

THESIS OF DOCTORAL (PhD) DISSERTATION

**INVESTIGATION OF SELENIUM CONTENT OF WHEAT CULTIVATED ON VARIOUS
SOIL TYPES LOCATED IN DIFFERENT REGIONS OF ROMANIA**

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Table of contents

1. Background and objectives of the doctoral thesis	3
2. Research methodology	5
3. The main findings of the thesis	7
3.1 The evaluation of the soil samples	7
<i>The selenium content of the soil samples</i>	7
<i>The determination of the pH value of the soil samples</i>	8
<i>The evaluation of the organic matter content of the soil samples</i>	9
3.2 The examination of wheat grass samples	9
<i>The selenium content of wheat grass samples</i>	10
<i>The selenomethionine content of wheat grass samples</i>	13
3.3 Examination of the wheat seed samples	15
<i>Dry matter of the wheat seed samples</i>	15
<i>Total selenium content of wheat seed samples</i>	15
<i>The selenomethionine content of wheat seeds</i>	19
4. New scientific results	21
5. Practical applicability of the results	22
6. Bibliography	23
7. Bibliography	27

1. Background and objectives of the doctoral thesis

The daily selenium requirement of an adult body is about 0.12 mg. This quantity of selenium can be provided through food only, because plants are able to absorb the plants are able to absorb an adequate amount of selenium from the soil. Due to acidic rainfalls, the selenium compounds in the soil convert to a form, from which the plants can absorb only small amounts. Selenium is present in the human diet mainly in the form of selenite, selenomethionine (SeMet) and selenocysteine (SeCys).

The biggest threat for Humans regarding selenium consumption is the consumption of selenium in the inorganic form, as an acute toxicity can develop easily and quickly this way. It is less dangerous when the selenium enters the body in an organic form like selenomethionine, but in this case, a potential danger is the accumulation of selenium, as SeMet is stored by the body for a longer time. It seems to be a better solution to consume selenium enriched plant nutrients, but perhaps it is even more advantageous when the necessary selenium enters the body through selenium enriched products of animal origin, as the animal organism performs such a conversion on selenium, which, assuming normal eating habits, can not cause any harm to the body.

Objectives

There is a lack of knowledge about the selenium supply of the population, the selenium content of the soils, the wheat crops and the wheat seeds in Romania. Because of this fact, my aim was to investigate:

- the total selenium content, the pH and organic compound content of the most important soil types in the Dobrogea-Bărăgan region (regosol, hydromorph waterlogged soil, podzolated brown forest soil, cambic brown forest soil, erodisol, raw alluvial soil, hydromorph grey meadow soil, redzina soil, humus alluvial soil, sandy soil-psamosol, cambic brown earth, luvisol, brown forest soil with albic horizon, hydromorph black meadow soil, mollic litic chernozem, mollic typical chernozem)
- the pH and organic compound content of the above soil types in the Ciuc basin
- the total selenium content and the SeMet content of wheat crops and wheat seeds of the aforementioned soil types originating from the Dobrogea-Bărăgan region (in 2008) and the Ciuc basin (in 2009 and 2010).
- it was my objective to evaluate the results of the samples originating from the Dobrogea-Bărăgan region regarding a correlation between the selenium content of the soil and the selenium content of the wheat crops and wheat seeds grown on the same soil type, as well as the correlation between the selenium and selenomethionine content of wheat crops and wheat seeds in each of the two regions.
- my aim was to determine the total selenium content and the selenomethionine content of one of the most important raw food material, the wheat seed, taking into account the selenium supply of the population in Romania, the Dobrogea-Bărăgan region and the Ciuc basin.

2. Research methodology

Sampling was performed on 16 different soil types in the Dobrogea-Bărăgan region located in the south-eastern part of Romania, and later on the same 16 different soil types in the Ciuc basin located in the south-east of Harghita county.

During the sampling we identified the place of origin using a GPS device, and there was care taken when collecting the wheat crop and wheat seed samples at the beginning of the harvest period, from the same regions where the soil and wheat grass were also sampled.

During our research we examined the selenium content and the content of different selenium forms of common wheat (or bread wheat, *Triticum aestivum*), as it is the most common wheat type grown in the Dobrogea-Bărăgan region as well as in the Ciuc basin.

The sampling of wheat was made at its maturation time (Dobrogea-Bărăgan region 25.06-10.07, Ciuc basin 20.07-01.08).

During the sampling the wheat grass samples were picked together with the soil. After the plant was pulled out by hand from the soil, the soil was placed into a plastic bag. The root of the plant was washed under running water, and the green wheat plants as well as the soil were transported into the laboratory immediately, where they were stored at -25 °C until preparation for further examination.

Wheat seeds were released from the plant by gloved hand. After removing the husk and the awn parts, the seeds were placed into a plastic bag, and stored at +5 °C until the beginning of the analysis examination. After releasing the seeds and removing the husk, there was no contamination which would have made washing the seeds necessary.

The amount of selenium in the soil is highly influenced by a number of important factors including the temperature or the amount of rainfall. For this reason, I monitored the changes of the climate with the support of the Romanian Meteorological Station during my research.

The analytical methods used for the analysis of the samples

Determination of the dry matter content of the samples

The dry matter content was determined according to the relevant Romanian Standard (STAS 9682-2-74).

Determination of the pH of the soil samples

To determine the selenium uptake capacity of the wheat plant and wheat seeds, it was necessary to determine the pH of the soil samples. The pH of the soil samples was determined in aqueous

suspension. 25 ml demineralized water was added to 1 g of soil sample. The mixture was shaken, and left overnight. After vigorous shaking, the pH of the samples was measured by a Consort C6010 pH measuring piece of equipment.

Determination of the organic matter content of the soil

The quantification of the organic matter content of the soil was determined according to the Tyurin method. A small amount of soil was sieved on a 0.25 mm mesh sieve to clear it from the root residues. To determine the organic matter content of the soil samples, the properly prepared soil samples were oxidized in a sulfuric acid solution of 0.2 M potassium dichromate under specified conditions. After oxidation, the excess potassium dichromate in excess was titrated with iron (II)-diamoniac sulfate solution, $(\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O})$ in the presence of a redox indicator (ISO 10390-2005).

Determination of selenium content by fluorimetric method

Selenium content is extremely sensitive to digestion conditions, therefore wet digestion was performed. The digestion was performed both with nitric acid and with royal water. The result of the three sample types (soil, wheat crop and wheat seeds) were appropriate only in the case where nitric acid was used for digestion, therefore only these results were considered for further evaluation.

2,3-diamino-naphthalene was added to the acidic solution of the digested samples, and the obtained piazo-selenol complex was measured fluorimetrically. The excitation wavelength was 380 nm and the measuring wavelength was 519 nm.

Determination of selenoamino acids by HPLC

The SeMet content was examined using a Varian Pro Star High Performance Liquid Chromatography, where o-phthalaldehyde 2-mercaptoethanol pre-column derivatization was performed after para-toluol-sulfonic acid hydrolysis (*Mándoki et al.*, 2008).

Statistical evaluation of the data

To find the relationship between the soil, the wheat plant- and the selenium content of the wheat a statistical analysis was performed with the Excel software package (standard deviation, mean, linear regression, correlation calculations) using the collected data. The results of the studies were evaluated with the Microsoft Office Excel 2003 program.

3. The main findings of the thesis

Considering that climate changes too, I came to the conclusion that the climatic conditions of the year 2008 and 2009 were the same. On the other hand, due to the geographic location of the Dobrogea-Bărăgan region, the average temperatures were higher here (approx. with 10°C higher) in the year 2008, than in the Ciuc basin in the year 2009. Comparing the weather conditions of the year 2009 and 2010 in the Ciuc basin, both the temperature and the amount of rainfall was higher in the year 2010.

3.1. The evaluation of the soil samples

The selenium content of the soil samples

At the beginning of our research we examined the selenium content of wheat plants grown on 16 different soil types in the Dobrogea-Bărăgan region, located in the south-eastern part of Romania.

In case of soil samples originating from the Dobrogea-Bărăgan region, the total selenium content ranged from the value of 52.0 µg/kg to 172 µg/kg. The average selenium content was about 119 µg/kg.

The lowest selenium content was measured in regosol (mean value 52.0 µg/kg), while the highest value was measured in the mollic (litic chernozem) soil sample (values between 170 and 172 µg/kg). We also detected low values in hydromorph waterlogged soil (65.9-67.1 µg/kg) and in case of podzolated brown forest soil (78.1-80.0 µg/kg). Higher values were detected of cambic brown forest soil (142-148 µg/kg), the cambic brown soil (149-150 µg/kg) and of hydromorph meadow soil (151-158 µg/kg). In the case of the other soil types, the selenium content of the samples was between 81.2-135 µg/kg. The value of the standard deviation for the total selenium content of the soil was 35.5 µg/kg.

The change of the selenium content of different soil types was it really identical or could you say it replicated the data cited in the literature (*Mayland et al.*, 1989). The volcanic rocks contained lower amounts of selenium than the sedimentary soil, especially compared with the clayey soils.

Based on the selenium content of the soil, there are four categories: incomplete (less than 75 µg/kg selenium), acceptable (75 to 175 µg/kg), high (175 to 1000 µg/kg) and specifically toxic (greater than 1000 µg/kg) (*Oldfield*, 1999, *Tan* 1989, *Kabata-Pendias and Pendias*, 1992, *Fordyce*, 2013). It can this be concluded, that the selenium content of these regions is deficient (lower than 0.75 µg/kg) and acceptable (between 75 to 175 µg/kg).

Comparing these results with those measured in other regions of Romania, it can be stated that the data is similar (Lăcătușu *et al.*, 2010, 2012 and 2013; Lungu, 2013).

The determination of the pH value of the soil samples

The pH of the soil samples varied between the value of 4.2 to 7.8 (Dobrogea Bărăgan- region 2008), 4.6 to 7.9 (Ciuc Basin 2009) and 5.4 to 8.3 (Ciuc Basin, 2010).

The lowest values in the Dobrogea Bărăgan region were pH 4.2 for the regosol soil type and 4.5 for the hydromorph waterlogged soil. The highest pH values were measured for the mollic (typical chernozem) 7.8, the mollic (lytic chernozem) 7.2. The standard deviation in the case of other soil types was 1.8.

The lowest values measured in the Ciuc basin both in 2009 and 2010, were pH 4.6 and 5.4 for the regosol soil type, and pH 4.6 and 5.7 for the hydromorph waterlogged soil type. The highest pH values were measured for the mollic (typical chernozem) and for the mollic (lytic chernozem) 7.2-8.3 and 7.1-8.2. In the case of other soil types, the standard deviation was 1.9 in 2009 and 1.5 in 2005.

I examined the relationship between the selenium content of the soil and the pH of the soil from the region of Dobrogea-Bărăgan. The value of the correlation coefficient (R^2) of the 70 samples was 0,879. This indicates a strong correlation between the selenium content and the pH of the soil.

I determined the relationship between the pH values of the soil samples for the three years. Comparing the soil samples from the Dobrogea-Bărăgan region with those from the Ciuc Basin, a strong correlation can be observed. In case of the Dobrogea-Bărăgan region 2008 and the Ciuc Basin 2009, the R^2 value is 0.834; in case of the Dobrogea-Bărăgan region 2008 and the Ciuc Basin 2010, the R^2 value is 0.841. On the other hand, there was a very strong correlation among the samples collected from the Ciuc basin in 2009 and 2010. The correlation coefficient of the 96 samples analyzed was $R^2 = 0.904$, indicating a strong correlation.

While analyzing the pH values of the soil samples there was a significant difference ($p < 0.05$) among the samples in all three years, but the pH of the same soil types did not change significantly during 2008, 2009 and 2010.

I found that the soils with a lower selenium content had a low pH, while the soils with a higher selenium content had a higher pH value. Comparing the collected data with the data in the literature, it can be stated, that the selenium content is greatly influenced by the pH values of the soil (Skinner, 1999; Ip and Ganther, 1992;. Kápolna *et al*, 2006; Cannon, 1964; Dhillon

and Dhillon, 1999, Barrow and Whelan , 1989; Széles et al, 2007;. Gissel-Nielsen et al, 1984; Mikkelsen et al, 1989; Patócs, 1990; Zhao et al 2007).

The evaluation of the organic matter content of the soil samples

The determination of the organic matter content of soil samples was carried out in order to check for correlation examine the relationship between the organic matter content and the selenomethionine content of the soil samples.

The soil samples collected in the Dobrogea Bărăgan region had an organic matter content of 0.70 to 3.67 %, the organic matter content of the soil samples collected in the Ciuc Basin was 2.63%-4.78% in 2009 and 0.95%-3.92% in 2010. The lowest organic matter content was measured in each of the three years from the regosol and the hydromorph waterlogged soil types, while the highest values were measured in case of the mollic (typical chernozem) and the mollic (lytic chernozem) soil samples.

Examined the correlation between the total selenium content and the organic matter content of the soil samples. In case of the 70 soil samples collected from the Dobrogea-Bărăgan region, the correlation coefficient (R^2) was 0.806, which indicates a moderate correlation.

The correlation between the organic matter content of the soil samples collected in the three years was also examined. Comparing the soil samples from the Dobrogea-Bărăgan region with those from the Ciuc Basin there is a moderate correlation (Dobrogea-Bărăgan region 2008 and the Ciuc Basin 2009 where the R^2 value was 0.811; for Dobrogea-Bărăgan region 2008 and the Ciuc Basin 2010 the R^2 was 0.821). There is a strong relationship between the samples collected in the Ciuc Basin in the years 2009 and 2010 collected from the same places. The correlation coefficient values of the 96 samples was 0, 807, which indicates a strong correlation.

Examining the organic matter content of the soils there was a significant difference ($p = 0.0001$) between the samples collected from the Dobrogea-Bărăgan region (in 2008) and the Ciuc Basin (2009 and 2010), but the organic matter content of the same soil types did not change significantly ($p < 0.05$).

3.2. The examination of wheat grass samples

During my research I examined the total selenium content and the SeMet content of wheat grass. The values were calculated on the basis of 100% dry matter, therefore I needed to determine the dry matter content of the samples.

The dry matter contents of wheat grasses originating from the Dobrogea-Bărăgan region were between 15.6% and 22.6%, while those originating from the Ciuc basin were between 15.6% and 22.4% in the year 2009 and between 16.2% and 23.1% in the year 2010.

The selenium content of wheat grass samples

The wheat grass samples originating from the Dobrogea-Bărăgan region showed a total selenium content with values ranging from 36.4 µg/kg to 122.4 µg/kg. The average selenium content was about 84.2 µg/kg.

The lowest selenium content was detected in the case of wheat grass cultivated on regosol (mean value 38.15 µg/kg), while the highest values were detected in the case of wheat grass cultivated on mollic (typical chernozem) soil type (121 µg/kg). Low values were also detected in the case of wheat grass samples cultivated on hydromorph waterlogged soil (49.6 µg/kg), erodisol (76.9 µg/kg) and the podzolated brown forest soil (63.8 µg/kg). Higher values were detected in the case of wheat grass cultivated on mollic redzina soil (89.1 µg/kg), mollic (litic chernozem) (108 µg/kg) and hydromorph meadow soil types (95.1 µg/kg). The wheat grass samples cultivated on other types of soil showed moderate selenium content. The standard deviation was 21.40 µg/kg.

According to the data in the literature, wheat grass does not belong to the selenium accumulating plant group, therefore the selenium content of the soil is proportional to that of wheat grass. It can be concluded that the total selenium content of wheat grass was deficient (less than 125 µg/kg) and acceptable (125-175 µg/kg) like in case of the soil (*Oldfield, 1999; Tan, 1989; Kabata-Pendias and Pendias, 1992; Fordyce, 2013*). My findings were similar to the data described in the literature (*Fordyce et al., 2000; Johnson et al., 2000; Wang and Gao, 2001, MacPherson et al., 1997, Fleming, 1980; Jacobs, 1989; Mayland, 1994; Neal, 1995*).

I found that the selenium deficient wheat grass samples originated from soils with lower selenium content (regosol 52.0 µg/kg, hydromorph waterlogged soil 66.8 µg/kg, erodisol 78.5 µg/kg, and the podzolated brown forest soil 79.9 µg/kg, while the wheat grass samples with acceptable selenium content originated from soil types with higher selenium content (mollic-typical chernozem 171 µg/kg, mollic-litic chernozem 169 µg/kg, hydromorph meadow soil 156 µg/kg).

The correlation between the total selenium content of soil types and wheat grass cultivated on it, was also examined. According to the results of the 70 different analyzes, the correlation coefficient (R^2 value) was 0.883, which indicates a strong correlation between the total selenium content of the soil and that of the wheat grass cultivated on it.

The total selenium content of wheat grass samples originating from the Ciuc Basin ranged from 70.6 µg/kg to 150 µg/kg in 2009. The average selenium content was about 117 µg/kg. The lowest value was detected in wheat grass samples cultivated on regosol (70.6 and 77.9 µg/kg), while the highest value was obtained in wheat grass cultivated on mollic (typical chernozem) soil (148 and 150 µg/kg). Low values have been also measured in wheat grass cultivated on hydromorph waterlogged soil (75.6 and 86.9 µg/kg) and podzolated brown forest soil (98.2 and 106 µg/kg). Higher values were obtained in wheat grass cultivated on mollic redzina soil type (129-132 µg/kg), the mollic-lytic chernozem (135-141 µg/kg) and sandy soil-psamosol (137-143 µg/kg). Wheat grass samples cultivated on other soil types showed moderate selenium content. The standard deviation was 20.4 µg/kg.

As a summary, it can be stated, that the selenium content of wheat grass is low, but it is higher than in the case of the samples originating from the same soil types of the Dobrogea Bărăgan region in 2008. Both the wheat grass samples with low selenium content as well as the wheat grass samples with high selenium content, originated from the same soil type.

I have compared the selenium content of wheat grass samples originating from the Dobrogea Bărăgan region (year 2008) and from the Ciuc basin (year 2009) from the same soil type. The selenium content of wheat grass samples originating from these two regions differs from each other at an average rate of 20-25 %. This difference is attributed to the climate differences in the two regions, as the average temperatures of the Dobrogea-Bărăgan region are approximately the same proportion higher than the temperatures in the Ciuc basin as the average selenium content difference. The selenium which can be mobilized is less bounded to wheat grass at higher temperature conditions.

I examined the correlation between the selenium content of wheat grass samples collected from the same soil type from the Dobrogea-Bărăgan-region (2008) and from the Ciuc Basin (2009). According to the statistical evaluation of the data from the 83 samples collected, the correlation coefficient (R^2) of the samples was 0.909, which indicates a strong correlation.

The average difference in the selenium content between the samples collected from the two different regions is 20-25%, which can be attributed to the climate differences, as the temperatures in the Dobrogea-Bărăgan region is approximately the same proportion higher than the temperature in the Ciuc Basin, so the mobile selenium binds less to the wheat grass samples at the higher temperatures.

The selenium content of wheat grass samples collected from the Ciuc basin in 2010 was between 48.7 µg/kg and 137 µg/kg. The average selenium content was about 97.6 µg/kg.

The lowest selenium content was measured from the samples cultivated on regosol (48.7 and 50.1 $\mu\text{g}/\text{kg}$), while the highest values were measured in case of samples cultivated on mollic (typical chernozem) soil (between 134 and 137 $\mu\text{g}/\text{kg}$). Low values have been measured in samples cultivated on hydromorph waterlogged soil (values between 52.3 and 56.7 $\mu\text{g}/\text{kg}$) and on podzolated brown forest soil (72.5 and 78.3 $\mu\text{g}/\text{kg}$). Higher values have been obtained in samples cultivated on mollic redzina soil (120 and 124 $\mu\text{g}/\text{kg}$), mollic (lytic chernozem) soil type (120-123 $\mu\text{g}/\text{kg}$) and sandy soil-psamosol (117-118 $\mu\text{g}/\text{kg}$). In the case of wheat grass samples cultivated on other soil types, the selenium content was moderate. The standard deviation was 22.9 $\mu\text{g}/\text{kg}$.

It can be concluded, that the selenium content of the wheat grass collected in 2010 was lower than in 2009, however it was higher than in the case of samples collected from the Dobrogea Bărăgan region in 2008. Both the wheat grass samples with low selenium content as well as the wheat grass samples with high selenium content originated from the same soil type.

I have examined the correlation between the 83 wheat grass samples cultivated on the same soil type but originating from the Dobrogea-Bărăgan region (2008) and the Ciuc basin (2010), and found that the correlation coefficient (R^2 value) of these samples is 0.814, suggesting a close correlation.

I compared the wheat grass samples collected from the same soil type of the Ciuc basin region (location determined with GPS) in 2009 and 2010. The selenium content of wheat grass samples collected in the second year was 5-10 % lower than those collected in the first year. As the samples originated from the same soil type and even the same places, the differences can be attributed to the weather condition, as both the average temperature as well as the amount of rainfall, were higher in 2010 compared to previous year.

According to the results of the analysis of the 96 wheat grass samples collected in 2009 and 2010, the correlation coefficient (R^2 value) was 0.927, which indicates a strong correlation. This was expected, as the samples have the same origin.

I examined the selenium content and the pH values of the samples collected from the same soil types in the two different regions (Dobrogea-Bărăgan-2008 Ciuc Basin, 2009 and 2010), and found a very strong correlation (Dobrogea-Bărăgan- (2008) -0.923, Ciuc Basin (2009) and -0.934 and (2010) -0.914). This explains the selenium content of the samples, as the literature refers also to the correlation between the pH value of the soil and the selenium content of the plants (*Ip and Ganther, 1992; Kápolna et al, 2006; Smith and Westfall, 1937; Scott Voegeli, 1961; Lindberg, 1968; Skinner, 1999; Dhillon and Dhillon, 1999; Nielsen et al Gissel, 1984; Mikkelsen et al, 1989*).

I analyzed the correlation between the organic matter content and the total selenium content of the wheat grass samples (for all samples), which lead to correlation coefficient values (R^2) of 0.889 in 2008; 0.885 in 2009 and 0.871 in 2010, indicating a strong relationship between the organic matter content and the total selenium content of the soil samples.

I examined the relationship between the pH values of the soil and the total selenium content of the wheat grass samples (for all three cases). The value of the correlation coefficient (R^2) was 0.845 in 2008, 0.864 in 2009 and 0.821 in 2010, indicating a close relationship between the pH value of the soil and the total selenium content of wheat and grass samples.

Looking at the total selenium content of the wheat grass samples from the Dobrogea-Bărăgan region (in 2008) and the Ciuc Basin (2009 and 2010) the total selenium content did not change significantly ($p < 0.01$) in the wheat grass samples collected from the same soil types in 2008, 2009 and 2010.

Regarding the selenium content of the wheat grass samples of all the three years, it can be concluded that the lowest values were always obtained in case of the samples cultivated on regosol (38.2 $\mu\text{g}/\text{kg}$ – 2008, 52.7 $\mu\text{g}/\text{kg}$ – 2009, 49.3 $\mu\text{g}/\text{kg}$ – 2010), while the highest values were obtained in case of wheat grass samples cultivated on mollic typical or litic chernozem soil (121 $\mu\text{g}/\text{kg}$ – 2008, 140 $\mu\text{g}/\text{kg}$ – 2009, 126 $\mu\text{g}/\text{kg}$ – 2010). As the regosol is a less cohesive soil type than the mollic type, it may be possible, that beside the weather conditions, the structure of the soil also plays an important role in the binding of selenium in wheat plants.

The selenium content of the soil and wheat plants originating from the Dobrogea Bărăgan region were 25 $\mu\text{g}/\text{kg}$ and 140 $\mu\text{g}/\text{kg}$ according to the data described in the literature (*Fordyce et al.*, 2000; *Johnson et al.*, 2000; *Wang and Gao*, 2001, *MacPherson et al.*, 1997, *Fleming*, 1980; *Jacobs*, 1989; *Mayland*, 1994; *Neal*, 1995), I therefore assume that the soil in the Ciuc basin (in the Ciuc basin the selenium content of the soil was not measured) from which the low selenium containing wheat grass samples was collected, is deficient in selenium. The soil from which the wheat grass samples with high selenium content were collected falls, according to this assumption, in the category of soils with acceptable selenium content (*Oldfield*, 1999, *Tan*, 1989, *Kabata-Pendias and Pendias*, 1992, *Fordyce*, 2013).

The selenomethionine content of wheat grass samples

According to the literature, the dominant form of selenium in foods of plant origin is SeMet (*Kryukov et al.*, 2003; *Stadlober, M. et al.*, 2001; *Beilstein and Whanger*, 1986; *Yasumoto et al.*, 1988). 56-83% of the selenium content of wheat is present in the form of SeMet, while other selenium containing compounds occur in a lower amount (such as selenate 12-19%,

SeCys 4-12%, Se-methyl-selenocysteine 1-4%, others 4-26%) (Whanger, 2002). Because of this reason, I considered it important to determine the SeMet content of wheat grass as well as that of the crop.

The selenomethionine content of wheat grass samples was 61.6-166 µg/kg (Dobrogea Bărăgan region, 2008), 110-189 µg/kg (Ciuc basin, 2009) and 97.7-173.3 µg/kg (Ciuc basin, 2010).

From the SeMet content, the organic selenium content could be also calculated. The values ranged in each sampling year from 48% to 66%. This was about 10-12% lower than the values stated in the literature.

The lowest SeMet content was measured in wheat grass samples cultivated on regosol (values between 61.6 and 66.6 µg/kg) and hydromorph waterlogged (64.6-67.21 µg/kg) soil collected from the Dobrogea-Bărăgan region. The highest amount of SeMet was measured in wheat grass samples cultivated on mollic (typical chernozem) (166-167 µg/kg), mollic (litic chernozem) (151-156 µg/kg) and mollic redzina (134-136 µg/kg) soil types. In the case of wheat grass samples collected from other soil types, the SeMet content was moderate. The standard deviation was 36.7 µg/kg.

The SeMet content of wheat grass samples collected from the Ciuc basin in 2009 was 20-25% higher than the SeMet content of wheat grass collected from the Dobrogea-Bărăgan region. The lowest SeMet content was measured from wheat grass cultivated on regosol (110 and 117 µg/kg) and the hydromorph waterlogged soil (115-125 µg/kg). The highest values were obtained from samples cultivated on mollic (typical chernozem) soil (187-189 µg/kg), mollic (litic chernozem) (174-180 µg/kg) and the hydromorph black meadow (171-175 µg/kg) soils. In case of wheat grass samples collected from other soil types, the SeMet content was moderate. The standard deviation was 20.4 µg/kg.

The SeMet content of wheat grass samples collected from the Ciuc basin in the year 2010 was 9-10% lower than the values measured in 2009. The lowest SeMet content was measured again in samples cultivated on regosol (98.3-101 µg/kg), and hydromorph waterlogged (103-105 µg/kg) soils. The highest values were obtained from samples cultivated on mollic (typical chernozem) soil (169-173 µg/kg), mollic (litic chernozem) (167-170 µg/kg) and the mollic redzina (160-167 µg/kg) soils. In the case of wheat grass samples collected from other soil types, the SeMet content was between 125-166 µg/kg, and the standard deviation was 21.0 µg/kg.

I have examined the correlation between the total selenium content of the soil in the Dobrogea-Bărăgan region (2008) and the SeMet content of wheat grass in the same region.

According to the results of the samples analyzed 70, the value of the correlation coefficient (R^2 value) was 0,862 in 2008, 0.874 in 2009 and 0.856 in 2010, which indicates a strong correlation between the Se content of the wheat grass and the SeMet content of the wheat grass. There was no significant difference between the SeMet content of the wheat grass samples of the three years ($p < 0.01$).

I have examined the correlation between the Se and SeMet content of wheat grasses in all three cases. As a result the correlation coefficient (R^2) was 0,825 in 2008, 0.865 in 2009 and 0.840 in 2010. According to these values, it can be concluded, that there is a strong correlation between the total selenium content and the SeMet content of wheat grasses. The very strong correlation was expected, as the selenium in the selenomethionine is part of the total selenium content, therefore the correlation between these values has to be high.

3.3. Examination of the wheat seed samples

Dry matter of the wheat seed samples

The dry matter content of wheat seeds was between 89.4% and 91.2%. The dry matter values were needed due to the fact, that the total selenium and SeMet content is reported against the total quantity of 100% dry matter.

Total selenium content of wheat seed samples

The selenium content of the wheat seeds varied between 29.9 $\mu\text{g}/\text{kg}$ and 134 $\mu\text{g}/\text{kg}$. The total selenium content of the wheat originating from the Dobrogea Bărăgan region (2008) was between 29.9 $\mu\text{g}/\text{kg}$ and 111 $\mu\text{g}/\text{kg}$. The average selenium content was about 72.8 $\mu\text{g}/\text{kg}$. The lowest selenium content was measured in wheat seed samples cultivated on regosol (29.9 $\mu\text{g}/\text{kg}$ and 30.4 $\mu\text{g}/\text{kg}$), while the highest values were obtained from wheat seed samples cultivated on mollic (typical chernozem) soil (108 and 111 $\mu\text{g}/\text{kg}$).

Low values have been also measured in the case of wheat seed samples cultivated on hydromorph waterlogged soil (37.4-38.7 $\mu\text{g}/\text{kg}$), on cambic brown forest soil (46.7-49.7 $\mu\text{g}/\text{kg}$), and on podzolated brown forest soil (52.6-52.6 $\mu\text{g}/\text{kg}$). Higher values have been obtained in samples cultivated on mollic (litic chernozem) soil (95.3-97.6 $\mu\text{g}/\text{kg}$ mollic redzina soil (74.5-79.6 $\mu\text{g}/\text{kg}$) and sandy soil-psamosol (94.7-96.1 $\mu\text{g}/\text{kg}$). In the case of wheat seed samples cultivated on other soil types, the selenium content was moderate. The standard deviation was 20.9 $\mu\text{g}/\text{kg}$.

I have examined the correlation between the selenium content of the soil in the Dobrogea-Bărăgan region (2008) and the selenium content of wheat seeds in the same region. According to the results, the correlation coefficient (R^2 value) was 0.876.

It can be concluded, that the selenium content of wheat seeds cultivated on the soils of these regions, is low (lower than 0.120 $\mu\text{g}/\text{kg}$) and acceptable (0.125-0.155 $\mu\text{g}/\text{kg}$) compared to the values stated in the literature (*Oldfield, 1999, Tan, 1989, Kabata-Pendias and Pendias, 1992, Gawalko et al., 2001, MacPherson et al., 1997, Broadley et al., 2010*).

The total selenium content of wheat seeds collected in the Ciuc basin (2009) was between 54.5 $\mu\text{g}/\text{kg}$ and 134 $\mu\text{g}/\text{kg}$. The average selenium content was 101 $\mu\text{g}/\text{kg}$.

The lowest selenium content was measured in wheat seed samples cultivated regosol (between the values 54.5 and 61.8 $\mu\text{g}/\text{kg}$), while the highest values were obtained from wheat seed samples grown on mollic (typical chernozem) soil (between the values 132 and 134 $\mu\text{g}/\text{kg}$). Low values have also been measured in the case of wheat seed samples cultivated on hydromorph waterlogged soil (59.5-70.8 $\mu\text{g}/\text{kg}$) and on podzolated brown forest soil (82.1-89.4 $\mu\text{g}/\text{kg}$). Higher values have been obtained in case of samples grown on mollic redzina soil (113-115 $\mu\text{g}/\text{kg}$), mollic (litic chernozem) soil (119-125 $\mu\text{g}/\text{kg}$) and sandy soil-psamosol (121-127 $\mu\text{g}/\text{kg}$). In the case of wheat seed samples cultivated on other soil types, the selenium content was moderate. The standard deviation was 20.4 $\mu\text{g}/\text{kg}$.

It can be concluded that the selenium content of wheat seeds is as low as that of the wheat grass samples in Ciuc region, but it is higher than that of wheat seed samples collected from the Dobrogea Bărăgan region (2008). These values were very similar to those obtained in the case of wheat grass samples, which can be explained by the weather conditions.

The lower selenium containing wheat seed samples were cultivated on regosol, such as the wheat grass samples collected from the Dobrogea Bărăgan region in 2008, while the higher selenium containing samples came from the mollic (litic chernozem) soil type.

I have examined the correlation between the wheat seeds collected from these two regions (Dobrogea Bărăgan region in 2008, Ciuc basin in 2009). According to the result of the 83 analyzes, the correlation coefficient (R^2 value) was 0.872. This means a strong correlation, from which it can be concluded, that the selenium content of the soils in the Ciuc basin could be proportional to the selenium content of the soils in the Dobrogea Bărăgan region.

The total selenium content of wheat seeds collected in the Ciuc basin in 2010 ranged from 34.1 $\mu\text{g}/\text{kg}$ to 122 $\mu\text{g}/\text{kg}$. The average selenium content was 82.9 $\mu\text{g}/\text{kg}$. The lowest selenium content was measured in wheat seed samples cultivated on regosol (34.1-35.5 $\mu\text{g}/\text{kg}$), while the highest values were obtained from wheat seed samples cultivated on mollic (typical

chernozem) soil (120-122 µg/kg). Low values have been also measured in case of wheat seed samples cultivated on hydromorph waterlogged soil (37.7-42.1 µg/kg) and on podzolated brown forest soil (57.9-63.7 µg/kg). Higher values have been obtained in case of samples cultivated on mollic redzina soil (105-109 µg/kg) and mollic (litic chernozem) soil (104-106 µg/kg). In the case of wheat seed samples cultivated on other soil types, the selenium content was moderate. The standard deviation was 22.9 µg/kg.

It can be concluded, that the total selenium content of the wheat seeds is lower than the samples collected in 2009, but it is higher than the wheat seed samples originating from the Dobrogea Bărăgan region in 2008. Both the wheat grass samples with low selenium content as well as the wheat grass samples with high selenium content originated from the same soil type. (The standard deviation is the same in case of wheat seeds collected in 2009 and 2010 as that of the wheat grasses collected in the same years).

Comparing the total selenium content of the wheat samples of the three years, it can be concluded, that the lowest values were measured in the Dobrogea Bărăgan region in 2008. These values are followed by the values obtained from measuring the samples of the Ciuc basin in 2010. The highest values were always obtained from the samples originating from the Ciuc basin in 2009.

The weather conditions in 2008 and 2010 were similar, while in 2009 the average temperatures and the amount of rainfall was extremely high, compared to previous years. The differences obtained in 2008 and 2010 can be attributed to the different geographic locations. The average temperature of the Dobrogea Bărăgan region is 10-12 °C higher during the whole year than the average temperature in the Ciuc basin. The variation in the selenium content can be explained by this difference in temperatures between the two regions.

I have observed that the selenium content of wheat grasses was in each year (2008-2010) the lowest when these were cultivated on the same soil type, and the highest when grown on other soil types. For this reason, I examined the correlation between the selenium content of wheat seeds collected in each of the three years.

I examined the correlation between the wheat samples collected from the same soil type in both regions (Dobrogea Bărăgan region 2008, and Ciuc basin 2010). According to the results obtained, the correlation coefficient (R^2 value) of the 83 samples was 0.906. This indicates a strong correlation.

At the same time I examined the correlation between the selenium content of wheat seeds collected always from the same places (location determination with GPS) of the Ciuc

basin in 2009 and 2010. According to the results of the 96 analyzes, the correlation coefficient (R^2 value) was 0.896, which means a strong correlation.

I examined the correlation between the organic matter content of the soil and the total selenium content of the wheat seed samples (in all three sampling years). The correlation coefficient (R^2) was 0.895 in the Dobrogea Bărăgan region in 2008, 0.891 in the samples from the Ciuc Basin in 2009 and 0.868 in 2010, which indicates a strong correlation between the organic matter content of the soil and the total selenium content of the wheat seeds.

I examined the relationship between the pH of the soil and the total selenium content of the wheat seeds (in all three years). The correlation coefficient (R^2) was 0.901 in 2008; 0.894 in 2009, and 0.897 in 2010, indicating a very strong correlation between the pH of the soil and the total selenium content of the wheat seeds.

Looking at the total selenium content of the wheat seed samples originating from the same soil types from the Dobrogea-Bărăgan region (in 2008) and the Ciuc Basin (2009 and 2010) the total selenium content did not change significantly ($p < 0.01$) in 2008, 2009 and 2010.

In the case of the selenium content of wheat seed samples, it can be concluded that the lowest values were measured on each year when the wheat grass samples were cultivated regosol (30.1 $\mu\text{g}/\text{kg}$ in 2008, 59 $\mu\text{g}/\text{kg}$ in 2009 and 34.7 $\mu\text{g}/\text{kg}$ in 2010), while the highest values were obtained when the wheat grass samples were cultivated on mollic (typical or litic chernozem) soil (109 $\mu\text{g}/\text{kg}$ in 2008, 133 $\mu\text{g}/\text{kg}$ in 2009 and 121 $\mu\text{g}/\text{kg}$ in 2010). Besides weather conditions, the structure of the soil and its type may also play an important role in the accumulation process of selenium in wheat seeds.

The selenium content of wheat seeds originating from the Dobrogea Bărăgan region was proportional to that of the soil, in the same manner as stated in the literature (*Fordyce et al.*, 2000; *Johnson et al.*, 2000; *Wang and Gao*, 2001; *MacPherson et al.*, 1997; *Fleming*, 1980; *Jacobs*, 1989; *Mayland*, 1994; *Neal*, 1995; *Adam et al.*, 2002; *Hahn et al.*, 1981; *Boila et al.*, 1993; *Gawalko et al.*, 2001; *Oldfield*, 1999; *Tan*, 1989; *Kabata-Pendias and Pendias*, 1992; *Fordyce*, 2005, 2013).

Tan (1989) found the following total selenium content ranges in China: lower than 25 $\mu\text{g}/\text{kg}$ means a selenium deficiency, 25-40 $\mu\text{g}/\text{kg}$ is an acceptable range, 40-1000 $\mu\text{g}/\text{kg}$ is a range between the acceptable and the high selenium content, while higher than 1000 $\mu\text{g}/\text{kg}$ represents a highly toxic value. *Gissel-Nielsen et al* (1984) reported low selenium concentration in the Scandinavian countries, where the selenium content of wheat ranged from 7-18 $\mu\text{g}/\text{kg}$. The wheat originating from Great Britain has also a low selenium content (*Barclay and Macpherson*, 1986). On the other hand, studies from the USA, reported an average selenium

content between 370 µg/kg and 475 µg/kg in the wheat originating from the USA (Wolnik *et al.*, 1983, Hahn *et al.*, 1981). According to recent experiments the selenium content of wheat produced in South America is between 5 to 720 µg/kg, with a mean value of 155 µg/kg (Lyons *et al.*, 2005). Adam *et al.* examined the selenium content of wheat originating from the United Kingdom in 2002. The average selenium content of these samples was 16-26 µg/kg. According to the findings of Boila *et al.* (1993) the selenium content of wheat in Canada is 760 µg/kg, while Gawalko *et al.* (2001) reports a selenium content of 280-770 µg/kg for the wheat from the same country. Wheat originating from Canada and from the USA derived from soils with higher selenium content, while the soils of the UK are mostly selenium deficient (50-100 µg/kg), for this reason the selenium seeds have also a lower selenium content (Fordyce, 2005; Boardley *et al.*, 2006).

Considering the, I assume that there is a strong relationship, between the soils selenium content in the Ciuc basin and the selenium content of the wheat seeds originated from this region. This is in accordance with the findings of the literature mentioned above. The low selenium wheat samples are coming from selenium poor soils and the higher selenium wheat samples were originated from selenium rich soils.

The selenomethionine content of wheat seeds

According to the data in the literature, 56-83% of the selenium content of wheat is SeMet (Kryukov *et al.*, 2003, Whanger, 2002). This is why I considered as important to examine the SeMet content of wheat seeds.

Comparing the SeMet content of wheat seed samples collected from Ciuc basin in the year 2009 to those collected from the Dobrogea Bărăgan region, the samples originating from the Ciuc basin contain 25-30 % more SeMet. The lowest SeMet content was measured in case of samples cultivated on regosol (89.9-105 µg/kg). The SeMet content was also low in case of samples grown on hydromorph waterlogged soil (99.9-123 µg/kg). The highest values were obtained from wheat seed samples cultivated on mollic (typical chernozem) soil (245-248 µg/kg), mollic (litic chernozem) soil (218-231 µg/kg) and hydromorph black meadow soil (213-221 µg/kg). In case of wheat seed samples cultivated on other soil types, the SeMet content was moderate. The standard deviation was 40,8 µg/kg.

The wheat seed samples collected from the Ciuc basin in the year 2010 had a 9-10% lower SeMet content than those collected in 2009 from the same region. This was the same in case of wheat grass cultivated in this region. The lowest SeMet content here was also measured in samples cultivated on regosol (48.1-51.2 µg/kg) and hydromorph waterlogged soil (55.6-64

µg/kg). Higher values were obtained from wheat seed samples cultivated on mollic (typical chernozem) soil (220-224 µg/kg), mollic (litic chernozem) soil (191-198 µg/kg) and mollic redzina (160-167 µg/kg) soil. In case of wheat seed samples cultivated on other soil types, the SeMet content was moderate, and reached values from 115 to 192 µg/kg. The standard deviation was 45.8 µg/kg.

I examined the correlation between the SeMet content of wheat seeds and total selenium content of the soil in the Dobrogea Bărăgan region (2008). According to the results, the correlation coefficient (R^2 value) was 0.907. This value indicates a strong correlation between the SeMet content of wheat seeds and the total selenium content of the soil.

I examined the correlation between the selenium content and SeMet content of the wheat seeds. In case of wheat seeds originating from the Dobrogea Bărăgan region the correlation coefficient (R^2) was 0.967, which indicates a strong correlation between the total selenium content of the wheat seed and its SeMet content.

In case of wheat seed samples collected from the Ciuc basin in 2009, the correlation coefficient (R^2 values) regarding the correlation between the selenium and SeMet content of wheat seeds was 0.932. This also indicates that there is a strong correlation between the selenium and SeMet content of wheat seeds originating from this region.

In case of wheat seed samples collected from the same region, but in the year 2010, the correlation coefficient (R^2 values) regarding the correlation between the selenium and SeMet content of wheat seeds was 0.935. This also indicates that there is a strong correlation between the selenium and SeMet content of the wheat seeds originating from this region.

The very strong correlations was expected, as the selenium in the selenomethionine is part of the total selenium content, on the other hand the samples were collected from the same soil types at the same stage of development and in the same time, therefore the correlation between these values has to be high.

4. New scientific results

- While examining the wheat grass, the wheat seed and the soil samples originating from the Dobrogea Bărăgan region, I concluded, that there is a very strong correlation regarding the selenium content between the soil and the wheat grass and wheat seeds cultivated on these soils.
- In case where the plants were grown on the same soil type, there is also a strong correlation between the selenium content and SeMet content of wheat grass and wheat seeds originating from the Dobrogea Bărăgan region and the Ciuc basin.
- I have concluded that there is a strong correlation between the total selenium content, the SeMet content of the wheat grass and wheat seeds and the pH of the soil as well in case of samples originating from the Ciuc Basin as in case of samples originating from the Dobrogea Bărăgan region.
- There was a strong correlation between the total selenium content and the SeMet content of wheat grass and wheat seeds and the organic matter content of the soil in both regions.
- I have concluded that both the order of SeMet content and the total selenium content is the same for both, the wheat grass and the wheat seeds originating from the Ciuc basin as in case of those originating from the Dobrogea Bărăgan region but grown on other soil types. Therefore the selenium content is only a function of the soil type, but not of the region.
- There was a strong correlation between the selenium content and SeMet content of wheat grass and wheat seeds in both regions. During the measurements carried out for two years in the Ciuc basin, I concluded that the amount of rainfall and the temperature has an influence on the total selenium content and SeMet content of wheat grass and wheat seeds cultivated on different soil types, but it has no influence on the order of the selenium content of wheat grass and wheat seeds cultivated on different soil types.

5. Practical applicability of the results

- There are very few data about the selenium content of soils come from Dobrogea-Bărăgan district, and there are absolutely no data about the selenium content of soils come from Csíki basin in the literature.
- According to my results there is a close correlation between the selenium content of the soil and the selenium content of the wheat grown on it, and it was established that both in Csíki basin and Dobrogea-Bărăgan region, as a result the selenium content of the wheat is small. By the consumption of the basic foodstuffs the humans do not get the sufficient amount of selenium at the region investigated by me. My results call the attention that it would be necessary to supplement the soils with selenium.
- Whereas the absorption of the selenium from different soil is influenced by many factors (the pH and the organic material of the soil was investigated by me), my results call the attention that before the enrichment of the soils with selenium, it is necessary to analyse the composition of the soils in more detail, or using another techniques (foliar application of the fertilizers) for optimisation of selenium supply for population of Seklerland and Romania.

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Subject: Ph.D. List of Publications

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List of publications related to the dissertation

Hungarian scientific article(s) in Hungarian journal(s) (3)

1. **Tamás M.**, Mándoki Z., Márton M., Mészáros S., Lányi S., Salamon R.V., Salamon S., Albert C., Csapó J.: Különböző őszibúza (*Triticum aestivum* L.) hajtás és búzaszem összesszelén- és szervesszelén-tartalma.
Acta agrar. Kvár. 15 (1), 67-84, 2011. ISSN: 1418-1789.
2. **Tamás M.**, Mándoki Z., Csapó J.: A szelén szerepe az emberi táplálkozásban: A búza szeléntartalmának elemzése (Irodalmi áttekintés).
Acta agrar. Kvár. 15 (1), 45-66, 2011. ISSN: 1418-1789.
3. **Tamás M.**, Mándoki Z., Márton M., Mészáros S., Lányi S., Csapó J.: Különböző talajtípusokon termesztett búzafű (*Triticum aestivum* L.) és búzamag szeléntartalma összefüggésének vizsgálata.
Agrártud. Közl. 41, 117-121, 2010. ISSN: 1587-1282.

Hungarian scientific article(s) in international journal(s) (2)

4. **Tamás M.**, Mándoki Z., Márton M., Mészáros S., Lányi S., Csapó J.: Különböző talajtípusokon termesztett búza (*Triticum aestivum* L.) összes és szerves szelén tartalma közötti összefüggés.
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5. **Tamás M.**, Mándoki Z., Márton M., Mészáros S., Lányi S., Csapó J.: Különböző talajtípusokon termesztett búzafű (*Triticum aestivum* L.) és búzamag szeléntartalma közötti összefüggés.
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