# COMPARISON OF DETERMINING METHODS REGARDING SELENIUM CONTENT IN WHEAT PLANT

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***Abstract***
*As a metallic chemical element, selenium has received special attention from biologists because of its dual role as a trace element essential and toxic. The important part of enzymes that protect cells against the effects of free radicals that are produced during normal metabolism of oxygen. Also, selenium is essential for normal immune system and thyroid gland, The concentration of selenium in the soil, which varies by region, determines the default concentration of selenium in plants growing in the soil.*
*The purpose of this paper is to present methods of comparison, dry oxidation at 450ºC and wet digestion – digestion with acids in high concentrations at microwave system digestion, for determining selenium content from wheat samples collected from the south-eastern part of Romania, namely Bărăgan Plain and Central-South Dobrogea. Selenium separation and dosage from obtained extracts carry out through a selective hydride generation atomic absorption spectrophotometry. With the software SURFER, a tendency map of selenium distribution was drawn.*

Keywords: wheat plant, selenium, hydride generation

## 1. INTRODUCTION

In 1817, JJBerzelius, following the manufacture of sulfuric acid, stops on waste sludge and discover a new element which he called "selenium" which in Greek meant earth satellite, the Moon, Berzelius published his next year in 1818, in "Annales de physique and chemistry", the new discovery. Later, this new element is found in some minerals by other researchers. [1]
Se has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the determined by the chemical form and concentration, soil factors such as pH, salinity and $CaCO_3$ content, the identity and concentration of competing ions, and the ability of the plant to absorb and metabolize selenium ( Kabata Pendias, 2001 [2]). Actively growing tissues usually contain the largest amounts of selenium (Kahakachchi et al., 2004 [3]). Plants usually accumulate more selenium in shoot and leaf than in root tissues (Zayed et al., 1998 [4]). Selenium concentration in soil, wich varies function by region, determines implicitly selenium concentration in plants that grows on respectiv soil. [5]
Selenium intake in humans is determined mainly by the level of available Se in the soil on which their food is grown, and by dietary composition, Se levels in major food classes usually occur within the following ranges: 0.10 – 0.60 mg/kg (fish), 0.05 – 0.60 (cereals), 0.05 – 0.30 (red meats), and 0.002 – 0.08 (fruit and vegetables) (Combs, 2001 [8]).

## 2. MATERIAL AND METHODS

A number of 49 wheat samples were collected from the south-eastern part of the Romanian territory, namely Bărăgan Plain and Central-South Dobrogea. Related to prelevating and preparation of plant samples for analysis, the samples are dried at the temperature of 30-40°C for 48 hours. The dried samples are pounded in mill plants.
This paper shows two analytical and estimation methods of selenium content from wheat plant, dry oxidation at 450ºC and wet digestion – digestion with acids in high

concentrations at microwave system digestion.

### 2.1. Samples preparation for analysis

To avoid losses of volatile selenium, since the preparation of samples for analysis taken some precautions. Thus, dried plant samples at 40º C were mortared and stored at room temperature.

### 2.2. Analytical methods and instrumentation

Two major groups of procedures may be distinguished: dry oxidation or ashing at elevated temperature 450ºC in a muffle furnace, method which is the most frequently applied. It ensures the quantitative removal of the organic matter; the mineral part of the matrix is dissolved in an appropiate acid, HCl. Compared to other digestion methods, the biggest advantage of dry oxidation is the possibility of ashing large sample amounts and dissolving the resulting ash by a simple acid to a small volume of final solution. As a consequence, this procedure allows the preconcentration of trace elements and is very useful in practice when low concentrations are to be determined. Wet digestion procedures, where the organic part of the sample is mineralized in the aqueous phase by a heating in the presence of oxidizing agents (usually combinations of acids and hydrogen peroxide). These methods generally do not totally decompose organic matter, which remains partly in the final solution and may impede determinations by particular analytical techniques together with the excess of various acid used.

Standard and reagents were products of Merck (Germany). Reference standard of Se (stock solution of 1000 mg/l $SeO_2$ in 0.5 m $HNO_3$) was used and selenium standard solutions were prepared from this solution. It pipettes appropriate amounts of standard solutions in 0.5 N HCl solution in 100 ml flasks. Such standard solutions prepared in 0.5 N HCl are stable for one week.

Mineralization of samples was carried out on the one hand by wet digestion with $HNO_3$ and $H_2O_2$ [6,9]. Samples were prepared by digesting 1 g of powdered and homogenized of each sample in a microwave furnace (Oven MILESTONE) with 7 ml $HNO_3$ 65% and 1 ml $H_2O_2$. Microwave furnace conditions are described in Table 1.

**Table 1. Sample burning procedure**

| Phase | Time (min) | Power (W) | Temp (°C) |
|---|---|---|---|
| 1 | 15 | 850 | 150 |
| 2 | 15 | 850 | 210 |
| 3 | 15 | 850 | 210 |

The digest was cooled and in order to facilitate the reduction of Se(VI) to Se(IV). 3 ml of concentrated HCl was added, then heated at 80ºC for 30 min [8]. After dilution to 15 ml with HCl 0.5n, Selenium content was determined on an aliquot by the hydride generation atomic absorption technique (a Thermo Electron Corporation SOLAAR S atomic absorption spectrometer equipped with a Thermo Electron Corporation VP 100 hydride generation system). 0.5% w/v $NaBH_4$ solution in 0.5% w/v NaOH was employed as a reducing agent that produced a volatile hydride of the analyte in contact with HCl.

Reductant is dispensed into the sample solution where it reacts to eliberate hydrogen; this in turn reduces the metal ions to volatile hydride, Se (IV) it is reduced to Se (-II). The hydrogen stream flushes the hydride into the heated quartz cell, where it is decomposed and the absorption of the metal measured. The detection limit of Se was 0.5 µg/l (ppb).

The content of selenium in plant, expressed in mg / kg (ppm) is calculated according to the formula:

$$Se\ (mg/kg\ s.u.) = \frac{a \times V \times F}{m \times 1000} \quad (1)$$

a = selenium content determined in the sample, readed the calibration curve, in mg/l

b = volume of sample obtained after calcination and filtration process, în ml;

F = dilution factor, taking into account the pre-reduction or dilution in case of high concentrations;

m = sample mass plant, in g;
1000 = conversion factor µg in mg;
The latter method, mineralization through dry oxidation was carried out in a muffle furnace [10]. 1 gram of the powdered dry plant is weighed in a platinum crucible and dried in an oven at 450ºC for 4 hours. After cooling, 1 ml HCl 6n are added to ashes. The sample is then slowly heated on a sand bath to dryness. This operation is repeated twice, with the addition of 1 ml HCl 6n. The dry residue is finally leached with 5 ml HCl 0.5n, and the solution is brought to 25 ml with HCl 0.5n too. From this flask, its taken 15 ml, 3 ml of concentrated HCl was added, then heated at 80ºC for 30 min. After cooling, the solution is brought to 25 ml with HCl 0.5n in order to facilitate the reduction of Se (VI) to Se (IV) same as it is described for the previous method. Selenium content was determined on an aliquot by the hydride generation atomic absorption technique.

## 3. RESULTS AND DISCUSSION

Data obtained about selenium content for these methods is showned in table 2.

The values of selenium content in plant obtained through *calcination* are situated between 0.006-0.065 mg/kg with average of 0.022 mg/kg, and those values of selenium content obtained through *microwave digestion* are situated between 0.002-0.042 mg/kg with average of 0.009 mg/kg**.**

It can be said that the values of selenium content from plant determined thorugh calcination were 2.4 times higher than those obtained through microwave digestion (the method through calcination did not involved losses of selenium, concordant the studies at the international level). In conclusion, to determine selenium content from plant, the calcination is favourably according these researchs. The averages of selenium content determined are size order according of *Kovalskii and Gololobov, 1969* [7] – ususally, in vegetal and animal organisms selenium content does not overtakes 0.6 mg/kg, on an average being $n \cdot 10^{-2}$ mg/kg.

The presented values in table 2 can be showned graphic in figures 1 and 2.

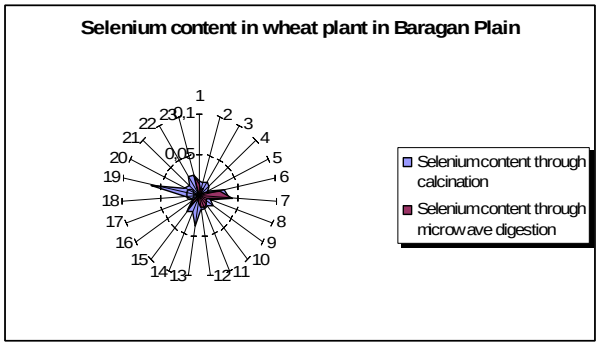


**Figure 1. Selenium content in wheat plant (mg/kg) in Bărăgan Plain**

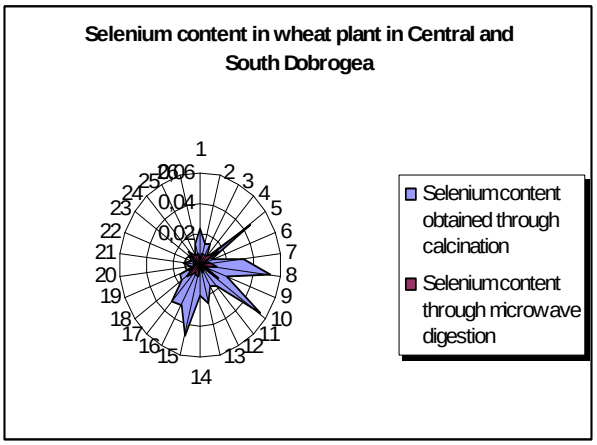


**Figure 2. Selenium content in wheat plant (mg/kg) in Central and South Dobrogea**

In figure 3 is showned a tendency map of selenium content distribution from green plant, map based on coordonates points, for these two studied areas, Bărăgan Plain and Central and South Dobrogea, map maded in acord with selenium content values obtained through calcination, method with good results. Points from coloured part of map, those from left side, represents selenium content values in wheat plant from Bărăgan Plain, and the more less coloured, right side of map, is represented by selenium content values in wheat plant from Central and South Dobrogea.

**Table 2 Selenium content in wheat plant prelevated from Bărăgan Plain and Central-South Dobrogea determined through these two method: calcination and microwave digestion**

| Nr. crt. | Localization | Calcination | Microwave digestion $HNO_3+H_2O_2$ |
|---|---|---|---|
| **Bărăgan Plain** | | | |
| 1 | S Slobozia | 0.017 | 0.015 |
| 2 | S Slobozia | 0.016 | 0.008 |
| 3 | S Slobozia 1 km N Drajna | 0.020 | 0.010 |
| 4 | E Drajna | 0.016 | 0.004 |
| 5 | E Drajna | 0.013 | 0.008 |
| 6 | Perişoru – Mărculeşti | 0.032 | 0.026 |
| 7 | Jegălia | 0.040 | 0.042 |
| 8 | E Ştefan cel Mare | 0.016 | 0.011 |
| 9 | 7 km before Feteşti | 0.021 | 0.011 |
| 10 | S Drajna to Călăraşi | 0.016 | 0.015 |
| 11 | S Drajna 2 | 0.018 | 0.016 |
| 12 | S Drajna 3 | 0.017 | 0.015 |
| 13 | N Călăraşi 15 km | 0.038 | 0.007 |
| 14 | N Călăraşi 7 km | 0.021 | 0.004 |
| 15 | NE Unirea | 0.025 | 0.021 |
| 16 | NE Unirea | 0.010 | 0.009 |
| 17 | NE Unirea | 0.011 | 0.002 |
| 18 | NE Unirea | 0.016 | 0.003 |
| 19 | NE Unirea | 0.065 | 0.004 |
| 20 | S Ţăndărei | 0.021 | 0.005 |
| 21 | S Ţăndărei | 0.018 | 0.002 |
| 22 | E Ţăndărei | 0.027 | 0.003 |
| 23 | V Giurgeni | 0.026 | 0.026 |
| **Central and South Dobrogea** | | | |
| 24 | SE Vadu Oii | 0.024 | 0.004 |
| 25 | N Hârşova | 0.014 | 0.008 |
| 26 | N Saraiu | 0.015 | 0.008 |
| 27 | Movilele Babei | 0.007 | 0.009 |
| 28 | SE Rahmanu | 0.046 | 0.016 |
| 29 | V Casimcea | 0.010 | 0.003 |
| 30 | V Sarighiol de Deal | 0.032 | 0.007 |
| 31 | S Râmnicu de Jos | 0.053 | 0.012 |
| 32 | V Cheia | 0.021 | 0.004 |
| 33 | E Cheia | 0.055 | 0.016 |
| 34 | N Mihail Kogălniceanu | 0.019 | 0.003 |
| 35 | V Sibioara | 0.015 | 0.006 |
| 36 | Ovidiu | 0.026 | 0.003 |
| 37 | Agigea V | 0.020 | 0.003 |
| 38 | Moviliţa | 0.047 | 0.007 |
| 39 | N Amzacea | 0.030 | 0.007 |
| 40 | SV Comana | 0.031 | 0.006 |
| 41 | N Negru Vodă | 0.018 | 0.013 |
| 42 | SE Movila Verde | 0.016 | 0.006 |
| 43 | S Negreşti | 0.006 | 0.004 |
| 44 | S Cobadin | 0.012 | 0.004 |
| 45 | E Pietreni | 0.006 | 0.004 |
| 46 | Adamclisi – vale | 0.011 | 0.003 |
| 47 | Ion Corvin – vale | 0.007 | 0.012 |
| 48 | S Alimanu | 0.017 | 0.007 |
| 49 | S Cochirleni | 0.014 | 0.007 |

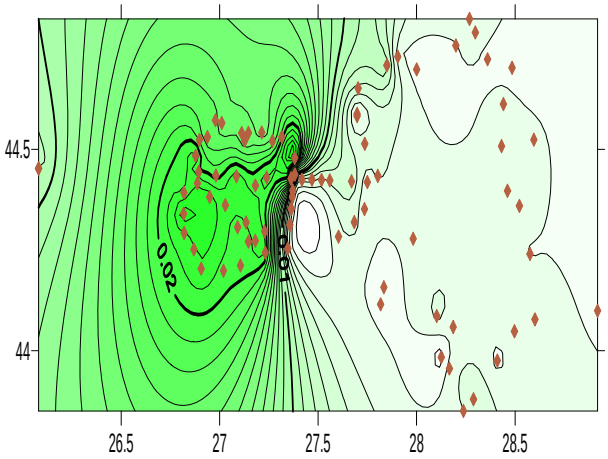


**Figure 3. Tendency map of selenium content distribution in green plant for these two studied zones**

## 3. CONCLUSIONS

For determining selenium content in plant, the method through calcination is better than microwave digestion because does not involved losses of selenium.

It can be said that the values of selenium content from plant determined though calcination were 2.4 times higher than those obtained through microwave digestion.

The selenium content in plant obtained through *calcination* had an average value of 0.022 mg/kg, and that obtained in microwave digestion had an average value of 0.009 mg/kg**.**

It was made a tendency map of selenium content distribution from green plant, map based on coordonates points, for these two studied areas, Bărăgan Plain and Central and South Dobrogea, map maded in acord with selenium content values obtained through calcination, method with good results.

## 4. ACKNOWLEDGEMENT

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