replications of the plots where grains were harvested.

Table 6. Microelement content of wheat grain samples from different production sites

Control samples			Treated samples		
Production site	Mean	$LSD_{5\%}$	Production site	Mean	$LSD_{5\%}$
Fe content (mg/kg)					
Karcag	33.0	a	Nagyhörcsök	41.2	A
Nagyhörcsök	40.2	a	Karcag	41.7	A
Iregszemcse	41.7	a, b	Kompolt	43.6	A
Kompolt	43.2	a, b	Iregszemcse	45.3	A
Putnok	48.4	c	Putnok	51.8	B
Mn content (mg/kg)					
Iregszemcse	12.9	a	Iregszemcse	13.4	A
Karcag	32.1	b	Karcag	32.6	B
Nagyhörcsök	36.8	c	Nagyhörcsök	41.0	C
Putnok	46.7	d	Kompolt	52.2	D
Kompolt	48.5	d	Putnok	58.2	E
Zn content (mg/kg)					
Karcag	21.3	a	Karcag	13.6	A
Nagyhörcsök	22.6	a, b	Iregszemcse	15.2	B
Iregszemcse	26.7	b,c	Nagyhörcsök	16.4	B
Kompolt	33.9	c	Kompolt	32.1	C
Putnok	53.8	d	Putnok	44.4	D
Cu content (mg/kg)					
Karcag	3.96	a	Karcag	3.40	A
Nagyhörcsök	4.24	a,b	Kompolt	4.21	B
Kompolt	4.52	b, c	Putnok	4.63	C
Iregszemcse	4.92	c	Nagyhörcsök	4.74	C
Putnok	4.94	c	Iregszemcse	5.30	D
Sr content (mg/kg)					
Kompolt	1.52	a	Kompolt	1.94	A
Nagyhörcsök	1.60	a	Karcag	2.05	A, B
Karcag	1.60	a	Nagyhörcsök	2.22	B, C
Iregszemcse	1.99	a, b	Iregszemcse	2.80	D
Putnok	2.49	b	Putnok	2.87	D

The K, S, Ca and Sr content sequence of samples which were collected from the control plots was the same as in the case of treated samples but the P, Mg, Mn, Fe, Cu and protein content sequence of wheat grains was different from the sequence of treated samples.

4.4. The effect of different production sites on the mineral element and protein content of wheat grain and flour

In this study we examined the influence of different growing sites on the mineral element and protein content of wheat grains and compared our results to those results which came from analysis of flour samples (Table 7 and 8). “Grain” samples were collected from the untreated control plots and all samples were derived in the year 2005.

Table 7. Macroelement content of wheat grain and flour samples from different production sites

Grain			Flour		
Production site	Mean	$LSD_{5\%}$	Production site	Mean	$LSD_{5\%}$
K content (mg/kg)					
Kompolt	2685	a	Putnok	1139	A
Nagyhörcsök	3016	a, b	Nagyhörcsök	1160	A
Putnok	3192	a, b	Karcag	1226	A
Karcag	3271	b	Kompolt	1342	B
Iregszemcse	3495	b	Iregszemcse	1360	B
P content (mg/kg)					
Nagyhörcsök	3714	a	Karcag	1141	A
Kompolt	3814	a	Nagyhörcsök	1162	A
Karcag	3934	a	Kompolt	1178	A
Iregszemcse	3978	a	Iregszemcse	1213	A
Putnok	4446	b	Putnok	1380	B
S content (mg/kg)					
Kompolt	1109	a	Karcag	885	A
Nagyhörcsök	1243	a, b	Nagyhörcsök	904	A
Putnok	1274	b	Kompolt	989	A
Iregszemcse	1477	b	Putnok	1008	A
Karcag	1719	c	Iregszemcse	1052	A
Mg content (mg/kg)					
Kompolt	1056	a	Karcag	210	A
Karcag	1162	a, b	Nagyhörcsök	216	A, B
Nagyhörcsök	1175	b	Kompolt	238	A, B, C
Iregszemcse	1207	b	Iregszemcse	252	B, C
Putnok	1234	b	Putnok	271	C
Ca content (mg/kg)					
Kompolt	420	a	Karcag	161	A
Putnok	547	b	Putnok	175	A, B
Karcag	562	b	Kompolt	185	A, B
Nagyhörcsök	595	b	Nagyhörcsök	189	A, B
Iregszemcse	615	b	Iregszemcse	207	B

During the statistical analysis, it was proved that there was significant (*P<0.01*) difference in the K, P, Mg, Ca, Mn, Zn, Cu and protein content (*P<0.05*) of samples, but there was no significant effect on the S, Fe and Sr concentration in flour.

Table 8. Microelement content of wheat grain and flour samples from different production sites

Grain			Flour		
Production site	Mean	$LSD_{5\%}$	Production site	Mean	$LSD_{5\%}$
Fe content (mg/kg)					
Kompolt	35.6	a	Karcag	7.75	A
Nagyhörcsök	40.2	a, b	Putnok	7.94	A
Karcag	45.1	a, b	Iregszemcse	8.29	A
Putnok	47.6	b	Kompolt	9.34	A
Iregszemcse	47.7	b	Nagyhörcsök	9.55	A
Mn content (mg/kg)					
Nagyhörcsök	31.8	a	Karcag	6.37	A
Putnok	32.7	a	Nagyhörcsök	7.08	A, B
Iregszemcse	33.1	a	Putnok	7.19	A, B
Karcag	37.1	a	Iregszemcse	7.58	A, B
Kompolt	46.4	b	Kompolt	9.02	B
Zn content (mg/kg)					
Iregszemcse	17.5	a	Karcag	5.41	A
Nagyhörcsök	24.3	b	Iregszemcse	5.59	A
Kompolt	28.4	c	Nagyhörcsök	5.69	A
Karcag	33.6	d	Kompolt	7.42	B
Putnok	36.3	d	Putnok	8.09	B
Cu content (mg/kg)					
Nagyhörcsök	4.03	a	Karcag	1.56	A
Putnok	4.33	a, b	Putnok	1.69	A, B
Karcag	4.68	b, c	Nagyhörcsök	1.80	A, B, C
Kompolt	4.69	b, c	Kompolt	2.00	B, C
Iregszemcse	5.01	c	Iregszemcse	2.05	C
Sr content (mg/kg)					
Kompolt	2.13	a	Putnok	0.376	A
Nagyhörcsök	2.36	a, b	Iregszemcse	0.382	A
Putnok	2.45	a, b	Kompolt	0.499	A
Iregszemcse	2.61	a, b	Karcag	0.512	A
Karcag	3.08	b	Nagyhörcsök	0.545	A

4.5. Examination of Net Protein Ratio

Those wheat samples which were examined during the 1st and 2nd animal feeding experiments were derived from the control plots (Wheat/2001/1, Wheat/2002/1) and those plots which were treated with 250 kg/ha N, 200 kg/ha P and 200 kg/ha K (Wheat/2001/21, Wheat/2002/21) in the year 2001 (Wheat/2001/1, Wheat/2001/21) and 2002 (Wheat/2002/1, Wheat/2002/21). Pea samples were collected from the control plots (Pea/1) and those areas which were received 125 kg/ha N, 180 kg/ha P and 150 kg/ha K fertilization (Pea/20) in 2003. The protein content of diet was 6% during the 1st feeding experiment. During the 2nd feeding experiment, rat food had 10% protein content and 50% of the protein was replaced by egg in those rat foods which were consumed in the test groups.

Table 9. Net Protein Ratio of tested samples in the 1st animal feeding trials

Groups	Protein consumption (g/rat/10 days)	Changes in body weight (g/rat/10 days)	Net Protein Ratio (NPR) (g/g)
EGGPRO	8.0	32.4±6.9	1.74
Wheat/2001/1	8.6	15.2±2.4	-0.37
Wheat/2002/21	6.4	7.6±2.9	-1.70
Wheat/2002/1	7.5	13.8±4.5	-0.61
Wheat /2002/21	6.9	7.8±1.5	-1.54

Table 10. Net Protein Ratio of tested samples in the 2nd animal feeding trials

Groups	Protein consumption (g/rat/10 days)	Changes in body weight (g/rat/10 days)	Net Protein Ratio (NPR) (g/g)
Pea/1	7.5	10.0	-1.09
Pea/20	7.2	8.6	-1.34
Wheat/2001/1	13.3	37.2	1.42
Wheat/ 2002/21	10.9	28.0	0.90
Wheat/2002/1	11.6	34.2	1.38
Wheat /2002/21	11.3	29.0	0.96

Despite of the fact that samples marked Wheat/2001/21, Wheat/2002/21 and Pea/20 had higher protein content like samples marked Wheat/2001/1, Wheat/2002/1 and Pea/1, those animals which were feed with rat food marked Wheat/2001/1, Wheat/2002/1 and Pea/1 had better feed intake, growth, weight gain and protein utilization (Table 9 and 10). These results were probably influenced by the unbalanced mineralelement and vitamin supply of animals.

5. NEW SCIENTIFIC RESULTS

1. The ammonium nitrate fertilizer stimulated the S, Cu, Mn and Sr uptake and enhanced the protein production of grain samples. N treatments significantly increased the S, Mn, Cu, Sr and protein content of flours. Samples were collected from Nagyhörcsök field station in the years 2001, 2002, 2004 and 2005.

2. Superphosphate fertilization enhanced the K, P, Mg, Mn and Sr concentration of grains but reduced the accumulation of Zn and Cu because of the antagonism between Cu, Zn and P. In the flour samples, superphosphate had positive significant effect only on the P, Mn, and Sr content but it had negative significant influence on the amount of Zn and Cu.

3. The potassium chloride fertilization reduced the accumulation of S, Ca and Sr in grains and flours.

4. The different experimental fields stations (Iregszemcse, Karcag, Kompolt, Nagyhörcsök and Putnok) of the Hungarian National Long-term Fertilization Trials have different soil types and climatic conditions which caused significant difference in the K, P, S, Mg, Ca, Fe, Mn, Zn, Cu, Sr ($P<0,01$) and protein content ($P<0,05$) of winter wheat samples in the year 2004. The NPK treatments had no influence on these differences.

5. Field stations with different soil types and climatic conditions such as Iregszemcse, Karcag, Kompolt, Nagyhörcsök and Putnok caused significant difference in the K, P, Mg, Ca, Mn, Zn, Cu and protein content ($P<0,05$) of flour samples, but there wasn't a significant effect on the S, Fe and Sr concentration of flours in the year 2005.

6. The content of every mineral element were higher in the whole grain than in the flour. The particle size of flour was 250 μm. The whole grain includes one and a half times as much S (141%) and protein (144%), more than twice as much K (252%) and Cu (247%), three times as much P (299%) and Ca (299%), more than four times as much Zn (435%) five times as much Mg (492%), Fe (504%) and Mn (485%) and more than five times as much Sr (546%) as the flour.

6. PRACTICAL USE OF RESULTS

1. The N treatments increased the S, Ca, Cu, Mn and Sr accumulation in flour and grain and the superphosphat application raised the P, Mn and Sr concentration of flour and helped K, P, Mg, Mn and Sr accumulation in grain so good fertilization strategies support the enhancement of mineral element content of crops which can be also measured in the processed food like flour.

2. The N treatments significantly enhanced the protein content of whole grain and flour, therefore the application of fertilizers could have positive influence on protein quantity.

3. Fertilization can also cause an opposite effect. In this study P and K treatments reduced the accumulation of some important microelements like Zn and Cu, so the determination of the accurate dose of mineral fertilizers has high importance during crop production.

4. Different growing sites caused significant difference in the mineral element content of wheat so geographical location may have great influence on the macro- and microelement content of our food. Responsible nutrition can be advanced if the location-specific mineral content of the produced food is known.

5. In whole grain, the content of every mineral element was the multiple of the flour, so those food products which containing bran or less refined flour could help to prevent the mineral element deficiency.

6. The results of animal feeding experiments shown that higher protein content of crops was not equal to better protein quality so the analysis of amino acid composition is very important. Sufficient microelement and vitamin supplement has a great role during animal feeding.

7. PUBLICATIONS IN THE TOPIC OF THE DISSERTATION

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2. Burján Zita, Győri Zoltán (2012): A különböző műtrágyakezelések hatása az őszi búza fehérje- és kéntartalmára. Agrártudományi közlemények. 48. 73-76.
3. Burján Zita, Győri Zoltán (2012): A gabonafélék molibdén felhalmozásának dinamikája karbonátos csernozjom talajon. Agrártudományi közlemények. 50. 81-86.
4. Puskás-Preszner Anita, Kovács Béla, Andrási Dávid, Burján Zita Kata (2012): Tartamkísérletben alkalmazott molibdén-kezelés hatása a növényi elemfelvételre. Agrártudományi Közlemények. 50. 121-126.
5. Zita Burján, Mariann Móré, Béla Kovács and Zoltán Győri (2012):The effect of different growing area ont he Cu, Mn and Zn content of winter wheat. Eur. Chem. Bull. 2012, 1(9), 375-377.
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7. Xénia Vágó, Kinga Nagy, Zita Burján, Dávid Andrási, Béla Kovács (2012): Oil seeds as natural resources of selenium. Eur. Chem. Bull. 2012, 1(12), 495-497.
8. Burján Zita, Móré Mariann, Czakóné Vágó Xénia, Győri Zoltán (2013): NPK kezelések hatása az őszi búza (*Triticum aestivum* L.) Cu- és Fe-tartalmára. Agrártudományi Közlemények. 52. 31-35.
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   Zita Burján, Zoltán Győri (2013): The effect of different production area and NPK fertilizer on Ca and Mg content of winter wheat (*Triticum aestivum* L.) grains. In.: Neményi, M., Varga, L., Facskó, F., Lőrincz, I. (szerk.): Science for Sustainability.

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4. Xénia Vágó, Kinga Nagy, Zita Burján, Dávid Andrási, Béla Kovács (2012): Oil seeds as natural resources of selenium. In: 4th International Symposium on Trace Elements in the Food Chain, Friends or Foes? 15-17.11.2012., Visegrád, Hungary. Abstracts. p. 60.
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2. Zita Burján, Mariann Móré, Béla Kovács, Zoltán Győri (2013): Changes in protein and sulphur content of winter wheat (*Triticum aestivum* L.) grains. In.: Csajbók, J. The influence of some technological elements over the wheat and corn grains quality stored in Bihor and Hajdu Bihar counties. University of Debrecen Centre for Agricultural and Applied Economic Sciences Faculty of Agricultural and Food Sciences and Environmental Management, Debrecen. ISBN-978-963-473-612-7. 8-13.