

Potential of multivariate analyses of X-ray fluorescence spectra for characterisation of the microchemical composition of plant materials

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Abstract. This work describes a method for the rapid element analysis of plant material using ED-XRF in conjunction with chemometrics. An effective analysis method is developed by measuring certified reference materials (CRM) of plant materials (algae, cabbage, lichen) covering major chemical elements with ED-XRF, to overcome the matrix effect. All samples have been measured additionally by ICP-MS. The ICP-MS analysis was used for missing information on the concentration of some elements in certificated standards. In addition, ICP-MS with CRM has been used to determine sample related element sensitivity for microelements for ED-XRF analyses.

The ED-XRF spectral patterns were used for multivariate principal component analyses by SIMCA strategy instead of each element concentration calculation. The model allows quickly analyse samples for similarity and differentiate them based on a little difference in spectral pattern, which corresponds to a minor difference in element concentration pattern. Samples with specific chemical composition could be easily spotted for in-depth analysis.

The proposed strategy for plant material sample chemical composition screening allows the quick method to improve laboratory work efficiency, reduce unnecessary analysis and rapid method for control reliability of results of more complex chemical methods, such as ICP-MS.

Key words: chemometrics, ED-XRF, ICP-MS, multivariate analysis, plants, screening analysis, SIMCA.

INTRODUCTION

Research on plants and medicinal herbs has a long tradition. However, plants' complex composition is a challenge for their analysis because plants contain both organic and inorganic constituents (Elzain et al., 2016; Pohl et al., 2018; Winkler et al., 2020). The main challenges in plant's multi-element analysis using analytical techniques are concentration variation (macro, micro and trace), content of water, spatial variation

of the composition, matrix effect (mainly organic substances), difficulties in sample preparation, digestion, extraction procedures (Bharti et al., 2017; Bharti et al., 2021).

The chemical composition of plants is affected by several factors: soil composition, climatic and environmental conditions, water quality, fertilisation and plant protection agents, and their ability to assimilate, accumulate and transfer elements (Laursen et al., 2011; Pytlakowska et al., 2012; Pohl et al., 2018; georgieva et al., 2020).

Previous studies have shown that plants are a link between soil quality and human and animal organisms. Therefore, they can be used as powerful indicators (Queralt et al., 2005; Malizia et al., 2010; Laursen, et al., 2011; Pytlakowska, et al., 2012; Remon et al., 2013).

Essential and trace elements play a significant role in plants (Vatansever, 2016). Several techniques, such as atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), neutron activation analysis (NAA), X-ray fluorescence (XRF) spectroscopy, energy dispersive X-ray fluorescence (ED-XRF) spectroscopy, synchrotron radiation X-ray fluorescence (SR ED-XRF) spectroscopy, laser-induced breakdown spectroscopy (LIBS), particle-induced X-ray emission (PIXE), etc. are generally used for the trace element analysis in plants and medicinal herbs (Başgel & Erdemoğlu, 2006; Babu et al., 2015; Elzain et al., 2016; Bharti et al., 2019; Lázaro et al., 2020; Winkler et al., 2020). The drawback of AAS, ICP-MS, ICP-AES techniques is the requirement of sample digestion and dissolution. These techniques are time-consuming and destructive. Non-destructive plant sample multi-elemental analysis techniques are LIBS, NAA and XRF (ED-XRF and SR ED-XRF). However, these methods also have some limitations and drawbacks. For example, the LIBS can be limited by sensitivity and reproducibility (Sharma et al., 2018; Lázaro et al., 2020). A serious concern related to NAA and SR ED-XRF techniques is synchrotron and nuclear reactor availability and cost for routine analysis and quality control of plants and medicinal herbs.

The present study describes a method for the rapid element analysis and profiling (fingerprinting) plant material using energy dispersive X-ray fluorescence spectroscopy (ED-XRF). The ED-XRF method is non-destructive, has minimal sample preparation, simple spectra, and is applicable as a multi-element method over a wide range of concentrations, and the equipment cost is low. This technique has been used for the micro-elemental qualitative and quantitative analysis (Ekinci et al., 2003; Mbaye et al., 2015). In this work, the ED-XRF patterns combined with multivariate principal component analysis (PCA) were used for fingerprinting, which implies the determination of combinations of elements (Djingova et al., 2004). A fingerprint is defined as a specific profile that visualises the chemical composition of a particular sample. Several studies suggest that the fingerprinting technique can be effectively used to construct a specific pattern of recognition. Fingerprints can be used for organic and inorganic matter characterisation in plants, such as ICP and XRF methods for inorganic, but FTIR and chromatography for organic composition. The ED-XRF fingerprinting technique combined with a multivariate statistical procedure is used to extract information from these fingerprinting profiles about the origin, quality and to compare fingerprinting profiles of different herbs (Laursen et al., 2011; Custers et al., 2016; Torres Astorga et al., 2018; Brangule et al., 2020).

The XRF method is strongly influenced by matrix effects (Guild & Stangoulis, 2016). In addition, light elements comprising water and organic matter also negatively affect measurements due to X-ray scattering and attenuation (Ravansari, 2020). To minimise the plants' matrix effect, four matrix-matched reference materials were used, covering a wide range of elements and strict reference values.

The objective of the present work is to evaluate: (i) the use of the ED-XRF method for fingerprinting medicinal herbs in combination with PCA and (ii) the effect of matrix-matched standards on result interpretation.

MATERIALS AND METHODS

Samples

The same certified reference materials and medicinal herbal samples were used for ICP-MS and for ED-XRF analysis.

Certified reference materials (CRM): *IAEA 336 Lichen*, *IAEA 392 Algae*, *IAEA 413 Algae*, *BCR 679 Algae* were used to standardise methods for the following elements:

Al, As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Se, Sr, V, Zn.

Plant Material – medicinal herbs (MH): 13 different Chamomile (*Matricariae flos*), 6 small-leaved Linden (*Tiliae flos*), 4 Calendula (*Calendulae flos*) and Hibiscus (*Hibisci sabdariffae flos*) commercial tea samples available in Latvia.

Sample preparation

All medicinal herb samples were ground to powder and sifted through a 2 mm sieve. Powders were stored at room temperature for further analysis.

For the XRF method samples were pressed in pellets using a manual hydraulic press. The diameter of the pellet disc was 10 mm and mass 0.2 g. The pellets reduce scattering, show a higher signal-to-noise ratio, and this allows the light elements to be detected above the background.

For the ICP method samples were prepared using the microwave-assisted acid digestion method. Samples were ground with a laboratory mill (*J13M, Russia*) and then sieved through a 0.2 mm sieve (*Rotilabo*). Approximately 0.2 g of each sample was weighed into Teflon vessel, then 6 mL of concentrated HNO₃ (TraceMetal grade, 69%, Fischer) and 2 mL of concentrated H₂O₂ (For Trace Analysis, 30%, Fischer) were added, and the vessel was tightly closed. Samples were heated in a microwave oven (Milestone Start E) under pressure conditions. The heating program was set as heating for 15 min to 160 °C and holding at 160 °C for 30 min. After heating, vessels were cooled to room temperature and deionized water (< 0.055 µS cm⁻¹, Adrona) was used to dilute samples to 50 mL.

Analytical methods

ED-XRF. Shimadzu EDX-8000 (furnished with a Rh anode, max power 50 kV, vacuum, no filter, 10 mm collimator, 600 s measurement time).

ICP-MS. Inductively Coupled Plasma Mass Spectrometer (Agilent 8900 ICP-MS QQQ) equipped with a micro-mist nebuliser and He collision cell was applied to determine the following elements: Na, Mg, Al, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, g a, g e, As, Se, Rb, Sr, Y, Cd, Ba, La, Ce, W, and Pb.

The following instrumental parameters of ICP-MS were set: RF power (1.550 W); sampling depth (8 mm); nebulizer gas flow rate (0.90 mL min⁻¹); plasma gas flow (15 L min⁻¹); He cell gas flow (5 mL min⁻¹); extraction 1 lens (-5.0 V); extraction 2 lens (7.0 V); omega lens 7.0 (-200 V); omega bias lens (-110 V); octupole bias (-3.0 V); cell gas flow rate (20% of full scale); axial acceleration (1.0 V).

The calibration graph was made using six standard solutions in the concentration range from 0.1 µg L⁻¹ to 500.0 µg L⁻¹. Analytical standard stock solutions were prepared from Certified Reference Material (HPS, ICP-MS-68A, 10 mg L⁻¹, traceable to NIST SRM 3100). Element concentrations in samples were calculated using the external calibration graph method, and the blank correction was applied. Internal standard solution (10 µg L⁻¹, Agilent) was used for system stability control during measurements. Two standard solutions (10 µg L⁻¹) were used between every ten samples to verify system stability.

Analysis of the spectra

The ED-XRF spectra were evaluated in order to select emission lines. Spectra were investigated, and normalisation was performed with the academic freeware software *SpectraGryph 1.2.14*. The spectra were normalised to the Rh tube emitted Rh K_α line.

The ICP-MS data procession, collection, and calculation of results were made by a *MassHunter* workstation program, including its subprograms - Instrument control and Offline data analysis.

Chemometrics

The principal component and hierarchical cluster analysis were performed using *SIMCA 14* software. Spectra were smoothed and denoised by a Savitzky - golay filter (polynomial order 5 and points 10), the second derivative of the samples was recorded. The component analysis was used to identify the dominant clusters in the data set. For the HCA, Ward's algorithm was used.

RESULTS AND DISCUSSION

Since the ICP-MS is suitable for a wide range of concentrations and elements, the ICP-MS method was chosen to fill the missing dates in creating a fingerprinting model. Theoretically, the ED-XRF method can measure a wide range of elements, from sodium Na (11) through uranium U (92). In practice, ED-XRF sensitivity is not sufficient for light elements such as sodium, magnesium, aluminium and silicon. The same certified reference materials (*IAEA 336 Lichen, IAEA 392 Algae, IAEA 413 Algae, BCR 679 Algae*) and medicinal herbal samples were used for both methods ICP-MS and ED-XRF.

Standard materials are usually certified for specific elements (10–15) with a specific concentration. Concentrations can vary significantly from one certified material to another. The ICP-MS method was chosen, to obtain information about the standards of uncertified components and their concentrations with high probability.

Analysing the ED-XRF spectra (Fig. 1) together with the quantitative results obtained by ICP-MS, the information about the sensitivity of the ED-XRF method and the detection limits of elements was obtained. For example, in certified standards, elements in very different concentration intervals were detected. For *K-elements*: magnesium Mg minimal concentration 4,000 mg kg⁻¹; aluminium Al and silicon Si –

600 mg kg⁻¹; phosphorus P and sulphur S – 200 mg kg⁻¹; titanium Ti to molybdenum Mo–10 mg kg⁻¹ and *L*-elements such as lead Pb and mercury Hg at least 50 mg kg⁻¹.

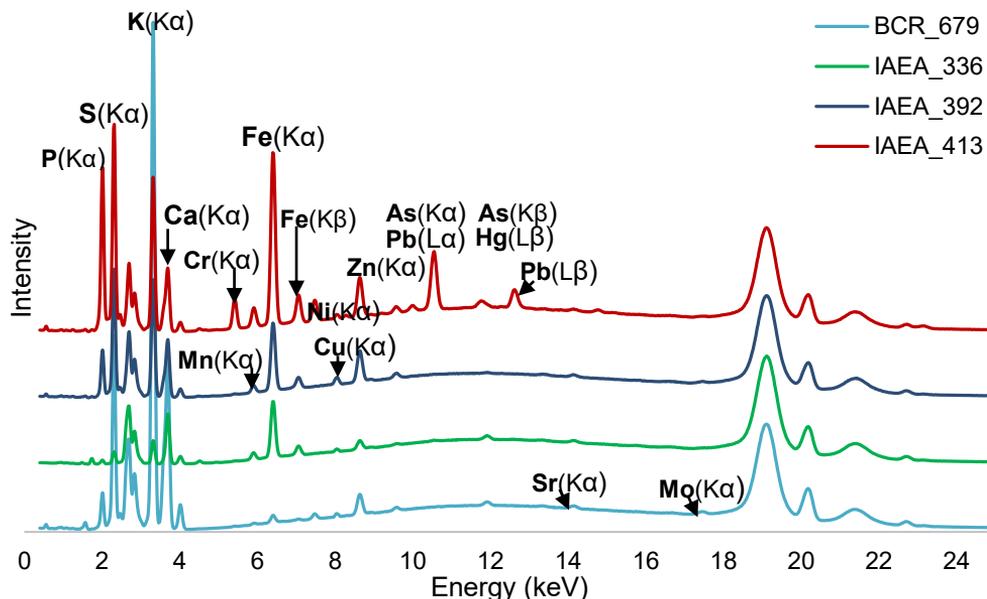


Figure 1. The ED-XRF patterns for certified reference standards (CRM).

It can be concluded that analysing different samples must consider that a high concentration of an element in a sample does not guarantee that these elements will have visible peaks in the spectrum. For example, magnesium - a small peak can be observed only in a sample with a concentration above 4,000 mg kg⁻¹. However, the peak is not visible in the sample with a magnesium concentration above 1,000 mg kg⁻¹.

Analysis of the data obtained by the ICP-MS and ED-XRF method shows that the intensity of spectral lines depends on the combination of elements in the samples. For example, a high concentration of potassium K in CRM 679 affects the sensitivity of aluminium Al. High concentration potassium reduces the possibility to identify aluminium as escape peaks partly overlap the spectral line of aluminium. Nevertheless, in CRM 336, potassium concentration is significantly lower, and aluminium is detectable at 680 mg kg⁻¹.

Fig. 2 shows an example of two elements Cu and Zn, with high sensitivity. Copper is detectable at low concentration ~ 3 mg kg⁻¹, but zinc makes intensity patterns proportionally to CRM certified concentrations.

The obtained data showed that very significant in the fingerprinting is the interference effect - the overlapping of spectral lines distorting results for one or more elements. For example, overlapping spectral lines for: lead Pb L α and arsenic As K α (10.55 keV); arsenic As K β and mercury Hg L β (11.80 keV) and chlorine Cl K α and rhodium Rh lamp L α and L β spectral lines (Fig. 3) were detected.

Literature shows that it is possible to build a fingerprinting model even if there are overlapping peaks. This is because the overlapping peak gives the spectrum a particular shape, making the spectrum unique for each sample.

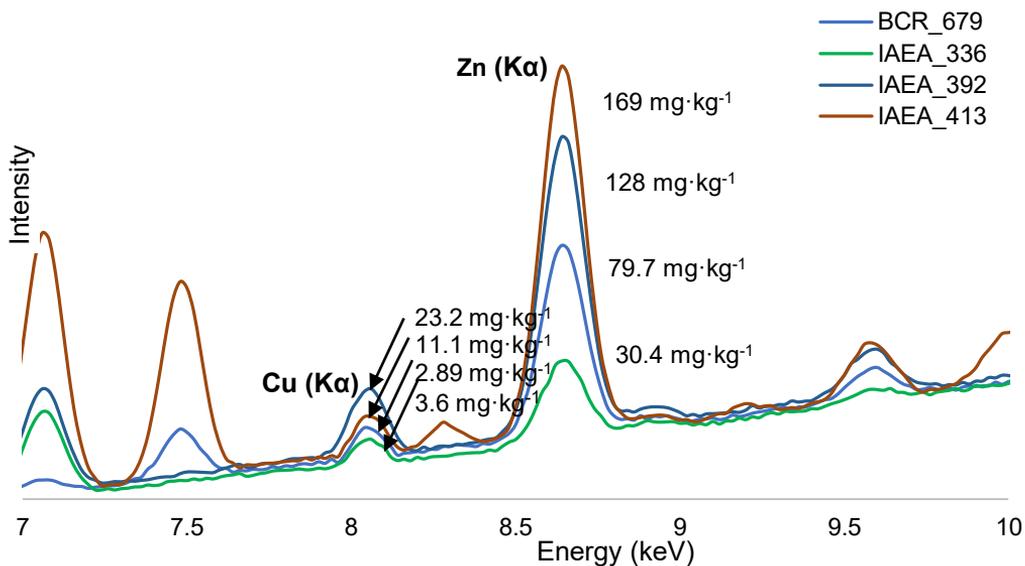


Figure 2. ED-XRF pattern intensities of Cu and Zn affected by the concentration in the certified reference material.

The next point of interest was to differentiate ED-XRF fingerprints of medicinal herbs MH and verify compliance with certificated reference materials. The conclusions about the fingerprinting method's effect were obtained by combining the ED-XRF and ICP-MS methods with unsupervised multidimensional statistical analysis.

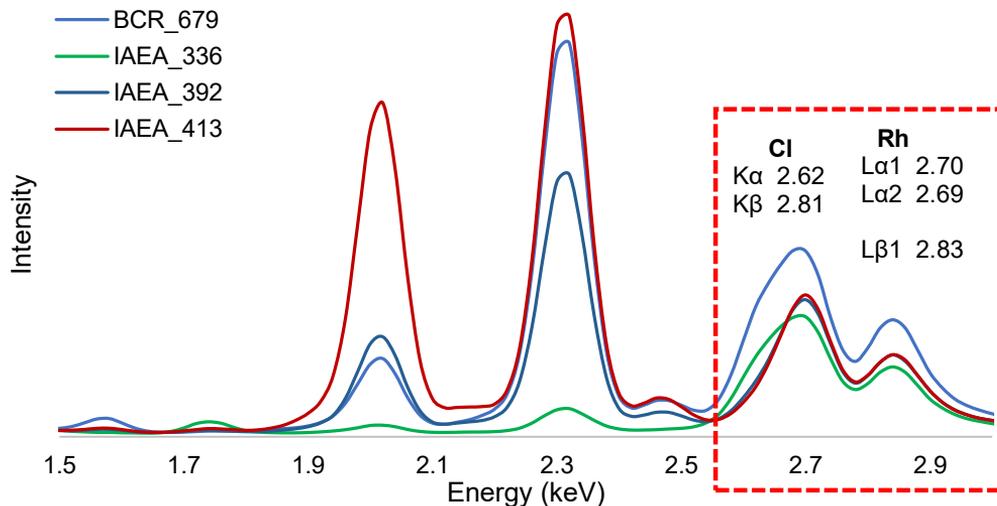


Figure 3. Overlapping element peaks for chlorine Cl and rhodium Rh.

The formation of clusters was depicted in diagrams and dendrograms. Four major clusters in PCA can be identified (Fig. 4): linden, chamomile, calendula and separate cluster for three reference materials – *IAEA 336 Lichen*, *IAEA 392 Algae*, *IAEA 413 Algae*.

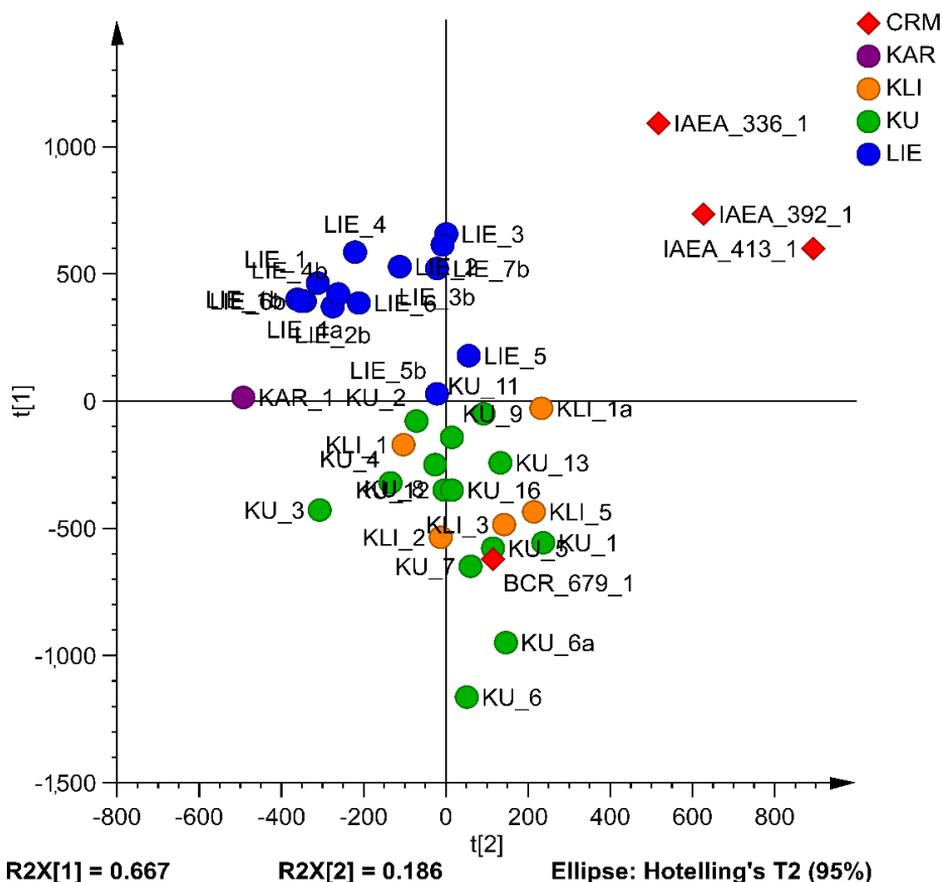


Figure 4. The PCA clusters for certified reference standards (CRM) and medicinal herbs. Red diamonds – CRM; blue – linden; green – chamomile; orange – calendula; violet – hibiscus.

PCA1 describes 67%, but PCA2 19%, forming 86% of spectral information. Loadings of PCA show a clear difference between PCA1 and PCA2: the potassium concentration describes dispersion across the PCA1 axis in samples, but across the PCA2 axis - by the ratio between calcium and iron concentration - dominant increase in calcium concentration and decrease in iron concentration. A combination of the first two components describes 85% of the composition of detectable elements. Cluster component PCA3 forms only 7% of the information, showing a dominant increase in iron concentration.

The PCA diagram shows that only one standard fits into the herbal cluster. The other three CRMs form a separate cluster. This leads to the conclusion that the matrix effect is essential using the fingerprint method and should be taken into account when choosing standards.

CONCLUSIONS

This work demonstrates the potential of the ED-XRF spectra fingerprinting method in combination with statistical analysis as a sensitive, rapid and non-destructive method for quality control in order to check trace element contents on this type of matrix.

The XRF method has improved during recent years by enhancing SDD detector resolution and increasing count rates. The current technical capabilities of XRF systems provide researchers with valuable information about major micro and macro elements. However, the use of XRF for the control of trace concentration of heavy elements is limited by current sensitivity, and more sensitive methods should be used.

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