# NEW APPROACH FOR WHEAT GRAIN ELEMENTAL ANALYSIS BASED ON ED(P)-XRFS METHOD

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*The paper addresses the concentration measurement of the essential and detrimental elements occurring into three Romanian wheat grain sample using energy dispersive polarized X-ray spectrometry (ED(P)-XRFS). The heavy metals (HMs) concentrations into Romanian wheat grain are below the limits specify by the Commission Regulation and the Food and Agriculture Organization. The paper underpins the adequacy of the ED(P)-XRFS method for assessing the wheat grain macro-mineral, micro-mineral and HMs contents. The novelties addressed are the method and technique used to measure the mineral contents into wheat grain and the data for wheat grains harvested from three areas of Baragan.*

**Keywords**: *ED(P)-XRFS, wheat,* macro-mineral, micro-mineral *heavy metals*

## 1. Introduction

The health risk assessment caused by dietary intake through wheat grain consumption has gained more attention during years [1-5]. It became a matter of evidence that food contamination is an important pathway for the entry of heavy metals (HMs) into the human body [1,2]. The wheat (*Triticum aestivum L.*) is one of the most consumed cereals by human beings and it is one of the main source of nutrients [3]. The global production of wheat grain was more than 750 million metric tons in 2016 [4] and more than 8 million metric tons in Romania [5]. Due to the high consumption of wheat in a variety of food all over the world, wheat gluten, proteins, fibers and elemental composition analysis are required to estimate if it is adequate for human consumption. Wheat flour is the basic ingredient for the preparation of bread, cakes, pasta and other bakery products.

The human life quality depends on the presence of minerals in the daily diet. Ca, Mg, P, K are considered macro minerals, whereas Fe, Zn Cu, Mn, Ni, Se,

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V are defined as essential micro minerals. Some elements are considered as non-essential trace elements e.g., B, Ti, Sb, As, Ba, Ce, Ge, Sr, Rb. The HMs at high concentrations exhibit toxic effects on human body [3, 7-9]. Elements such as P, Ca, Mg, K and Cl are required in large quantities (grams) for a healthy nutrition because they are essential for disease prevention. The K and Mg are essential for maintaining normal cardiac rhythm and normal fluid balance in cells [10]. Ca is involved in contraction and relaxation of muscles while P is essential for growth and renewal of tissues [10]. Fe, Cu, Mn and Zn are essential in the enzymes metabolism. Fe is responsible for good health maintenance and Zn acts as an antioxidant, while Mn plays a vital role in diabetes control [11-13]. Co deficiency cause the decreasing of immune system function [11]. Excessive quantities of Fe, Cu, Mn, Zn, Co can have toxic effects [12-14]. Pb, Hg and Cd are reported as toxic even in trace amounts and they are considered as high-risk factors to human health in general, also it has been shown to have carcinogenic effects [2, 14].

Several papers reported the HMs measurements in wheat grains [15-20], but none of them used ED(P)-XRFS technique. XRFS is a rapid method for accurate and precise elemental analysis used in geochemistry, cement and fuel industries [21-23]. Thus, ED(P)-XRFS has become a reliable, sensitive, quantitative multi-elemental analysis and non-destructive technique. Also, ED(P)-XRFS is suitable for grain food analysis due to its analytical performances and minimal sample preparation. The limit of detection and the limit of quantification of the ED(P)-XRFS technique are greater than that of ICP or AAS, therefore special care should be paid to the measurement uncertainty in the case of ED(P)-XRFS. This study aims to demonstrate the adequacy of the ED(P)-XRFS method for the measurement of the wheat grain mineral composition. Also, this study should be considered as a preliminary endeavor as there is no literature on elemental concentration measurements carried out on wheat grain using ED(P)-XRFS. Besides, there is a lack of information about heavy metal occurrence into Romanian wheat grains. This gap will be easily filled if ED(P)-XRFS will be adopted as a standard method because it is the most efficient one among AAS, ICP, ICP-MS methods.

The novelty addressed by the paper are the method and technique used to measure the heavy metal in wheat grain harvested form the main area of wheat crop in Romanian i.e *Baragan*.

## 2. Materials and methods

The wheat grain samples were collected from *Baragan*, which is the main wheat-growing area of Romania known. The exact mention of the sampling places is not important for this study and, from commercial reasons, the names of the sampling areas denotes as A1, A2 and A3. The wheat harvesting practice consists

in a preliminary deposition of wheat crop as a batch that contains the crop of about 10 ha. The samples take advantage of this practice and drew a 10x10 mesh whose rectangles were numbered from 0 to 99. Ten rectangles were randomly chosen based on ASTM E 826-85 recommendations [24]. 1 kg of grains was collected from each sampled rectangle using a Vintage sampling probe. Wheat samples were dried in an oven at 105 °C until constant mass was achieved. The wheat specimens were grinded in a Retch type ball mill. Grist specimens were prepared in the form of pressed powder pellets using 12.5g of grist that was mixed with 2.8g of Cereox binder. Subsequently, the mixture was homogenized in the Retch mill using specific balls. 7.65g of mixture was poured in a Specac press die. The die was vibrated to ensure the uniform distribution of the grist at bulk level. The mixture was pressed into a 40mm die diameter using a Specac equipment at 15 tf pressure for 60s.

A Spectro XEPOS equipped with a 3D X-ray polarizing geometry, provided by AMETEK Materials Analysis Division, was used to perform the elemental analysis. The XEPOS analytical domain rages from Z=11 to Z=92. In this regard, some specimens were analyzed using the Turboquant, FP and Lucas-Tooth analytical method to establish which one is the best. Finally, the Turboquant was selected. Standard deviation and LOQ were the criteria for establishing the best calibration method and the associated specimen sample preparation procedure. The ED(P)-XRFS reported results for each measured concentration is the average of three measurements carried out, in reproductive conditions, on three pellets of the same wheat grain sort.

The Student test was used to estimate the significance of the differences among the concentrations of the same element in different wheat grain sorts. A difference has to be considered as significant if the calculated t-value is greater than the tabulated value for the 0.05 significance level.

The expanded measurement uncertainty (U) was estimated taking into account the GUM procedure [23,25]. The U of each measuring was estimated using an extended coefficient k = 2 for the 0.95 confidence level.

## 3. Results and discussions

The ED(P)-XRFS results obtained on $A_1$, $A_2$ and $A_3$ specimens support the existence of the major elements (Ca, Cl, K, Mg, Si, S, P) and minor elements (Cu, Fe, Mn, Mo, Nb, Ni, Pb, Rb, Sn, Sr, Y, Zn and Zr). In this regard for a better interpretation, the obtained ED(P)-XRFS results were divided into four categories depending on the classification of elements as: 1) macro-minerals; 2) micro-minerals; 3) non-essential trace elements; 4) heavy metals. The concentration values of the Ca, Mg, K, P, Na support their classification as macro-minerals which is in accordance with the literature data [19, 25-28]. The Si, S, Cl have to

be included in this category as their concentrations are comparable to the above-mentioned elements. The micro-minerals are Fe, Zn, Cu, Mn, Se, V. The non-essential trace elements are: As, B, Ti, Sb, Ba, Ce, Ge, Sr, Rb.

Taking into account their toxic effect similarity As, Cr, Ni, Cu, and Zn, where included in the HMs category even that they do not belong to the seventh group of the Mendeleev's Table [29]. Thus, the conventional HMs group consists of: As, Cd, Cr, Cu, Hg, Ni, Pb and Zn. Based on the preceding, the obtained results are given for each category in separate tables. Thus, the concentration of the major elements Ca, Cl, K, Mg, Si, S and P occurring into each wheat grains samples are given in Table 1.

*Table 1.*

**The concentrations of the macro-elements in wheat grains collected from Romania, *Baragan* and the assigned measurement uncertainties (U)**

| Elements | Sampling area | | | | | |
|---|---|---|---|---|---|---|
| | $A_1$ | | $A_2$ | | $A_3$ | |
| | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** |
| **Ca** | 830 | 21 | 910 | 20 | 890 | 22 |
| **Mg** | 420 | 16 | 405 | 14 | 438 | 17 |
| **K** | 6780 | 60 | 7350 | 58 | 7250 | 64 |
| **P** | 3460 | 62 | 3610 | 66 | 3820 | 64 |
| **Si** | 1040 | 28 | 950 | 28 | 1110 | 27 |
| **Cl** | 540 | 14 | 570 | 13 | 530 | 14 |
| **S** | 1190 | 24 | 1070 | 22 | 1280 | 22 |

Grain cereals taken from zone A3 showed the highest concentrations of Mg (438 µg / g), P (3820 µg / g), Si (1110 µg / g) and S (1280 µg / g) as compared to the other two areas. Wheat grain taken from the second zone provided the highest concentration of K (7350 µg / g), Cl (570 µg / g) and Ca (910 µg / g) and the lowest concentration of Mg, S and Si. The t test indicates significant differences in the wheat grain types with respect to the concentration of Ca, K, Mg, Si and P. The concentrations of the micro mineral elements are given in Table 2.

The HMs concentrations into wheat samples are quite similar as shown in Table 2. Wheat sampled from 3$^{rd}$ area has the highest concentration values for the following elements: Cu (17 µg/g), Fe (52 µg/g), Mn (22 µg/g), and Zn (27 µg/g). Wheat sampled from 2$^{nd}$ area shows the highest values for the concentration of Sn (16 µg/g) and the smallest values for the concentration of Fe (41 µg/g), Mn (18 µg/g) and Zn (27 µg/g). The Mn, Ni, Fe, Sn, Zn and Zr concentrations do not differ significantly between wheat grains from different areas (Table 2). The concentration values of As, Cd and Hg were below quantification limits. The t test applied to the HMs concentrations has shown that the concentration values of the same element into three samples do not significantly differ.

*Table 2*

**The concentrations of the micro-elements in wheat grains collected from Romania, *Baragan* and the assigned measurement uncertainties (U)**

| Elements | $A_1$ | | $A_2$ | | $A_3$ | |
|---|---|---|---|---|---|---|
| | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** |
| **Cu** | 15 | 3 | 14 | 3 | 17 | 8 |
| **Fe** | 46 | 2 | 41 | 3 | 52 | 10 |
| **Mn** | 19 | 3 | 18 | 3 | 22 | 3 |
| **Sn** | 15 | 4 | 16 | 3 | 14 | 3 |
| **Zn** | 28 | 3 | 27 | 3 | 31 | 4 |
| **Zr** | 32 | 0.5 | 31 | 1 | 29 | 1 |

*Table 3*

**The concentrations of the heavy metals in wheat grains collected from Romania, *Baragan* and the assigned measurement uncertainties (U)**

| Elements | $A_1$ | | $A_2$ | | $A_3$ | |
|---|---|---|---|---|---|---|
| | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** | **Mean [µg/g]** | **U [95%]** |
| **As** | 0.45 | 0.10 | 0.51 | 0.09 | 0.43 | 0.09 |
| **Cd** | 0.10 | 0.05 | 0.11 | 0.05 | 0.10 | 0.05 |
| **Cu** | 3.4 | 0.15 | 3.5 | 0.1 | 3.4 | 0.15 |
| **Hg** | <0.05 | - | - | - | - | - |
| **Ni** | 1.2 | 0.5 | 1.3 | 0.7 | 1.7 | 0.7 |
| **Pb** | 0.3 | 0.1 | 0.34 | 0.07 | 0.3 | 0.1 |
| **Zn** | 28 | 8 | 27 | 8 | 31 | 7 |

HMs average concentrations in wheat samples do not exceed the tolerance limits (Table 4) [1]. In this case, further measurement has to be performed. According to the international legislation on wheat grain and foodstuff (Table 3) [2, 3, 29, 30], the Ni, Pb, Sn concentrations were below the tolerance limits.

*Table 4*

**Permitted levels of heavy metals in wheat (µg/g)**

| Criteria | Hg | As | Cd | Cr | Pb | Cu | Zn | Ni |
|---|---|---|---|---|---|---|---|---|
| European Commision (EC 2001) | - | - | 0.24 | - | 0.24 | - | - | - |
| Food and Agriculture Organization and World Health Organization (FAO/WHO 1984) | - | - | 0.21 | 0.02 | 0.43 | 3.0 | 27.4 | 1.63 |
| Agricultural standard in China (NY861-2004) | 0.02 | 0.7 | 0.1 | 1.0 | 0.4 | 10 | 50 | - |
| Chinese Hygiene Standard for wheat (GB2762-2012) | 0.02 | 0.5 | 0.1 | 1.0 | 0.2 | - | - | 1.0 |

The average elemental concentration of tested wheat samples is in fair agreement with those reported by different surveys conducted in different countries (Table 5).

*Table 5*

**Reported data for wheat grain concentration [µg/g]**

| Fe | Mn | Zn | Cu | As | Cd | Pb | Literature source |
|---|---|---|---|---|---|---|---|
| 41-52 | 18-22 | 27-31 | 3.4-3.5 | 0.43-0.51 | 0.10-011 | 0.3-0.34 | This study |
| - | - | 12.06-80.33 | 2.43-6.83 | 0.029-0.086 | 0.006-0.179 | 0.017-1.158 | [2] |
| 28.8-50.8 | - | 13.5-34.5 | - | - | - | - | [27] |
| 8.5-84.1 | - | 4.6-41.4 | - | | | | [28] |
| 21.4–62.5 | 28.4–83.7 | 7.8–56.4 | 1.25–6.93 | - | - | - | [3] |

Fe, Cu, Zn and Mn concentrations are close to the values reported in the literature as shown in Table 3, The Cu, Mn and Fe concentrations measured in this study definitely fall in the high concentration range while Zn falls in the middle.

The average concentration values of HMs into analyzed sampled are of the same order with the quantification limits of the ED(P)-XRFS calibration curves. Therefore, the comparison of the measured values to the permitted limits (Table 4) is critical due to the great Us assigned to these results. Albeit with, the ED(P)-XRFS results show that the HMs contents are of the same order to the analyzed samples, but more important, the HMs contents curve measured more accurate by improving the LOQ of the XEPOS calibration curves.

## 6. Conclusions

The occurring pattern for wheat grains from all sites is Mn > Fe > Zn > Sn > Cu > Zr > Y > Ni > Rb > Sr > Mo > Nb > Pb.

The concentrations of the essential trace elements Zn, Cu, Mn and Fe which are necessary for maintain the life process are consistent with the allowable limits.

The achieved knowledge and results attest the ED(P)-XRF as a candidate for the most efficient method for fast screening of the mineral content into wheat grains.

The t tests show that there are significant differences between the sites regarding the concentration of Ca, K, Mg, Si and P. The test carried on concentration of heavy metals attest that there are no significant differences among the tested wheat grains.

The measured concentrations of HMs in wheat grains collected from three different sites.

Further work has to be done to improve the limit of quantification and the exactness of the ED(P)-XRF for the HMs measurement developing of a new

specimen preparation method of the melted bead type. Also, the developing of a new specimen preparation method dedicated to wheat have to be considered. Secondly, a more adequate calibration method dedicated to wheat grains have to be developed.

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