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1 *Artificial cellulose standards as calibration standards for wavelength-dispersive*  
2 *X-ray fluorescence analysis of elements in plant samples*

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14 **Highlights**

- 15 • Synthetic cellulose standards can be successfully used for calibration  
16 • Thin layer method is equally good as other methods (for higher concentrations)  
17 • The accuracy of standardless method is similar to other methods (for higher conc.)  
18 • The addition of wax binder improves the precision  
19 • The best results were obtained for 4th period elements of the Periodic Table

20 **Abstract**

21 This research explores the possibilities and limitations of WD-XRF, applied as a method for  
22 quantification of 20 elements in plant material, using spiked cellulose standards for calibration. Three  
23 different analytical methods were investigated: 1) standards created from pure spiked cellulose; 2) spiked  
24 cellulose mixed with 20 % of binder and 3) spiked cellulose applied as a thin layer on an inert carrier.  
25 Sensitivity, linearity, limit of detection, limit of quantification, repeatability, intralaboratory  
26 reproducibility, and accuracy were determined and compared. The accuracy of the investigated methods  
27 was tested by analysis of standard reference materials and comparison with other routinely used analytical  
28 techniques (ICP-OES and ICP-MS). The comparison included real plant samples which were collected  
29 from the environment characterized by different pollution levels. The accuracy of the semiquantitative  
30 standardless method was also considered and compared with other investigated methods. Tested methods  
31 can be very precise, with good intralaboratory reproducibility over wide linear range.

32 **Keywords**

33 Green chemistry, accuracy, precision, sample preparation, WD-XRF, method comparison

34

# 1. Introduction

Wavelength-dispersive X-ray fluorescence (WD-XRF) is a non-destructive analytical technique with a wide analytical range (from  $\text{mg}\cdot\text{kg}^{-1}$  concentration up to 100 %)(1,2,11–15,3–10). It is based on irradiation of the sample with X-rays and measurement of the resulting fluorescence. Since every element exhibits characteristic fluorescence, this analytical method can be used for the determination of almost all elements of the periodic table. Metals, nonmetals, and/or metalloids can be analyzed at the same time, regardless of the chemical composition of the sample (1,2,8). It is important to mention that the sample matrix has a strong influence on the results, and thus requires a careful calibration strategy, which is especially important when *e.g.*, plant samples, containing a high amount of organic matter, are analyzed.

WD-XRF is usually utilized for analysis of solid samples. Samples are often prepared as a pressed pellet, which is practical and easy to use. It requires a minimal amount of chemicals (compared to wet chemical analysis), which represents an important advantage from economic point of view, as well as from green chemistry standpoint (7). The interaction of X-ray fluorescence applied to solid samples is rather predictable. Therefore, semiquantitative calibration methods, based on fundamental parameters (8,11,16–21) with appropriate matrices, have been developed. These methods are standardless and allow a fast estimation of the composition of a sample, ranging from Na to U, without requiring application of calibration standards.

WD-XRF has a high potential as a fast and affordable direct screening method for the analysis of plant samples, especially for the recognition of bioaccumulation species (9,13). On the other side, it can also be applied in agriculture for measurement of nutrients such as K and P, without any demanding sample preparation procedures (18).

Since plant materials have a high content of cellulose, it is possible to prepare standards for quantitative analysis by spiking crystalline cellulose with known amounts of standard solutions (2,5,22–27). This approach was already tested and proved as feasible, but only for a limited number of elements (*e.g.* Mg, Al, K, Ca, Mn, Fe, Cu, Sr, and Pb) (28). We investigated the quantification performance of WD-XRF, by using cellulose standards containing twenty elements, whereby the specific elements were chosen according to their relevance for environmental studies, but also representative for the whole periodic table. Sensitivity, linearity, limit of detection, limit of quantification, precision (repeatability and intralaboratory reproducibility), and accuracy were determined for these elements. Na, Mg, and Al served as a representative for light elements, whereas Cr, Mn, Fe, and Cu, as atypical examples for transition metals, together with Pb and Bi represented the heavy metals. Compared to other studies, that have investigated only a few elements, we have performed a comprehensive study on twenty elements, aiming to determine the possibilities and limitations of WD-XRF for plant analysis, and moreover evaluated three different ways for plant sample preparation.

Plant samples have been prepared in three different ways (29): i) direct press of plant material without binder; ii) direct press with addition of a binder and iii) preparation of a thin sample layer on the surface of boric acid (30). Thin layer preparation can be advantageous when only a limited amount of sample is available.

Accuracy was assessed by analysis of certified reference materials, and comparison of the results obtained with inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled

1 plasma mass spectrometry (ICP-MS). Additionally, in present study we included real plant samples with  
2 different pollution levels, from several plant species.

3 Finally, we investigated the internal standardless method UniQuant (UQ), based on fundamental  
4 parameters, which in our case uses UQ software (21). This method is fast, rapid and applicable for all  
5 elements heavier than Na. It is considered to be only semiquantitative, but has not been tested in  
6 environmental studies.

## 7 **2. Materials and methods**

8 Three types of pressed pellets were investigated. The first pellet type was prepared solely from spiked  
9 cellulose material, and it was used for the creation of Pure Cellulose (PC) method. Although the physical  
10 properties of the crystalline cellulose allow the preparation of pellets which are stable under high vacuum  
11 and intensive X-ray radiation, it is less likely that pellets prepared from plant material will be equally  
12 stable. It is of great importance for matrices of analyzed samples to match as much as possible the  
13 calibration standards. Therefore, the second type of pellets was prepared from the mixture of spiked  
14 cellulose (or sample material) with binder (wax). They were used to create Cellulose Wax (CW) method.  
15 Pellets prepared in this way have better stability and much smoother surface, which is important for the  
16 quality of the measurements. The third pellet type requires a smaller amount of sample, since sample  
17 material is being evenly applied as a thin layer onto the surface of a much larger mass of boric acid,  
18 which serves as the inert carrier (30). This type of synthetic pellets was used for the creation of Thin  
19 Layer (TL) method. Figures of sample preparation procedure are provided in the Supplementary  
20 Materials.

### 21 **2.1 Preparation of synthetic reference material powders and pressed pellets**

22 Synthetic reference materials were prepared using pure cellulose powder (Acros Organics C.A.S. number:  
23 9004-34-6), spiked with an appropriate volume of Multi-Element Plasma Standard Solution 4, Specpure®  
24 (Alfa Aesar), in order to obtain the desired concentration levels (0, 10, 20, 50, 100, 200 and 500 mg·kg<sup>-1</sup>).  
25 For each concentration, three batches of 8.0 g cellulose were spiked with an appropriate volume of 1000  
26 mg·kg<sup>-1</sup> standard solution that contained 20 elements (Ag, Al, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Mg,  
27 Mn, Na, Ni, Pb, Sr, Tl, and Zn). After the addition of liquid standard solution, the changes of the cellulose  
28 matrix were minimal and no significant weight loss was observed (16). Spiked cellulose was dried  
29 overnight at 60 °C, in order to evaporate excess moisture. Afterward, it was homogenized in an agate  
30 mortar and dried again at 60 °C, until the constant weight was obtained.

31 Each batch of spiked cellulose was divided into 3 parts. The first two parts were used for the preparation  
32 of the pellets for Pure Cellulose (PC) and Cellulose Wax (CW) methods. The third part of each batch was  
33 saved for ICP-OES analysis, in order to confirm that the analyte concentrations in the reference materials  
34 matched the expected concentrations. Amounts of spiked cellulose were approximately: 4.0 g, 3.0 g, and  
35 0.70 g respectively. CW pellets contained 20 % weight (wt) of binder (Hoechst wax C micropowder,  
36 Merck, C.A.S. number: 110-30-5).

37 Another series of in-house reference material was prepared by the Thin Layer (TL) method. Pellets were  
38 prepared from batches containing 2.0 g of spiked cellulose with the same concentrations as for the PC and  
39 CW methods. Each batch was divided into three replicates containing approximately 0.70 g of spiked  
40 cellulose. Stated quantity of spiked cellulose was mixed with 20 % wt Hoechst wax C micropowder.

1 Homogenized mixtures of wax and spiked cellulose were carefully placed as a thin layer onto the surface  
2 of 3 g of boric acid, which served as an inert carrier (Supplementary material).

3 Pellets with certified reference materials, NIST SRM 1573a Tomato leaves and NIST SRM 1575a Pine  
4 needles, were prepared according to CW and TL preparation procedures, in order to investigate the  
5 accuracy of the methods. Further investigation of accuracy relied on a comparison of results gained from  
6 the WD-XRF and ICP-MS analyses of real samples, collected from the environment. For this purpose,  
7 each of the 33 conifer plant samples was divided into two aliquots, one for the preparation of pellets and  
8 the other for digestion, necessary for ICP-MS analysis. The selection of plant samples aimed to provide a  
9 wide range of concentrations and physical properties of the sample matrices. Samples were collected from  
10 locations with different pollution levels (urban and rural environments). The age of the needles varied  
11 from a few weeks to one year old, and in addition, 3 different plant species were included in the study.

12 The conifer samples, *Pinus nigra* and *Abies alba*, were dried at room temperature and homogenized with  
13 an ultra-centrifugal mill (Retsch ZM 200, Retsch GmbH, Haan, Germany), equipped with a 12-tooth  
14 titanium rotor and a 1.0 mm titanium sieve. The pellets for WD-XRF analysis were prepared with the  
15 same procedure as the in-house reference material, containing 20 % wt of wax binder.

16 All pellets (standards and samples) were 32 mm in diameter. They were created by exposure to a pressure  
17 of 20 t, applied for 5 minutes using a pellet press (Retsch PP 25, Retsch GmbH, Haan, Germany).

## 18 **2.2 Digestion of plant samples**

19 Digestion of spiked cellulose aliquots was completed in an open system, on a water bath by gradual  
20 addition of 35 cm<sup>3</sup> concentrated nitric acid (Sigma-Aldrich, C.A.S. number: 7697-37-2) and 8 cm<sup>3</sup> of 30  
21 % hydrogen peroxide (Sigma-Aldrich, C.A.S. number: 7722-84-1).

22 For ICP-MS analysis, conifer samples were digested in a microwave digestion system (MLS  
23 UltraCLAVE) with 5 cm<sup>3</sup> of distilled concentrated nitric acid (65% p.a., Carl Roth). All samples were  
24 analyzed in triplicate as well as blanks and certified reference materials, NIST SRM 1573a Tomato leaves  
25 and NIST SRM 1575a Pine needles, which were used for quality control.

## 26 **2.3 Analytical apparatus**

### 27 **2.3.1 Wavelength dispersive X-ray fluorescence spectrometer (WD-XRF)**

28 An ARL™ PERFORM'X Sequential X-Ray Fluorescence Spectrometer (Thermo Fisher Scientific,  
29 Switzerland), equipped with a 4.2 kW Rh X-ray tube with a 50 µm Be window, was used. During the  
30 analysis, four different crystals (AX03, LiF200, LiF220, and PET), two collimators (0.4 and 1), and a  
31 tandem of detectors (Flow proportional counter-FPC and Scintillation counter-SC) were applied. The  
32 analysis was performed in a high vacuum (< 1 Pa). Table 1. shows the analytical lines and parameters for  
33 the analyzed elements.

34 Table 1. Analytical lines and parameters of analyzed elements

| <i>Element</i> | <i>Line</i> | <i>Wavelength (Å)</i> | <i>Crystal</i> | <i>Detector</i> | <i>Count time (s)</i> | <i>Filter</i> |
|----------------|-------------|-----------------------|----------------|-----------------|-----------------------|---------------|
| <i>Na</i>      | Ka 1,2      | 11.9101               | AX03           | FPC             | 40                    | None          |
| <i>Mg</i>      | Ka 1,2      | 9.89                  | AX03           | FPC             | 40                    | None          |
| <i>Al</i>      | Ka 1,2      | 8.3401                | PET            | FPC             | 24                    | None          |

|           |        |        |        |     |    |            |
|-----------|--------|--------|--------|-----|----|------------|
| <b>K</b>  | Ka 1,2 | 3.7424 | LiF200 | FPC | 24 | None       |
| <b>Ca</b> | Ka 1,2 | 3.3595 | LiF200 | FPC | 24 | None       |
| <b>Cr</b> | Ka 1,2 | 2.291  | LiF200 | FPC | 24 | None       |
| <b>Mn</b> | Ka 1,2 | 2.1031 | LiF200 | FPC | 24 | None       |
| <b>Fe</b> | Ka 1,2 | 1.9374 | LiF200 | FPC | 24 | None       |
| <b>Co</b> | Ka 1,2 | 1.7903 | LiF200 | FPC | 24 | None       |
| <b>Ni</b> | Ka 1,2 | 1.6592 | LiF200 | SC  | 24 | None       |
| <b>Cu</b> | Ka 1,2 | 1.5418 | LiF200 | SC  | 24 | None       |
| <b>Zn</b> | Ka 1,2 | 1.4364 | LiF200 | SC  | 24 | None       |
| <b>Ga</b> | Ka 1,2 | 1.3414 | LiF200 | SC  | 24 | None       |
| <b>Sr</b> | Ka 1,2 | 0.8766 | LiF200 | SC  | 16 | None       |
| <b>Ag</b> | Ka 1,2 | 0.5609 | LiF200 | SC  | 40 | Cu 0.27 mm |
| <b>Cd</b> | Ka 1,2 | 0.5365 | LiF200 | SC  | 40 | Cu 0.27 mm |
| <b>In</b> | Ka 1,2 | 0.5136 | LiF200 | SC  | 24 | Cu 0.27 mm |
| <b>Tl</b> | La 1   | 1.2074 | LiF200 | SC  | 24 | None       |
| <b>Pb</b> | La 1   | 1.175  | LiF200 | SC  | 24 | None       |
| <b>Bi</b> | La 1   | 1.1439 | LiF200 | SC  | 24 | None       |

1

## 2 **2.4 Calculation of limit of detection and limit of quantification**

3 The limits of detection (LoD) were calculated (Eq. 1) as a quotient of three standard deviations of the  
4 ordinate intercepts, divided by the slope of the regression line (34) (Table 3):

$$5 \quad LoD = \frac{3\sigma}{slope} \quad (Eq. 1)$$

6 The limits of quantification (LoQ) were calculated (Eq. 2) as a quotient of ten standard deviations of the  
7 ordinate intercepts, divided by the slope of the regression line (Table 2):

$$8 \quad LoQ = \frac{10\sigma}{slope} \quad (Eq. 2)$$

9 The only difference between equations 1 and 2 is in the factor multiplying the standard deviation,  
10 therefore the same observations and conclusions can be drawn for quantification limits as for limits of  
11 detection.

## 12 **2.5 Determination of accuracy**

13 The first step in the characterization of the accuracy was to estimate the rate of accuracy deterioration, in  
14 case when analyte concentrations approach the detection limit. For this purpose, calibration pellets were  
15 analyzed again as unknown samples. They were chosen because they contain all elements of interest in a  
16 wide range of concentrations, which gradually decrease in regular order (from 500 mg·kg<sup>-1</sup> to 10 mg·kg<sup>-1</sup>).

17 In the next step, it was confirmed that the spiked cellulose material indeed contained the desired levels of  
18 analytes. This was achieved by digesting aliquots of spiked cellulose, which were later analyzed by ICP-  
19 OES.

20 In the final step, it was necessary to verify that the tested methods (CW, TL, and UQ) produce results  
21 with satisfying accuracy, when they are applied to the real plant material. This was accomplished by two

1 means: 1. Two types of SRM were analyzed by CW, TL and UQ methods; 2. A large number of plant  
2 samples was divided into aliquots which were later analyzed by ICP-MS as well as with WD-XRF.

3 The accuracy of an analytical procedure is calculated according to the criteria expressed by Eq. 3 (35).  $C_x$   
4 is the measured concentration on the analyte,  $C_w$  is the true (reference) value and  $\sigma$  is the standard  
5 deviation of  $C_x$ . The formula quantifies systematic errors ( $C_x - C_w$ ) as well as random errors ( $2\sigma$ ) and  
6 produces results given as a percentage of the sum of errors, compared to the reference value ( $C_w$ ). Since  
7 the results are expressed as relative values (%), the accuracies for different elements and concentrations  
8 can be mutually compared.

9 
$$TE = \frac{|C_x - C_w| + 2\sigma}{C_w} * 100 \quad (\text{Eq. 3})$$

10  
11

1

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### 3 **2.3.2 UniQuant**

4 UniQuant (21) (ThermoFisher Scientific, Integrated version) is a standardless semi-quantitative to  
5 quantitative method for XRF analysis. It is based on a software program that relies on fundamental  
6 parameters and proprietary algorithms for data processing. Calculations are based on physical equations  
7 and other parameters, such as elementary mass absorption coefficients and X-Ray tube spectral  
8 distribution. These complex algorithms can make matrix and inter-elemental effect corrections in order to  
9 obtain accurate results.

### 10 **2.3.3 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)**

11 Multi-elemental analysis of digested aliquots of synthetic reference materials was carried out with an  
12 Ultima 2 ICP-OES spectrometer (Horiba Scientific, France). The elements were observed in radial mode.  
13 A dual photomultiplier was used as detection system. A total of 20 elements were analyzed: Na (588.995  
14 nm), Mg (285.213 nm), Al (396.152 nm), K (766.490 nm) Ca (422.673 nm), Cr (205.552 nm), Mn  
15 (257.610 nm), Fe (259.940 nm), Co (228.616), Ni (341.476), Cu (327.396), Zn (206.191 nm), Sr (421.552  
16 nm), Ag (328.068 nm), Cd (214.438 nm), In (303.936 nm), Tl (190.800 nm), Bi (223.061 nm) and Pb  
17 (216.999 nm). The optical part of the instrument (slits, photomultiplier voltage) was tuned by the  
18 instrument autotuning procedure based on the measurement of real samples.

### 19 **2.3.4 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)**

20 ICP multi-element standard solution VI for ICP-MS CertiPUR® (Merck) was used for the preparation of  
21 calibration standards. Solutions of Ge, In, and Lu were used as internal standards. Measurements were  
22 performed with an ICP-MS/MS (Agilent 8800 ICP-QQQ).

23

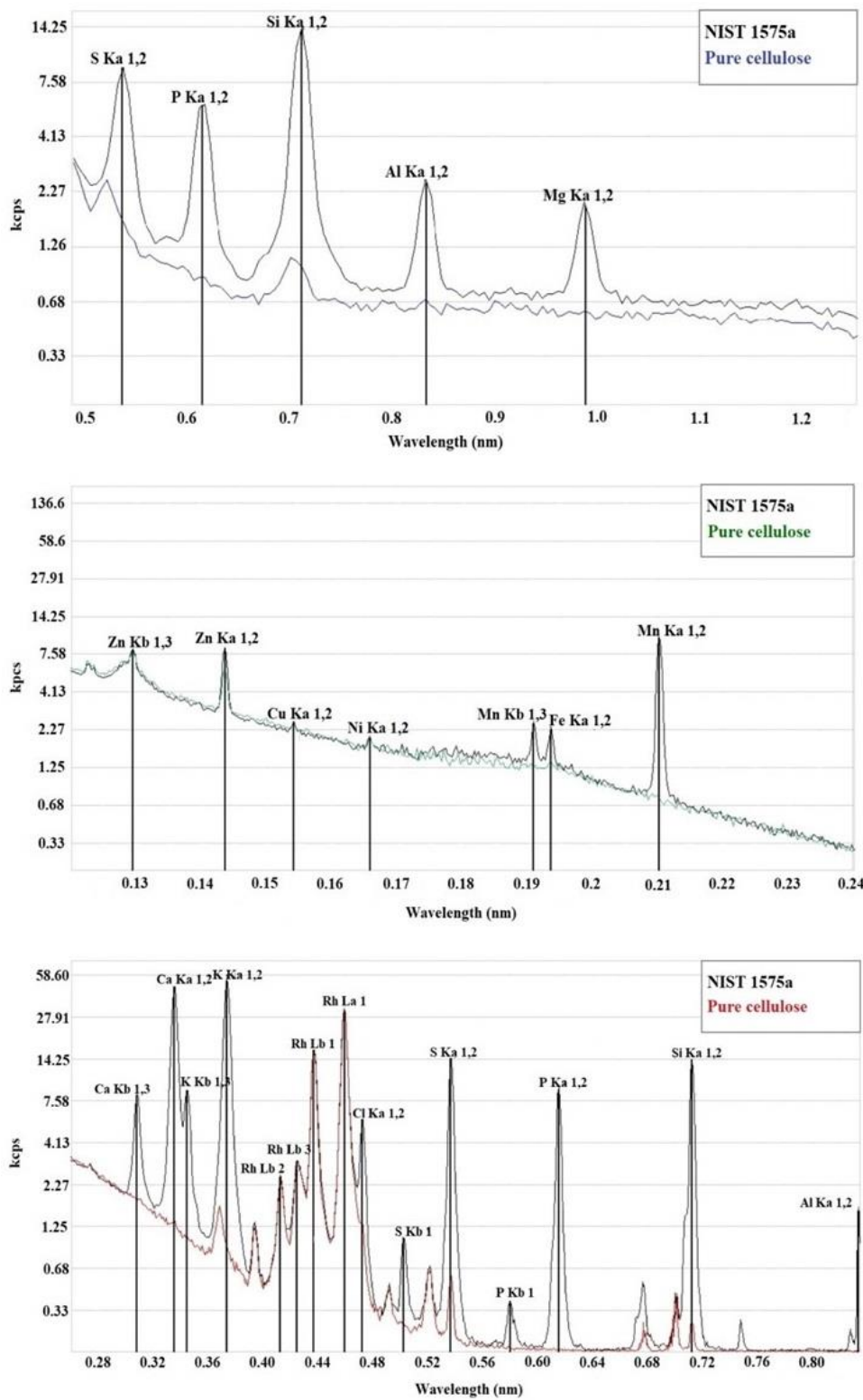
## 24 **3. Results and discussion**

### 25 **3.1 Qualitative analysis**

26 Cellulose and plant materials consist mainly of light elements (C, N, H, O), and therefore they should  
27 most likely exhibit similar matrix effects (31). This assumption was first tested by overlapping (Fig. 1)  
28 the spectra of pure cellulose and NIST SRM 1575a, which was chosen as a representative example. As it  
29 can be seen from the Fig. 1, plant material and cellulose are characterized with baselines with the same  
30 shape and often even with identical intensities.

31 According to obtained qualitative analysis spectra it can be observed that the chosen analytical spectral  
32 lines are well defined and without significant overlaps. It also demonstrated that cellulose, which was  
33 used for the preparation of synthetic reference materials, does not contain any significant amount of  
34 impurities. Zn and Cu peaks originate from instrument sample holders. Replacing them with holders  
35 created from different materials, it would be possible to avoid occurrence of observed peaks.





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Fig. 1. Comparison of WD-XRF spectra of pure cellulose and certified reference material NIST 1575a (a LiF220, b AX03, c PET). Ordinates are given on a logarithmic scale, in order to obtain better peak definition for less abundant elements.

1 The region between 0.37 and 0.49 nm is burdened with the interferences attributed to the discrete spectral  
2 lines of Rh that are present in the anode of the X-ray tube. These interferences can be omitted by the use  
3 of Cu filters, but their use will negatively affect the determination of several elements that are heavier  
4 than Rh (*e.g.*, Ag, Cd, In).

### 5 **3.2 Sensitivity**

6 Sensitivity was obtained as the slope by plotting the response of the instrument (kilo counts per second)  
7 against the analyte concentration (Table 2). Fig. 2 presents the sensitivities of PC, CW, and TL methods  
8 for all observed elements. Na, Mg, and Al have similar low sensitivities due to decreasing fluorescent  
9 yield and increasing analyte line absorption which occurs in the matrix and instrumentation. K and Ca  
10 have remarkably higher sensitivities than the previous three investigated elements, and this trend  
11 continues through the whole 4<sup>th</sup> period (Fig. 2). Elements with the atomic number in the range from 20 to  
12 40 have the highest sensitivity due to a favorable combination of efficient excitation, high fluorescent  
13 yield, low analyte line absorption, high reflectivity of crystals, and high efficiency of used detectors (32).

14 In the group of Ag, Cd, and In, sensitivity values suffer from a sharp decline, and then they rapidly regain  
15 higher values with heavier elements, but never reach the extent seen among elements of the 4<sup>th</sup> period  
16 (Fig. 2). Much lower sensitivity occurs because these elements require the use of a primary beam filter,  
17 which leads to a significant decrease in the primary beam intensity. The heaviest elements (Tl, Pb, and Bi)  
18 do not have the highest sensitivity due to increasing excitation potential and high spectral background,  
19 which consists mostly of the first order scattered radiation. Another important cause is that the L lines  
20 have significantly weaker intensities, compared to the K lines which are used for the lighter elements.

21 Comparison of the PC, CW, and TL methods' sensitivities revealed that TL does not differ from other  
22 methods until the middle of the 4<sup>th</sup> period is reached. For heavier elements (Ag, Cd, In, Tl, Pb, and Bi),  
23 the sensitivity of the TL method falls behind the PC and CW method sensitivities (Fig. 2), sometimes  
24 even to just one-third of their values. This trend is a consequence of the higher penetration depths of  
25 primary X-rays, which exceed the depths of sample layers in pellets prepared by the TL method.  
26 Furthermore, the CW method sensitivity is higher compared to the PC method. The reason for this trend  
27 is that the addition of binder affects the physical characteristics of the pellet and provides a flatter surface  
28 with less influence of shadow effect (33).

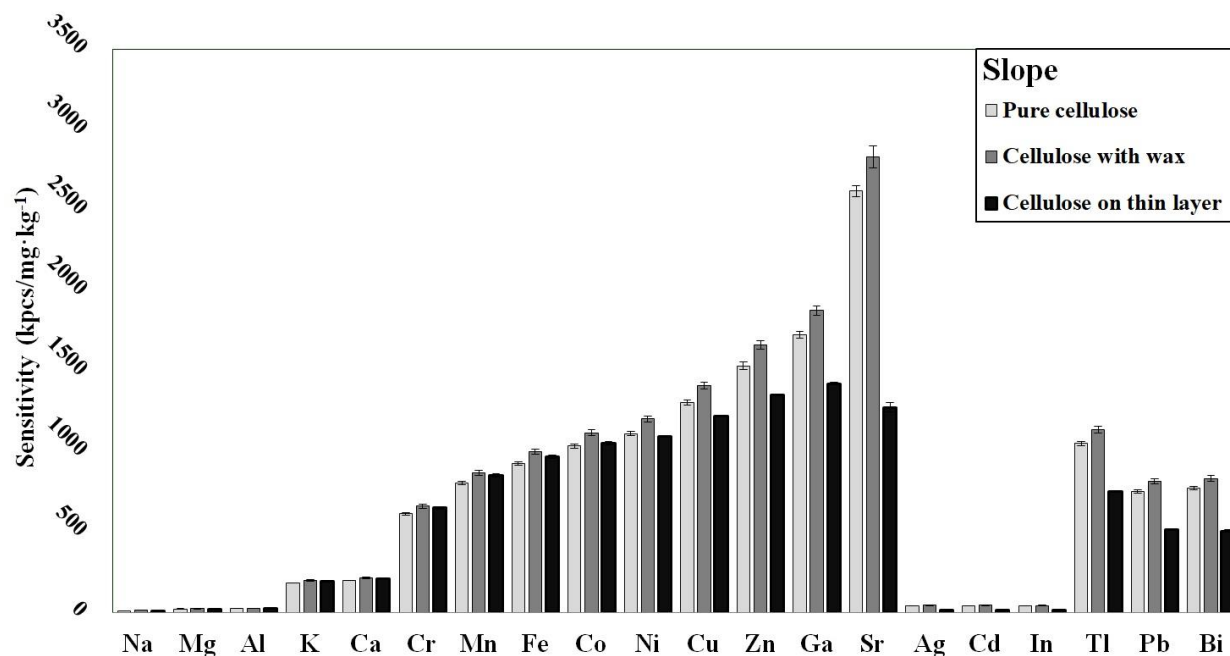


Fig. 2. The sensitivity of PC, CW, and TL methods.

### 3.3 Range and Linearity

The linearity of the analytical procedure was tested by applying linear regression to the measured standards. To quantify potential deviation from linearity, the coefficients of determination ( $R^2$ ) were calculated and are presented in Table 2, along with the slope coefficients and y-intercepts, and standard deviations. Calibration lines were generated in triplicate. The arithmetic means of the regression parameters are given in Table 2. Visual inspection of the plots confirmed that a linear model best describes relationship between intensities and concentrations, while high values of coefficients of determination quantitatively confirmed observed linear relationship. The only exception was observed for Na, which was hard to quantify in concentrations lower than  $100 \text{ mg}\cdot\text{kg}^{-1}$ . It was noticed that the highest measured standards ( $500 \text{ mg}\cdot\text{kg}^{-1}$ ) deviate from the regression lines, yet did not produce residuals larger than 10 %.

For the Pure Cellulose (PC) method, average coefficients of determination ( $R^2$ ) are ranging from 0.9837 for Na to 0.9994 for Ca. Regression coefficients are better than 0.9900 for all elements, except for Na, and mostly better or close to 0.9990, which confirms good linearity for all investigated elements.

The addition of binder did not significantly affect neither method linearity nor the order in which the coefficients of analyzed elements descend (Table 2). The exception was Na and to a lesser extent K, whose average  $R^2$  values were 0.8171 and 0.9944, respectively. These deviations are most likely the consequence of trace amounts of Na and K which were present in the wax.

Compared to the other two methods, the Thin Layer method (TL) contains a significantly lower amount of spiked cellulose in each calibration standard pellet. It was expected that a small amount of spiked cellulose and a more demanding sample preparation procedure might negatively affect the calibration of the method, nevertheless, the coefficients of determination were only slightly lower, compared to PC and CW. For all elements, they were better than 0.9900 (Table 2). The weakest linearity was observed for Ag

1 and In (average  $R^2$  equals 0.9927 and 0.9911, respectively), which is a consequence of the applied filter  
2 used to prevent interferences originating from the anode of the X-ray tube.

3 The analyzed SRM samples (NIST SRM 1573a Tomato leaves and NIST SRM 1575a Pine needles) have  
4 concentrations of Mg, Ca, and K which are multiple times higher compared to the maximum  
5 concentration of the standards used for calibration ( $500 \text{ mg}\cdot\text{kg}^{-1}$ ). Nevertheless, the calibration methods  
6 have quantified these elements accurately and precisely, although these values are significantly outlying  
7 above the calibration range. Investigating the highest limits of linearity was out of the scope of this  
8 research and it will be further explored in a series of more complex experiments, yet the SRM results  
9 suggest that linearity is still present at very high concentrations (8) (see Section 3.6.4).

Table 2. Coefficients of determination  $R^2$ , slopes, and y-intercepts with standard deviations (SD) of PC, CW, and TL methods

| <i>Element</i> | <i>Pure cellulose</i> |                                  |     |                                      |       | <i>Cellulose with wax</i> |                                  |     |                                      |       | <i>Cellulose on thin layer</i> |                                  |      |                                      |       |
|----------------|-----------------------|----------------------------------|-----|--------------------------------------|-------|---------------------------|----------------------------------|-----|--------------------------------------|-------|--------------------------------|----------------------------------|------|--------------------------------------|-------|
|                | $R^2$                 | <i>Slope <math>\pm</math> SD</i> |     | <i>Intercept <math>\pm</math> SD</i> |       | $R^2$                     | <i>Slope <math>\pm</math> SD</i> |     | <i>Intercept <math>\pm</math> SD</i> |       | $R^2$                          | <i>Slope <math>\pm</math> SD</i> |      | <i>Intercept <math>\pm</math> SD</i> |       |
| <i>Na</i>      | 0.9837                | 11.3                             | 0.4 | 1.38                                 | 0.01  | 0.8171                    | 14.1                             | 0.5 | 1.44                                 | 0.02  | 0.9940                         | 11.55                            | 0.07 | 1.394                                | 0.005 |
| <i>Mg</i>      | 0.9951                | 23.3                             | 0.7 | 1.51                                 | 0.02  | 0.9960                    | 24.3                             | 0.9 | 1.55                                 | 0.01  | 0.9956                         | 22.3                             | 0.1  | 1.525                                | 0.002 |
| <i>Al</i>      | 0.9958                | 24.1                             | 0.3 | 0.04                                 | 0.02  | 0.9972                    | 25.2                             | 0.9 | 0.048                                | 0.005 | 0.9950                         | 24.3                             | 0.3  | 0.035                                | 0.004 |
| <i>K</i>       | 0.9992                | 184                              | 1   | 0.55                                 | 0.03  | 0.9944                    | 198                              | 6   | 0.76                                 | 0.08  | 0.9950                         | 195                              | 3    | 0.59                                 | 0.03  |
| <i>Ca</i>      | 0.9994                | 200                              | 2   | 0.52                                 | 0.03  | 0.9994                    | 215                              | 5   | 0.60                                 | 0.02  | 0.9956                         | 212                              | 3    | 0.58                                 | 0.03  |
| <i>Cr</i>      | 0.9992                | 613                              | 6   | 2.18                                 | 0.03  | 0.9988                    | 661                              | 12  | 2.33                                 | 0.02  | 0.9968                         | 652                              | 9    | 2.2                                  | 0.1   |
| <i>Mn</i>      | 0.9990                | 807                              | 10  | 3.37                                 | 0.04  | 0.9987                    | 869                              | 15  | 3.59                                 | 0.02  | 0.9967                         | 854                              | 11   | 3.4                                  | 0.1   |
| <i>Fe</i>      | 0.9986                | 926                              | 11  | 5.32                                 | 0.03  | 0.9983                    | 1000                             | 15  | 5.61                                 | 0.02  | 0.9965                         | 969                              | 11   | 5.3                                  | 0.1   |
| <i>Co</i>      | 0.9982                | 1035                             | 14  | 5.77                                 | 0.05  | 0.9980                    | 1119                             | 17  | 6.10                                 | 0.03  | 0.9965                         | 1054                             | 10   | 5.7                                  | 0.2   |
| <i>Ni</i>      | 0.9977                | 1114                             | 16  | 6.44                                 | 0.05  | 0.9975                    | 1205                             | 18  | 6.769                                | 0.009 | 0.9964                         | 1096                             | 6    | 6.3                                  | 0.2   |
| <i>Cu</i>      | 0.9970                | 1305                             | 18  | 8.35                                 | 0.05  | 0.9970                    | 1412                             | 21  | 8.79                                 | 0.01  | 0.9964                         | 1224                             | 4    | 8.1                                  | 0.3   |
| <i>Zn</i>      | 0.9961                | 1535                             | 22  | 10.80                                | 0.05  | 0.9963                    | 1664                             | 27  | 11.35                                | 0.04  | 0.9963                         | 1352                             | 4    | 10.3                                 | 0.3   |
| <i>Ga</i>      | 0.9953                | 1729                             | 22  | 14.45                                | 0.06  | 0.9958                    | 1879                             | 29  | 15.14                                | 0.03  | 0.9960                         | 1424                             | 9    | 13.6                                 | 0.4   |
| <i>Sr</i>      | 0.9946                | 2621                             | 33  | 43.1                                 | 0.3   | 0.9953                    | 2832                             | 69  | 43.9                                 | 0.4   | 0.9947                         | 1276                             | 28   | 40.5                                 | 0.4   |
| <i>Ag</i>      | 0.9989                | 43.8                             | 0.3 | 0.682                                | 0.003 | 0.9967                    | 46                               | 2   | 0.67                                 | 0.01  | 0.9927                         | 15.8                             | 0.5  | 0.649                                | 0.006 |
| <i>Cd</i>      | 0.9983                | 43.7                             | 0.9 | 0.630                                | 0.008 | 0.9969                    | 46                               | 2   | 0.61                                 | 0.01  | 0.9942                         | 15.0                             | 0.5  | 0.599                                | 0.006 |
| <i>In</i>      | 0.9989                | 41.5                             | 0.8 | 0.82                                 | 0.01  | 0.9967                    | 44                               | 2   | 0.80                                 | 0.01  | 0.9911                         | 14.3                             | 0.4  | 0.789                                | 0.002 |
| <i>Tl</i>      | 0.9946                | 1051                             | 14  | 19.22                                | 0.08  | 0.9950                    | 1138                             | 20  | 20.32                                | 0.02  | 0.9955                         | 752                              | 7    | 18.7                                 | 0.3   |
| <i>Pb</i>      | 0.9938                | 755                              | 11  | 21.11                                | 0.07  | 0.9943                    | 817                              | 14  | 22.35                                | 0.05  | 0.9951                         | 516                              | 7    | 20.8                                 | 0.2   |
| <i>Bi</i>      | 0.9939                | 771                              | 11  | 23.41                                | 0.07  | 0.9945                    | 834                              | 16  | 24.75                                | 0.06  | 0.9942                         | 507                              | 9    | 23.0                                 | 0.2   |

1

### 2 **3.4 Limit of detection and limit of quantification**

3 Light elements (Na, Mg, Al, K, and Ca) have higher LoDs compared to the other elements. For the CW  
 4 method, LoDs are in the range from 47 to 2.3 mg·kg<sup>-1</sup> (Table 3). Among transition metals (from Cr to  
 5 Ga), detection limits are lower than 1 mg·kg<sup>-1</sup>, Fe and Ni have the lowest LODs (0.24 and 0.23 mg·kg<sup>-1</sup>).  
 6 Elements that had to be measured with the use of filters (Ag, Cd, and In) have significantly higher  
 7 detection limits, but they are still below concentrations of 10 mg·kg<sup>-1</sup>. The heaviest analyzed elements (Tl,  
 8 Pb, and Bi) have slightly higher detection limits compared to lighter transition metals (Table 3). All 3  
 9 methods have similar trends among the LoD and LoQ values. CW has similar detection limits compared  
 10 to the PC method, with values usually just 0.5 to 1 mg·kg<sup>-1</sup> lower. The TL method has a few times higher  
 11 detection limits than other two methods. Exceptions from this rule can be seen among the lightest  
 12 elements (from Na to K), where TL actually outperformed PC and CW (Table 3). Such results are not  
 13 surprising because the penetration depth of X-rays for light elements is extremely small, thus the total  
 14 amount of the analyte which is effectively measured is the same in all 3 methods. High energy X-rays,  
 15 necessary for excitation of heavy elements, penetrate much deeper into the pellets (up to several cm),  
 16 which negatively affects TL method.

17 Table 3. Limits of detection (LoDs) and limits of quantification (LoQs) for  
 18 PC, CW, and TL methods (mg·kg<sup>-1</sup>)

| <i>Elements</i> | <i>Pure cellulose</i> |            | <i>Cellulose with wax</i> |            | <i>Cellulose on thin layer</i> |            |
|-----------------|-----------------------|------------|---------------------------|------------|--------------------------------|------------|
|                 | <i>LoD</i>            | <i>LoQ</i> | <i>LoD</i>                | <i>LoQ</i> | <i>LoD</i>                     | <i>LoQ</i> |
| <i>Na</i>       | 32                    | 107        | 47                        | 157        | 13                             | 43         |
| <i>Mg</i>       | 26                    | 87         | 16                        | 54.8       | 2.7                            | 8.9        |
| <i>Al</i>       | 22                    | 75         | 6.1                       | 20.2       | 4.7                            | 16         |
| <i>K</i>        | 5.0                   | 17         | 11                        | 38.0       | 3.9                            | 13         |
| <i>Ca</i>       | 4.8                   | 16         | 2.3                       | 7.7        | 3.7                            | 12         |
| <i>Cr</i>       | 1.7                   | 5.6        | 1.1                       | 3.5        | 4.7                            | 16         |
| <i>Mn</i>       | 1.4                   | 4.6        | 0.59                      | 2.0        | 5.0                            | 17         |
| <i>Fe</i>       | 1.0                   | 3.4        | 0.46                      | 1.6        | 4.5                            | 15         |
| <i>Co</i>       | 1.4                   | 4.5        | 0.71                      | 2.4        | 5.2                            | 17         |
| <i>Ni</i>       | 1.4                   | 4.6        | 0.23                      | 0.78       | 5.5                            | 18         |
| <i>Cu</i>       | 1.1                   | 3.7        | 0.24                      | 0.80       | 6.5                            | 22         |
| <i>Zn</i>       | 1.1                   | 3.5        | 0.68                      | 2.3        | 7.0                            | 23         |
| <i>Ga</i>       | 0.97                  | 3.2        | 0.55                      | 1.8        | 7.6                            | 25         |
| <i>Sr</i>       | 3.2                   | 11         | 4.0                       | 13.2       | 10                             | 34         |
| <i>Ag</i>       | 1.9                   | 6.3        | 8.8                       | 29.3       | 11                             | 36         |
| <i>Cd</i>       | 5.7                   | 19.07      | 8.8                       | 29.5       | 12                             | 41         |
| <i>In</i>       | 8.4                   | 28         | 9.4                       | 31.3       | 3.2                            | 11         |
| <i>Tl</i>       | 2.2                   | 7.4        | 0.46                      | 1.6        | 10                             | 34         |
| <i>Pb</i>       | 2.6                   | 8.7        | 1.9                       | 6.4        | 9.8                            | 33         |
| <i>Bi</i>       | 2.8                   | 9.3        | 2.0                       | 6.7        | 13                             | 44         |

### 19 **3.5 Precision: repeatability and intralaboratory reproducibility (ILR)**

20 Precision was characterized by investigating repeatability and intralaboratory reproducibility.  
 21 Repeatability was determined by measuring the same pellets within the same day in triplicates and  
 22 expressed as the relative standard deviation (RSD) of the repeated measurements. Intralaboratory

1 reproducibility aims to assess the robustness of the method under different conditions, which might vary  
 2 under regular analytical work. Therefore, the investigation of intralaboratory reproducibility covered the  
 3 whole analytical process: the production and pelleting of synthetic reference materials, as well as  
 4 measurements on the instrument. Table 4 presents the repeatability and intralaboratory reproducibility at  
 5 low and high concentration levels (20 and 500 mg·kg<sup>-1</sup>). Analysis of reproducibility also verified the  
 6 reliability of the spiking procedure, since each of the three analyzed pellets was created from different  
 7 batches of spiked cellulose.

8

9 Table 4. Repeatability and intralaboratory reproducibility (ILR) at low (20 mg·kg<sup>-1</sup>) and high (500 mg·kg<sup>-1</sup>)  
 10 concentration levels.

|           | <u>PC method</u>         |             |                |             | <u>CW method</u>         |             |                |             | <u>TL method</u>         |             |                |             |
|-----------|--------------------------|-------------|----------------|-------------|--------------------------|-------------|----------------|-------------|--------------------------|-------------|----------------|-------------|
|           | <u>Repeatability (%)</u> |             | <u>ILR (%)</u> |             | <u>Repeatability (%)</u> |             | <u>ILR (%)</u> |             | <u>Repeatability (%)</u> |             | <u>ILR (%)</u> |             |
|           | <i>Low</i>               | <i>High</i> | <i>Low</i>     | <i>High</i> | <i>Low</i>               | <i>High</i> | <i>Low</i>     | <i>High</i> | <i>Low</i>               | <i>High</i> | <i>Low</i>     | <i>High</i> |
| <i>Na</i> | 27.8                     | 3.8         | 318            | 15.5        | 19.7                     | 1.4         | /              | 4.7         | 39.5                     | 1.7         | 32.6           | 3.6         |
| <i>Mg</i> | 3.0                      | 2.1         | 5.0            | 30.5        | 3.6                      | 1.4         | 4.2            | 6.1         | 8.6                      | 1.8         | 24.4           | 2.9         |
| <i>Al</i> | 12.6                     | 2.6         | 3.5            | 28.7        | 2.0                      | 1.7         | 9.2            | 6.0         | 10.5                     | 2.4         | 10.4           | 1.7         |
| <i>K</i>  | 2.5                      | 1.2         | 1.0            | 17.0        | 0.0                      | 0.9         | 4.2            | 4.6         | 4.3                      | 1.2         | 15.1           | 1.8         |
| <i>Ca</i> | 2.5                      | 1.2         | 5.0            | 14.9        | 0.5                      | 0.8         | 9.2            | 4.0         | 0.6                      | 1.3         | 9.2            | 2.2         |
| <i>Cr</i> | 0.5                      | 0.9         | 10.0           | 9.4         | 0.5                      | 0.8         | 3.5            | 2.5         | 1.2                      | 0.8         | 3.5            | 1.1         |
| <i>Mn</i> | 1.5                      | 1.0         | 7.6            | 8.9         | 0.0                      | 0.7         | 0.0            | 2.4         | 1.9                      | 0.8         | 3.5            | 0.7         |
| <i>Fe</i> | 2.0                      | 0.8         | 7.6            | 8.1         | 0.0                      | 0.6         | 0.0            | 2.1         | 1.9                      | 0.8         | 0.0            | 0.6         |
| <i>Co</i> | 2.0                      | 0.8         | 5.0            | 7.6         | 1.0                      | 0.5         | 3.5            | 2.1         | 1.2                      | 0.6         | 3.5            | 0.3         |
| <i>Ni</i> | 1.5                      | 0.8         | 5.0            | 6.1         | 1.5                      | 0.5         | 3.5            | 1.8         | 1.9                      | 0.6         | 3.5            | 0.3         |
| <i>Cu</i> | 1.5                      | 0.7         | 2.9            | 5.9         | 0.5                      | 0.5         | 0.0            | 1.7         | 0.6                      | 0.5         | 3.5            | 0.7         |
| <i>Zn</i> | 9.1                      | 0.8         | 2.9            | 8.0         | 7.1                      | 0.6         | 0.0            | 1.7         | 7.4                      | 0.7         | 2.3            | 1.4         |
| <i>Ga</i> | 2.0                      | 0.7         | 2.9            | 5.6         | 1.5                      | 0.5         | 0.0            | 1.5         | 1.9                      | 0.4         | 6.0            | 1.7         |
| <i>Sr</i> | 1.0                      | 0.5         | 2.9            | 5.6         | 1.0                      | 0.1         | 6.9            | 2.1         | 3.1                      | 0.4         | 19.3           | 3.3         |
| <i>Ag</i> | 3.5                      | 0.7         | 2.9            | 7.1         | 7.1                      | 0.1         | 4.2            | 3.6         | 19.1                     | 0.3         | 35.6           | 3.7         |
| <i>Cd</i> | 3.5                      | 0.2         | 3.5            | 6.6         | 1.5                      | 0.5         | 8.5            | 3.3         | 2.5                      | 0.3         | 33.4           | 4.4         |
| <i>In</i> | 2.5                      | 0.4         | 29             | 7.9         | 7.6                      | 0.4         | 4.2            | 3.5         | 17.9                     | 1.1         | 46.0           | 3.3         |
| <i>Tl</i> | 2.0                      | 0.8         | 2.9            | 5.5         | 0.5                      | 0.4         | 3.5            | 1.7         | 1.9                      | 0.4         | 15.9           | 2.2         |
| <i>Pb</i> | 2.0                      | 0.6         | 5.0            | 5.8         | 1.0                      | 0.4         | 6.0            | 1.7         | 2.5                      | 0.2         | 25.0           | 2.5         |
| <i>Bi</i> | 2.0                      | 0.8         | 2.9            | 5.9         | 3.0                      | 0.5         | 6.9            | 1.7         | 5.6                      | 0.2         | 31.2           | 2.8         |

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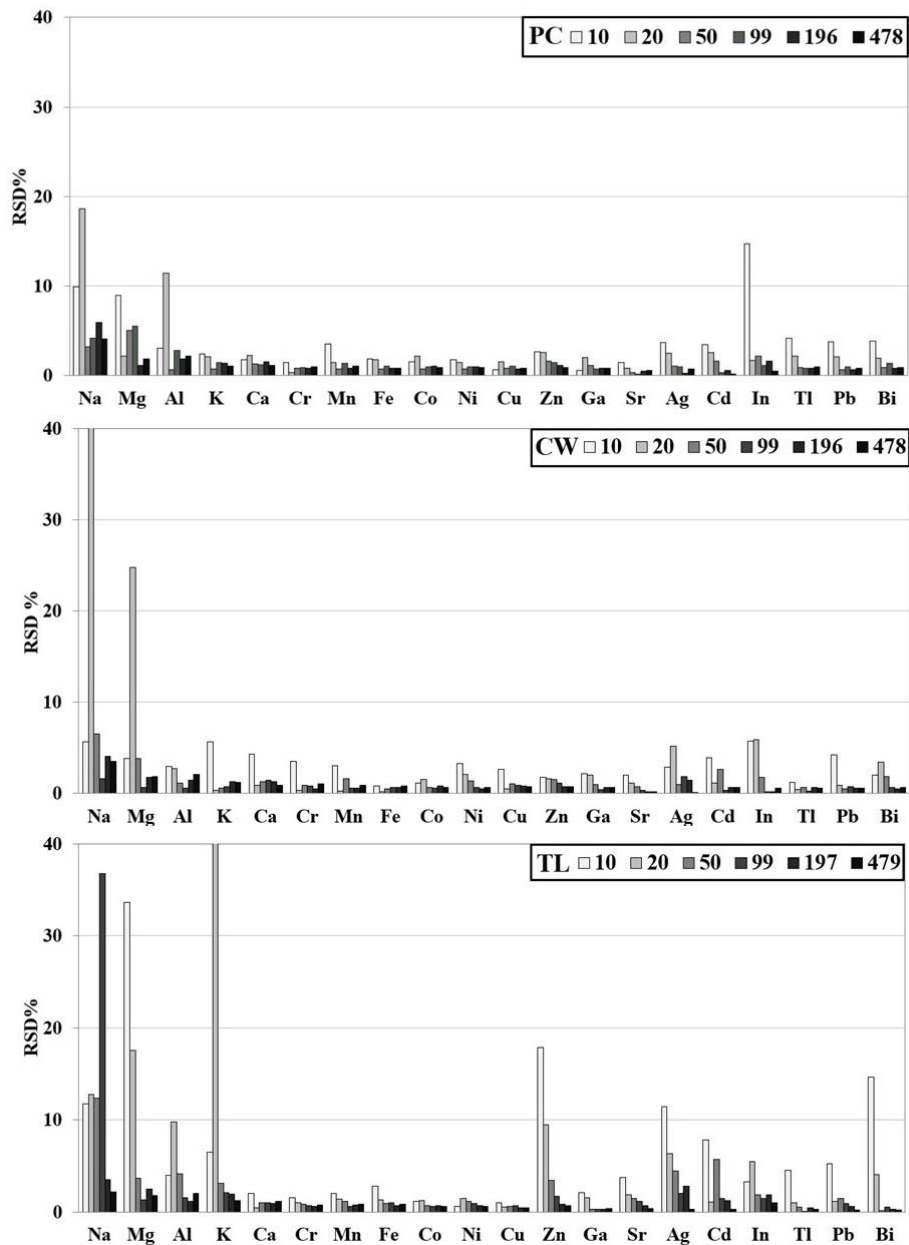
12 ILR was best for elements in the 4<sup>th</sup> period of Periodic Table of Elements, in some cases, the RSDs were  
 13 equal to 0. The use of the binder improved the reproducibility (Table 4), especially for the higher  
 14 concentrations. Within the TL method, ILR was unfavorable for elements lighter than Ca and heavier than  
 15 Sr when these elements were present in low concentrations (20 mg·kg<sup>-1</sup>). Nevertheless, among higher  
 16 concentrations, the ILR of the TL method was at a similar level compared to the other two methods  
 17 (Table 4). This finding is important because it implies that the manual step in which the sample is applied  
 18 as a thin layer on top of the mass of boric acid does not significantly affect the TL method.

1 Contrary to the TL method, the ILR of the CW and even more the PC method at the 500 mg·kg<sup>-1</sup> level  
2 was similar or worse (respectively), compared to the ILR observed at 20 mg·kg<sup>-1</sup>. These findings are most  
3 prominent for light elements analyzed by the PC method. It was also observed that the RSD values for  
4 repeatability and ILR were much closer to each other at 20 mg·kg<sup>-1</sup> level than among the higher  
5 concentrations of the investigated elements. The RSD of ILR was a few times higher than the RSD of the  
6 repeatability when they were compared at the highest concentration level. The observed trends might be a  
7 consequence of imperfect homogenization within the process of cellulose spiking. Homogeneity of the  
8 sample could be further improved by automatization of the homogenization process. Homogeneity of the  
9 sample is even more important for the light elements because they are being measured mostly within the  
10 top layers of the pellets. X-rays of higher energy penetrate deeper layers of the pellets, and therefore  
11 outstanding homogeneity is not of utmost importance for heavier elements.

12 It is valuable to understand how repeatability changes as the concentrations of the analytes decline among  
13 the samples, because this information may have additional value for proper estimation of detection limits.  
14 The change of repeatability as a function of concentration is presented in Fig. 3. A rapid deterioration of  
15 repeatability was observed when lower concentrations (below 20 mg·kg<sup>-1</sup>) of the light elements (Na, Mg,  
16 Al) were analyzed. A similar but much less pronounced trend was observed for the heaviest elements,  
17 especially if they require the use of X-ray filters (Fig. 3). Although both trends were observed within all  
18 three investigated methods, the CW method has the best performance while TL is the worst regarding this  
19 issue. Nevertheless, the differences do not seem to be very significant.

20 All of investigated methods proved to have excellent repeatability, which is very often below 1%. Even  
21 among lower concentrations it rarely exceeds 5% for most of the elements. The addition of the binder  
22 improves repeatability, especially at higher concentrations.





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Fig. 3. Repeatability (RSD %) as a function of different concentrations for Pure Cellulose (PC) method, Cellulose with Wax (CW) method, and Thin Layer (TL) method.

3

4

### 3.6 Accuracy

5

In the previous sections, the properties of the CW and TL methods (sensitivity, limits of detection and quantification) were compared with the PC method. Comparisons with the PC method were necessary in order to investigate the existence of potential negative effects of binder addition, as well as the changes of pellet properties which are introduced by the different way of pellet preparation. The PC method has limited practical value because pellets prepared exclusively from the plant material are often not stable

6

1 enough, showing poor physical properties and surface geometry. On the other hand, the UniQuant (UQ)  
2 method is standardless, and it has great practical value as a fast-screening method. Since it is only a  
3 semiquantitative method, it is important to properly characterize its accuracy.

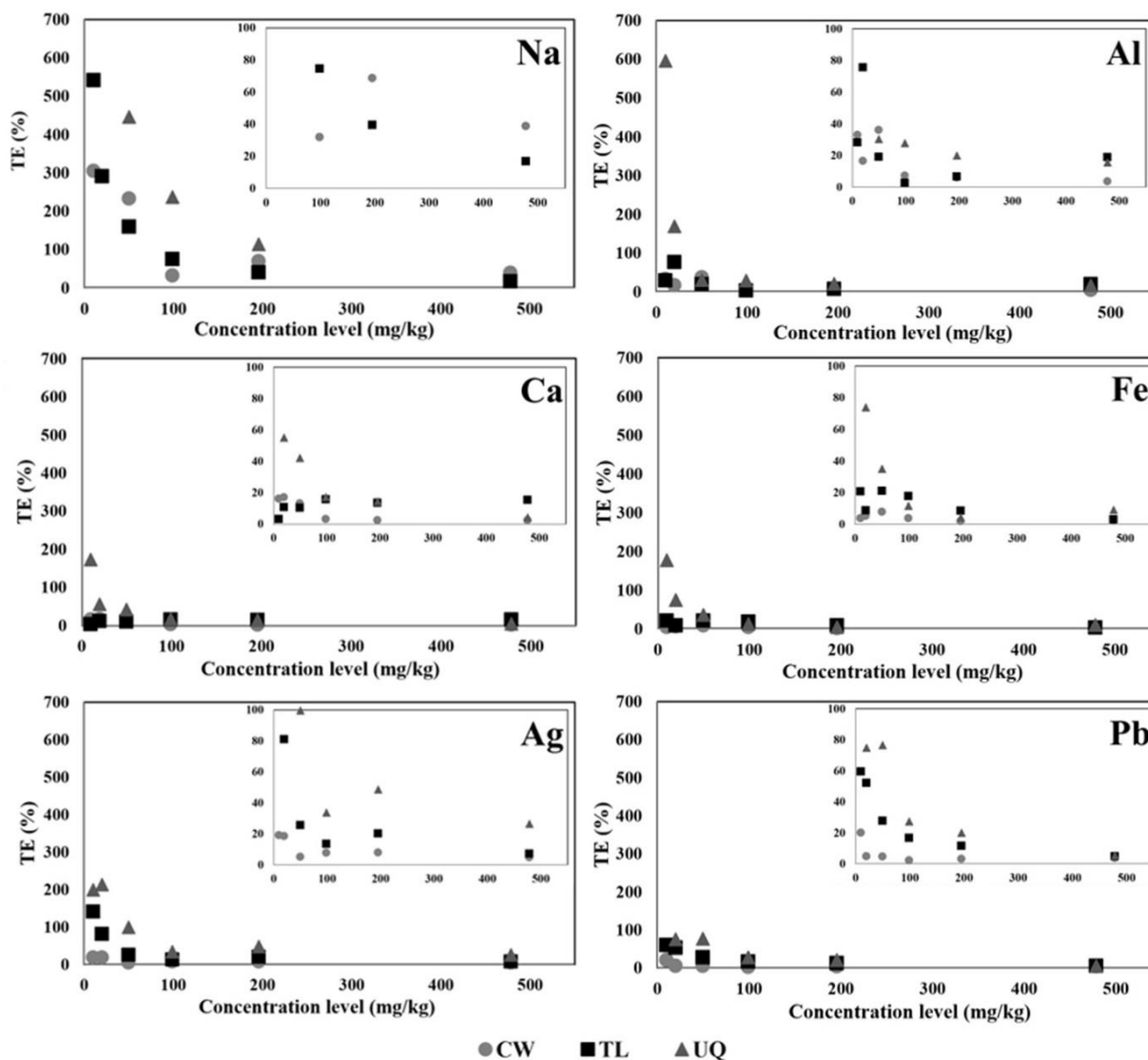
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### 5 **3.6.1 Characterization of accuracy based on spiked cellulose reference material**

#### 6 **3.6.1.1 Comparison of CW, TL, and UQ methods**

7 It was observed that CW method has the best accuracy, and the UQ method the worst, which is expected  
8 because UQ is semiquantitative (Fig. 4). Nevertheless, among higher concentrations differences between  
9 all 3 methods become insignificant, since they all have TE values lower than 25%, very often even lower  
10 than 5%. CW and TS methods achieved good accuracy even with the lowest concentrations, while the UQ  
11 method's accuracy deteriorates exponentially in the region of 20 to 50 mg·kg<sup>-1</sup>. The decline of accuracy  
12 within the TL method is much more rapid for the elements with higher atomic numbers, especially  
13 elements heavier than Tl, such as Pb and Bi.

14 Application of X-ray filters, which was necessary for the analysis of Ag, Cd, and In, will also lead to very  
15 inaccurate results if the concentrations of these elements drop below 40 or 50 mg·kg<sup>-1</sup>, because the filters  
16 significantly decrease the amount of primary X-ray radiation which can reach these elements.



1  
 2 Fig. 4. TE values (Eq. 3) calculated for different concentration levels. Chosen elements are representative  
 3 of the heaviest elements (Pb), elements that require the use of X-ray filters (Ag), transition metals (Fe),  
 4 and light elements (Na, Al, and Ca).

5 **3.6.1.2 Characterization of accuracy as a function of the change of concentration**

6 Since XRF is not well suited for the lightest elements, it was difficult to achieve accurate measurements  
 7 of Na, even in higher concentrations (higher than  $100 \text{ mg}\cdot\text{kg}^{-1}$ ). Nevertheless, the accuracy was drastically  
 8 better for other light elements (Mg, Al, K, and especially Ca). It was estimated that Ca can be very  
 9 accurately measured on the lowest tested values ( $10 \text{ mg}\cdot\text{kg}^{-1}$ ), while the other 3 elements required  
 10 concentrations higher than  $50 \text{ mg}\cdot\text{kg}^{-1}$ . Both CW and TL methods proved to have excellent accuracy for  
 11 transition metals from Cr to Cu, even for the lowest investigated concentrations, yet UQ can reach similar  
 12 accuracies as the other two methods with concentrations higher than  $50 \text{ mg}\cdot\text{kg}^{-1}$ .

13 For elements heavier than Cu, the accuracy becomes worse, particularly among elements that use filters  
 14 during measurements (Ag, Cd, In). For this group of elements, the CW method could reach  $\text{TE} < 25 \%$   
 15 when the concentrations were in a region between 15 and  $20 \text{ mg}\cdot\text{kg}^{-1}$ . For similar accuracy, the TL

1 method required concentrations between 40 and 80 mg·kg<sup>-1</sup>, and UQ around 100 mg·kg<sup>-1</sup>. The heaviest  
2 investigated elements (Tl, Pb, and Bi) demonstrated excellent accuracy similar to the transition elements,  
3 but only for the CW method.

#### 4 **3.6.2 Characterization of accuracy by linear regression**

5 TE values (Eq. 3) are a good tool for the depiction of accuracy on a single concentration level, but linear  
6 regression can offer a more comprehensive analysis for the overall characterization of accuracy.  
7 Measured values were plotted against reference values for the calculation of linear regression parameters:  
8 slope (a) and Pearson's coefficient (R).

9 The first step was to plot the expected concentrations vs. measured values. The results presented in Table  
10 5 are relevant for the analysis of random errors for CW, TL, and UQ methods, but also the systematic  
11 errors of UQ method. UQ is a standardless method, therefore the analyzed pellets were not used for its  
12 calibration. Pearson's coefficient squares are higher than 0.9900 for all investigated elements (except Na).  
13 Random errors are the highest for the light elements, but their size drops rapidly from Na to Ca. R<sup>2</sup> for the  
14 CW method goes up to 0.9999 for most of analyzed elements (Table 5). It can also be noticed that the CW  
15 method shows higher precision than the TL and the UQ method.

16 The UQ method tends to overestimate the concentrations of the light elements, but the direction  
17 coefficients rapidly approach the ideal value (a = 1) as we move from Na to K (Table 5). Development of  
18 a slight positive systematic error can be observed for elements heavier than Co, but it is not observed for  
19 the heaviest analyzed elements (Tl, Pb, and Bi).

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1 Table 5. Slopes ( $a$ ) and coefficients of determination ( $R^2$ ) from linear regression,  
 2 applied to measured values and plotted against expected values

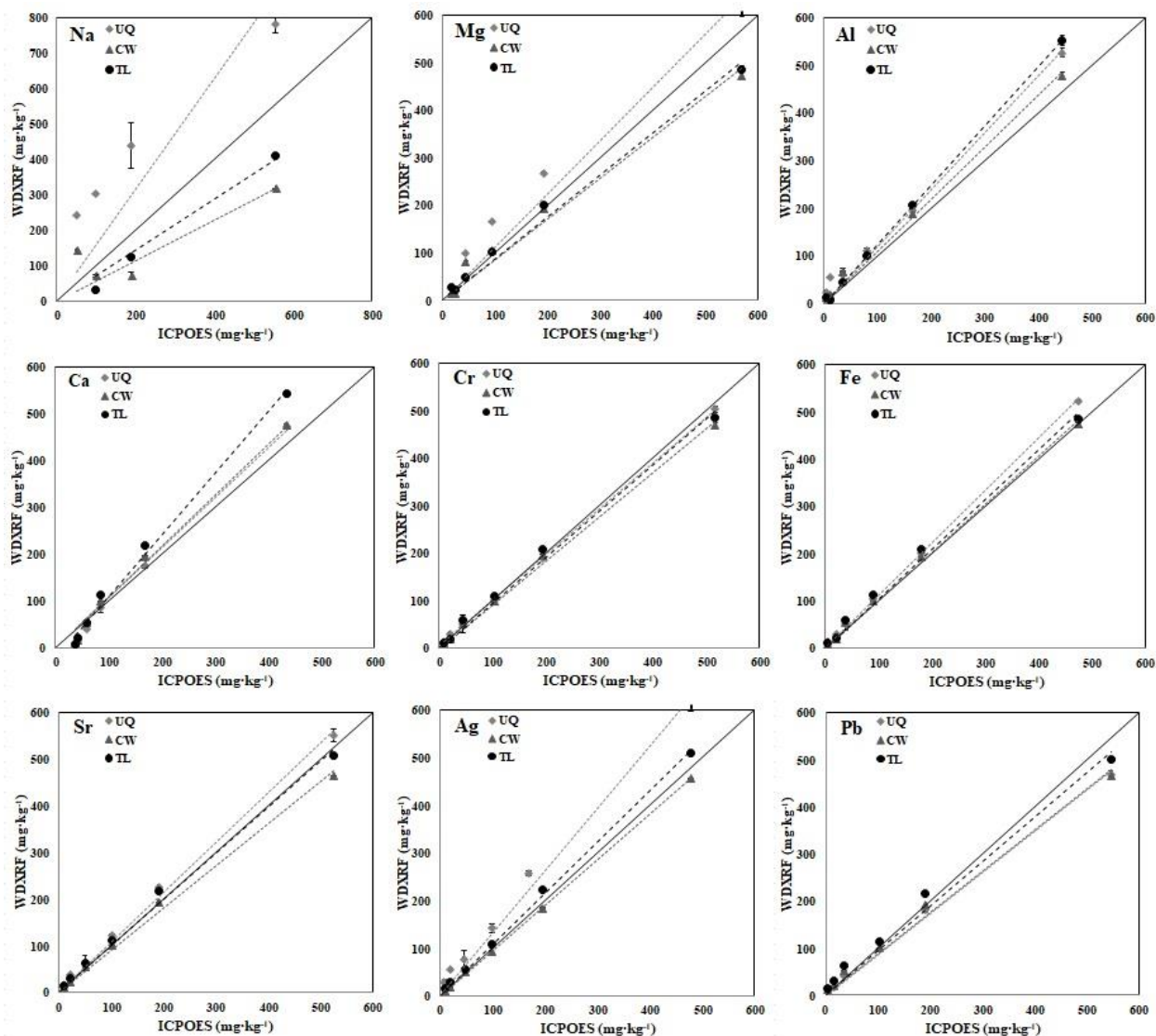
| <i>Element</i> | <i>a</i>  |           |           | <i>R</i> <sup>2</sup> |           |           |
|----------------|-----------|-----------|-----------|-----------------------|-----------|-----------|
|                | <i>CW</i> | <i>TL</i> | <i>UQ</i> | <i>CW</i>             | <i>TL</i> | <i>UQ</i> |
| <i>Na</i>      | 0.6433    | 0.8100    | 1.7906    | 0.6149                | 0.9462    | 0.8756    |
| <i>Mg</i>      | 1.0155    | 1.0155    | 1.2937    | 0.9981                | 0.9981    | 0.9848    |
| <i>Al</i>      | 0.9983    | 1.1309    | 1.0908    | 0.9975                | 0.9957    | 0.9913    |
| <i>K</i>       | 0.9458    | 0.9681    | 1.0469    | 0.9941                | 0.9944    | 0.9966    |
| <i>Ca</i>      | 0.9949    | 1.1316    | 0.9700    | 0.9997                | 0.9999    | 0.9964    |
| <i>Cr</i>      | 0.9873    | 1.0291    | 1.0413    | 0.9998                | 0.9987    | 0.9980    |
| <i>Mn</i>      | 0.9969    | 1.0274    | 1.1981    | 0.9999                | 0.9985    | 0.9988    |
| <i>Fe</i>      | 0.9936    | 1.0311    | 1.0855    | 0.9998                | 0.9980    | 0.9985    |
| <i>Co</i>      | 0.9930    | 1.0331    | 0.9745    | 0.9999                | 0.9983    | 0.9986    |
| <i>Ni</i>      | 0.9926    | 1.0370    | 1.0982    | 0.9998                | 0.9983    | 0.9989    |
| <i>Cu</i>      | 0.9905    | 1.0391    | 1.1168    | 0.9998                | 0.9984    | 0.9989    |
| <i>Zn</i>      | 0.7472    | 1.0011    | 1.1017    | 0.9434                | 0.9988    | 0.9988    |
| <i>Ga</i>      | 1.0231    | 1.0486    | 0.9744    | 0.9926                | 0.9987    | 0.9861    |
| <i>Sr</i>      | 0.9749    | 1.0683    | 1.1542    | 0.9995                | 0.9988    | 0.9982    |
| <i>Ag</i>      | 0.9550    | 1.0788    | 1.2873    | 0.9999                | 0.9983    | 0.9929    |
| <i>Cd</i>      | 0.9558    | 1.0852    | 1.2507    | 0.9995                | 0.9968    | 0.9927    |
| <i>In</i>      | 1.0094    | 1.0748    | 1.1528    | 0.9975                | 0.9914    | 0.9937    |
| <i>Tl</i>      | 0.9842    | 1.0537    | 1.0515    | 0.9997                | 0.9988    | 0.9969    |
| <i>Pb</i>      | 0.9761    | 1.0588    | 0.9694    | 0.9998                | 0.9979    | 0.9981    |
| <i>Bi</i>      | 0.9823    | 1.0779    | 0.9854    | 0.9997                | 0.9974    | 0.9987    |

3 **3.6.3 Characterization of accuracy based on comparison of WD-XRF with ICP-OES**

4 Aliquots of synthetic reference materials were digested and analyzed with ICP-OES. The accuracy of the  
 5 ICP-OES measurements was controlled by analysis of SRMs. The purpose of the comparison of ICP-OES  
 6 and WD-XRF results was to provide additional proof regarding the accuracy of the investigated XRF  
 7 methods.

8 The results for the most typical elements are presented in Fig. 5. Elements of the 4<sup>th</sup> period had very good  
 9 correlations between ICP-OES and all 3 WD-XRF methods (CW, TL, and UQ). The TL method is more  
 10 prone to systematic errors than the CW method when light elements are analyzed, and neither of these  
 11 methods is suited for accurate quantification of Na.

12 The UQ method has a positive bias when light elements were analyzed as well as elements that require X-  
 13 ray filters (Ag, Cd and In). Bias is most pronounced for Na, but it rapidly decreases for heavier elements  
 14 (Mg and Al).



1

2

3

Fig. 5. Comparison of ICP-OES and WD-XRF measurements of cellulose synthetic reference materials.

#### 4 3.6.4 Assessment of accuracy based on analysis of SRM

5 Table 6 contains the concentrations gained from the measurement of two SRMs and TE values that  
6 combine contributions from the systematic and random errors (Eq. 3).

7 Accuracy was satisfying for all elements analyzed within pine needles SRM (except for Na). The CW and  
8 TL methods had significantly better accuracy than the UQ method. Nevertheless, UQ performed very  
9 well, if we consider its semiquantitative character. Some of the elements had larger concentrations than  
10 the highest standards used for calibration. Nevertheless, the concentrations of these elements were still  
11 quite accurately determined, which confirms that XRF methods have a very wide linearity.

12 Analysis of tomato leaves SRM acquired results with acceptable but less favorable accuracy. This is a  
13 material with a very difficult matrix since it contains very high concentrations (1 to 5 %) of macro  
14 elements such as Ca, Al, Mg, and Si. These results indicate that for highly accurate measurements it is

1 necessary to prepare special calibration standards suited for matrices with extremely high concentrations.  
 2 They would be able to take into account all spectral interferences which may occur (change of the  
 3 baseline, enhancement effects, etc.). It is important to notice that the accuracy of the UQ method was  
 4 similar for both SRMs, which confirms that UniQuant software is well adjusted and able to deal with all  
 5 concentration levels and interelement interferences.

6 Table 6. Accuracy (TE) values of SRMs NIST SRM 1573a Tomato leaves and NIST SRM 1575a Pine  
 7 needles, obtained with three different XRF methods

| <i>Pine needles</i> |   |               |           |           | <i>Tomato leaves</i> |   |               |           |           |
|---------------------|---|---------------|-----------|-----------|----------------------|---|---------------|-----------|-----------|
| <i>Element</i>      | <i>Reference concentration (mg·kg<sup>-1</sup>)</i> | <i>TE (%)</i> |           |           | <i>Element</i>       | <i>Reference concentration (mg·kg<sup>-1</sup>)</i> | <i>TE (%)</i> |           |           |
|                     |   | <i>CW</i>     | <i>TL</i> | <i>UQ</i> |                      |   | <i>CW</i>     | <i>TL</i> | <i>UQ</i> |
| <i>Na</i>           | 63  | 97.9          | 75.1      | 585       | <i>Na</i>            | 136   | 106           | 577       | 501       |
| <i>Mg</i>           | 1060  | 22.7          | 10.5      | 20.6      | <i>Mg</i>            | 12000   | 29.6          | 11.3      | 18.7      |
| <i>Al</i>           | 580   | 12.8          | 17.7      | 26.1      | <i>Al</i>            | 598   | 21.6          | 22.1      | 31.2      |
| <i>K</i>            | 4170  | 13.8          | 4.9       | 19.3      | <i>K</i>             | 27000   | 37.7          | 29.1      | 12.0      |
| <i>Ca</i>           | 2500  | 7.3           | 7.2       | 13.4      | <i>Ca</i>            | 50500   | 43.9          | 35.4      | 4.4       |
| <i>Mn</i>           | 488   | 6.7           | 4.0       | 29.8      | <i>Mn</i>            | 246   | 59.1          | 63.3      | 20.5      |
| <i>Fe</i>           | 46  | 6.9           | 5.3       | 18.8      | <i>Fe</i>            | 368   | 60.4          | 63.0      | 5.9       |
| <i>Sr</i>           | not certified                                       | /             | /         | /         | <i>Sr</i>            | 85  | 62.6          | 193       | 27.6      |

8

### 9 3.6.5 Assessment of accuracy based on the analysis of real samples

10 The final test of the XRF measurements accuracy was performed by measuring the concentrations in real  
 11 samples. Concentrations determined with WD-XRF were compared to the results acquired by ICP-MS.  
 12 For this purpose, 33 samples of three different conifer species were analyzed by both techniques

13 Before the ICP-MS analysis, plant samples were digested in triplicate. ICP-MS analysis was controlled  
 14 with drift standards at 2 concentration levels, blanks, and with 2 different certified reference materials,  
 15 therefore it provided the results of high reliability and accuracy, suitable for the comparison with WD-  
 16 XRF method. The results were compared by plotting them on graphs and applying linear regression (Fig.  
 17 6). Statistical parameters of linear regression (correlation (R) and slope (a) coefficients) are given in Table  
 18 7.

19 Correlation between CW and ICP-MS methods was the highest for Ca, K, Fe, and Mn ( $0.95 < R < 0.99$ ).  
 20 CW and ICP-MS measurements of lighter elements were also in very good compliance ( $0.80 < R < 0.97$ ).  
 21 The only exception was Na, but it remains unclear if this is a consequence of low concentrations or  
 22 inadequacy of the CW method. It is worth mentioning that some macro elements had concentrations that  
 23 exceeded the calibration range of the CW method, nevertheless, this fact did not negatively affect  
 24 compliance of the measured values with ICP-MS method. These results confirmed that CW and UQ  
 25 methods have good linearity even in ranges that exceed the tested values.

26

27

Table 7. Comparison of CW and UQ methods with ICP-MS. R – correlation coefficient, a – slope, M - median value of ICP-MS measurements

| <i>Element</i> | <i>UQ and ICP-MS</i> |          | <i>CW and ICP-MS</i> |          | <i>M (mg·kg<sup>-1</sup>)</i> |
|----------------|----------------------|----------|----------------------|----------|-------------------------------|
|                | <i>a</i>             | <i>R</i> | <i>a</i>             | <i>R</i> |                               |
| <i>Na</i>      | 1.38                 | 0.8192   | -0.07                | 0.0883   | 10.7                          |
| <i>Mg</i>      | 0.80                 | 0.6977   | 0.38                 | 0.8001   | 986                           |
| <i>Al</i>      | 2.87                 | 0.9157   | 1.52                 | 0.9061   | 29.3                          |
| <i>K</i>       | 1.32                 | 0.8444   | 0.84                 | 0.9680   | 9883                          |
| <i>Ca</i>      | 1.33                 | 0.8665   | 0.91                 | 0.9842   | 3128                          |
| <i>Mn</i>      | 1.42                 | 0.8720   | 0.71                 | 0.9923   | 75.3                          |
| <i>Fe</i>      | 1.89                 | 0.9204   | 0.62                 | 0.9495   | 41.3                          |
| <i>Sr</i>      | 0.97                 | 0.9234   | 0.73                 | 0.8006   | 5.90                          |
| <i>P</i>       | 0.87                 | 0.9042   | /                    | /        | 2953                          |

The accordance between ICP-MS and UQ methods was worse compared to CW, but still remarkably good ( $0.70 < R < 0.92$ ), especially if it is kept in mind that UQ is a semi-quantitative method. Since UQ is a standardless method, it was possible to extend the comparison of methods even to the elements which were not included in the preparation of spiked cellulose standards materials, such as phosphorus. The results have indicated that UQ measurements of P were quite accurate ( $a = 0.87$ ;  $R = 0.9042$ ).

Analysis of slope coefficients indicates that the UQ method tends to overestimate concentrations for most of the investigated elements, while the CW method tends to underestimate them, but this systematic error can be easily corrected since the very good correlations between ICP-MS and XRF measurements suggest that the error is highly predictable.



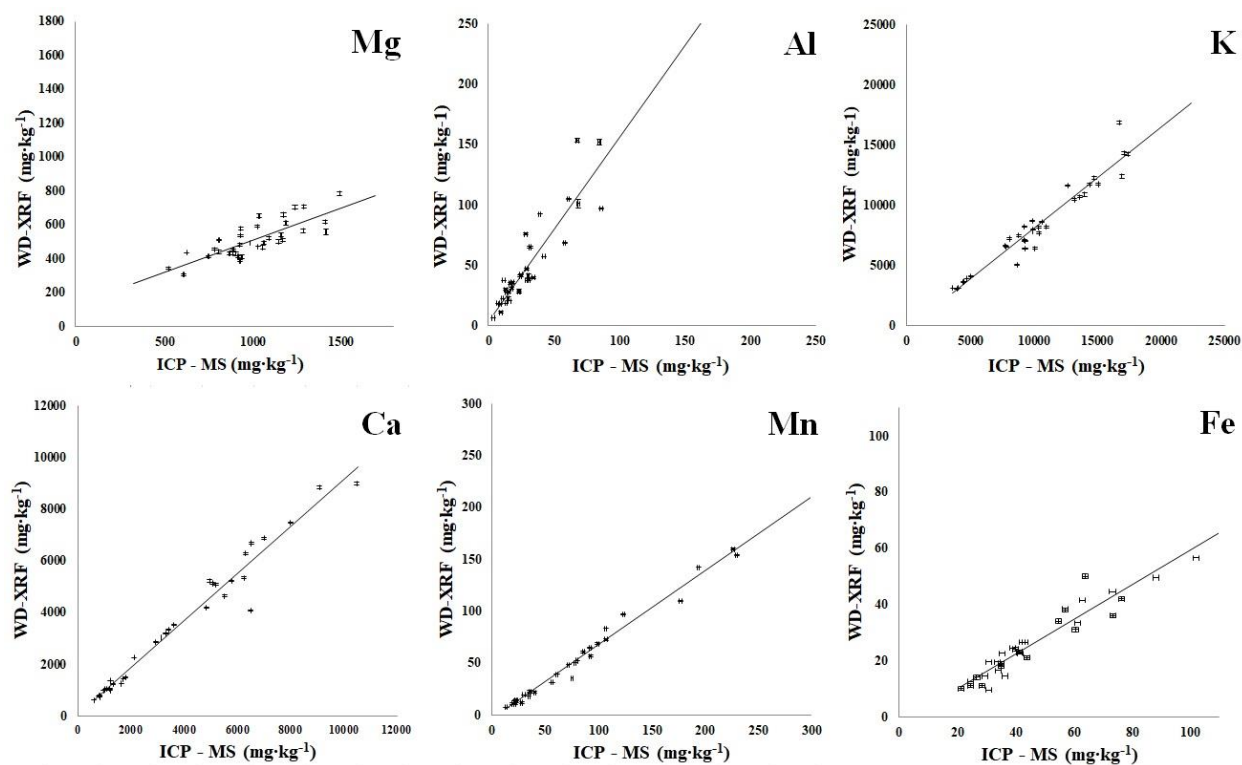


Fig. 6. Comparison of concentrations acquired by CW method and ICP-MS.

#### 4. Conclusion

All investigated methods, using synthetic standard materials created from spiked cellulose, are suitable for the analysis of heavy and light elements in plant material. These methods offer a simple, inexpensive, eco-friendly procedure for the preparation of the samples, even when they are available in limited amounts. All tested methods (PC, CW, and TL) can be very precise, with good repeatability, intralaboratory reproducibility and a wide linear range. The best results were obtained for the elements from the 4<sup>th</sup> period of the Periodic Table of Elements, and the worst for light elements.

The good precision of the TL method indicates that the applied procedure for pellet preparation can be successfully used for sample preparation, and the manual preparation of the sample as a thin layer does not represent a significant source of random errors.

The addition of wax binder did not produce negative effects, on the contrary it has slightly improved the precision, detection, and quantification limits. The TL method is often performed at the same level as the other two methods, although it operates with significantly smaller quantities of analytical material and it has an additional manual step in standard and sample preparation. Nevertheless, in some aspects the TL method was not equally efficient as other investigated methods. The sensitivity of the TL method was less satisfactory when heavier elements were analyzed. The detection limits were higher for all elements heavier than Ca, and the repeatability was not satisfactory if heavy elements were present in low concentrations, but the general conclusion is that the TL method was not significantly falling behind the CW and PC methods.

1 Analysis of accuracy concluded that the CW method has the best accuracy among the compared methods  
2 and UQ has the worst, but when the concentrations of the analytes are sufficiently high, the accuracy of  
3 all methods is similar and very good. The accuracy of the UQ method experienced a sharp decline when  
4 the concentrations of light elements fell below  $50 \text{ mg}\cdot\text{kg}^{-1}$ , while the TL method did not perform as well  
5 as the other methods during the analysis of the heaviest elements. The accuracy of both methods (UQ and  
6 TL) suffered when X-ray filters had to be applied.

7 Elements such Ga, Cd, In, and Tl are usually present in low concentrations in plants, and obtained LoQ  
8 values are high. For that reason, the XRF method would not be recommended for the analysis of those  
9 elements in plant material.

10 It was observed that the UQ method tends to experience positive systematic errors, while CW is prone to  
11 negative errors, but the comparisons with other methods proved that these errors are highly predictable,  
12 and therefore they could be corrected. The UQ method could be the most accurate method when samples  
13 with certain difficult matrices are analyzed (*e.g.* samples with an extremely wide concentration range of  
14 elements).

15

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17

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