

# LIBS analysis of crop plants†

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A fast and cheap method for the analysis of macronutrients (K, P, Mg and Ca) in leaves of crop plants (wheat, poppy, barley and rape) using double pulse laser induced breakdown spectrometry (LIBS) is suggested. A commercially available LIBS spectrometer was calibrated with the aid of authentic plant samples previously analysed by AAS and ICP-OES after microwave digestion. The concentration ranges of K, P, Mg and Ca in the calibration standards were 30–66; 2.8–6.6; 1.0–5.6 and 6–24 g kg<sup>-1</sup>, respectively. Cryogenic grinding and powder pressing to pellets were used as sample preparation steps. Obtained LIBS/ICP-OES recovery values for authentic crop plant samples were 93–109% (K); 93–114% (P); 89–109% (Mg) and 94–110% (Ca). Six certified reference materials (CRMs) of plant tissues were used for the LIBS method validation. Recovery values of 96–101% were obtained for K and P, when the certified concentration of the element in the CRM was inside the concentration range of the used calibration standards. Substantial matrix differences of the CRMs and LIBS calibration standards seem to be a probable reason for the worse recovery results (58–99%) obtained for Mg and Ca.

## Introduction

Laser induced breakdown spectroscopy (LIBS) is a novel dynamically developing method of atomic spectroscopy with a wide range of applications. In LIBS, a high power laser pulse is focused on a small spot of the sample where it ablates the surface layer and successively heats and ionises the vaporised matter and produces plasma. The spectral emission, which occurs as a result of the subsequent relaxation of the constituent excited species, is measured by an appropriate spectrometer.<sup>1</sup>

An application of the LIBS technique for plant tissue analysis was described by Sun *et al.*<sup>2</sup> A direct multi-elemental analysis of P, Al, Ca, Cu, Mg, Zn, Mn, and Fe in solid plant samples was performed. Dry powders of NIST standard reference materials, including apple, peach, spinach and tomato leaves, were directly applied onto a double stand tape and then measured. The precision for the intra-measurements was about 8–15% and for the inter-measurements, 5%. Femtosecond LIBS technique was used for the investigation of Pb and Cd accumulation in different parts of leaf samples by Kaiser *et al.*<sup>3</sup> Spatial information about the distribution of heavy metals within particular parts of leaves was obtained. A similar application was described by Galiova *et al.*<sup>4</sup>

An experimental setup consisting of a Nd:YAG laser operating at 1064 nm and an Echelle spectrometer with an ICCD detector was used for the analysis of P, K, Ca and Mg in crop

plants by Trevizan *et al.*<sup>5</sup> The particular calibration curves were obtained using a limited set of four (or six) certified reference materials (CRMs). The concentration ranges of the calibrations for P, K, Ca and Mg were 1.0–3.4; 1.3–32.5; 0.1–16.8 and 0.4–4.3 g kg<sup>-1</sup>, respectively. The coefficients of variation of the LIBS results ( $n = 10$ ) varied from 5% to 25% depending on the type of sample and the analysed element. The recovery, expressed as a ratio of LIBS and ICP-OES results, varied from 35% to 145%.

The main aim of our project was to develop a fast, cheap, and effective methodology for the analysis of real crop plant samples using laser induced breakdown spectroscopy. We intended to prepare a routine method with minimal sample preparation steps, maximal throughput of samples through laboratory processing and negligible influence of operators on analytical results. The utilisation of a commercially available instrument instead of an experimental set-up was necessary for the applicability of our results in a regular agrochemical laboratory.

## Experimental

### Sample preparation

The aim of the sample preparation step was to unify the particle size distribution of particular samples and thereby eliminate the influence of micro-heterogeneity of pellets and avoid excessive sputtering of dust in the sample chamber. Plant samples and CRMs were cryogenically ground using Freezer/Mill 6750 with Model 6751 grinding vials (Spex, Ceritprep, USA). A pre-cooling time of 7 min with two grinding steps 2 min long interrupted by a 2 min re-cooling period were applied. The frequency of steel impactor was 10 Hz. The obtained powder was pressed into pellets by a force of 100 kN for 1 min using an evacuable KBr die set with an inner diameter of 16 mm (ICL, Garfield, USA) and a manual hydraulic press (Maassen, Germany).

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## LIBS analysis

The LIBS spectrometer (LEA S500, Solar TII Ltd., Belarus) used is fully described elsewhere.<sup>6</sup> The instrument has a dual pulse Q-switched Nd:YAG laser operating at 1064 nm. The laser emits two colinear pulses of about 12 ns duration with an energy per pulse typically variable between 80–150 mJ at a maximum repetition rate of 20 Hz. The inter-pulse delay can be set from 0 to 20  $\mu$ s. The Czerny Turner spectrograph, with a focal length of 500 mm and grating of 1800 lines  $\text{mm}^{-1}$ , provides a dispersion of 1 nm  $\text{mm}^{-1}$ . The wavelength range of the spectrograph is 170–800 nm. Recording of the spectra is carried out by means of a back thinned and front illuminated CCD-camera (2048  $\times$  14 pixels) with minimal integration time of 1  $\mu$ s. The spectra are measured in arbitrary setting 30 nm regions.

For all measured lines, a double pulse mode with an inter-pulse delay of 7  $\mu$ s was adopted. The focal spot diameter was set up on the minimal value of 200  $\mu$ m to minimize the sample sputtering and dusting of the entrance window of the spectrometer. All measurements were performed in quiescent air atmosphere with normal pressure. Other experimental parameters were adjusted separately for each particular element to optimise the analytical line intensity and the measurement reproducibility, and to obtain sufficiently a wide dynamic range of calibrations. Optimised parameters are summarised in Table 1.

At each measuring point, 5 blank (non-registered) laser shots were first performed followed by 30 analytical (registered) shots. Each spectral region for each particular sample was measured at sixteen separate points placed in a square with a side-length of 2 mm. Afterwards, all sixteen individual spectra were averaged into one which was then used for the equipment calibration or the sample analysis.

## Results and discussion

### Calibration of the LIBS spectrometer

Seven authentic samples of barley leaves and three samples of poppy leaves were used as calibration standards. Concentrations of the selected elements in the above mentioned samples were measured in three independent accredited laboratories by AAS and ICP-OES after microwave digestion. The concentration ranges of K, P, Mg and Ca in the calibration standards were 30–66; 2.8–6.6; 1.0–5.6 and 6–24  $\text{g kg}^{-1}$ , respectively.

The integral area of the 404.72 nm line of K was calculated after Gaussian fitting of the peak in the integration window 404.67–404.78 nm (8 pixels). The left background point was calculated as an average intensity in the integration window 404.64–404.66 nm (1 pixel) and the corresponding integration window for the right background point was 404.79–404.81 nm (1 pixel). The analytical line of P with wavelength 213.61 nm was

measured in the window 213.53–213.74 nm (15 pixels) and the corresponding background points in the windows 213.44–213.47 nm (2 pixels) and 213.78–213.79 (1 pixel). The spectral window for the Mg measured at 428.94 nm was 277.94–278.07 nm (9 pixels) and for the background 277.90–277.92 nm (1 pixel) and 278.10–278.12 nm (1 pixel). Ca was measured at 428.94 nm in the window 428.91–429.02 nm (8 pixels) and the background points at 428.72–428.79 nm (3 pixels) and 429.10–429.14 (3 pixels).

The integral peak areas of the selected analytical lines previously corrected for the background intensity were plotted against the corresponding concentration values, and the following regression model was suggested for the description of experimental data

$$C_X = \beta_0 + \beta_1 I_X + \beta_2 I_X^2 + \beta_3 I_{\text{BGR}}$$

where  $C_X$  is the concentration of element X expressed in  $\text{g kg}^{-1}$ ,  $I_X$  is the analytical line intensity and  $I_{\text{BGR}}$  is the background intensity (in cps) calculated for the corresponding analytical line. Using the Student's t-test, the terms with the statistically insignificant parameters  $\beta$  were stepwise removed from particular equations and, therefore, the obtained simplified calibration models were then employed for the analysis of crop plant samples. OPstat software, designed by Prof. Oldřich Pytela (University of Pardubice, Czech Republic), was used for all statistical calculations. The resulting calibration equations for particular elements are summarised in Table 2.

### Analysis of authentic crop plant samples

Seven samples of wheat leaves and three samples of rape leaves were analysed by the proposed LIBS method using the above mentioned calibration models. The results of the LIBS technique were compared with average results from three independent accredited laboratories where these samples were analysed by ICP-OES or AAS after microwave digestion.

The results of the LIBS summarised in Table 3 represent average values,  $X$ , and the corresponding standard deviations,

**Table 2** LIBS calibration models

Element	Model	$R^2$ (%)
K	$C_K = 1.1 \times 10^{-2} + 4.08 \times 10^{-4} I_K - 5.88 \times 10^{-8} I_K^2$	98.51
P	$C_P = 1.38 \times 10^{-3} + 1.86 \times 10^{-5} I_P$	98.43
Mg	$C_{\text{Mg}} = -1.249 \times 10^{-3} + 1.63 \times 10^{-5} I_{\text{Mg}} - 3.86 \times 10^{-6} I_{\text{BGRMg}}$	99.18
Ca	$C_{\text{Ca}} = -1.72 \times 10^{-2} + 4.07 \times 10^{-5} I_{\text{Ca}} + 3.194 \times 10^{-4} I_{\text{BGRCa}}$	98.91

**Table 1** LIBS experimental conditions

	Ca	K	Mg	P
Wavelength/nm	428.94	404.72	277.98	213.61
Spectral region/nm	415–445	385–415	270–300	195–225
Spectrograph slit width/ $\mu$ m	20	15	15	15
Laser energy/mJ	68	65	68	78

**Table 3** Results of LIBS analysis of real crop plant samples

<b>K</b>					
LIBS					
	$X/g\text{ kg}^{-1}$	<i>SD</i>	<i>RSD</i> (%)	AAS/ICP-OES	Recovery (%)
Rape 1	36.3	1.67	4.6	37.7	96.2
Rape 2	36.8	1.43	3.9	34.3	107.2
Rape 3	28.1	1.23	4.4	27.6	101.8
Wheat 1	50.4	0.50	1.0	49.9	101.1
Wheat 2	40.6	1.39	3.4	39.7	102.1
Wheat 3	30.7	1.05	3.4	33.1	92.7
Wheat 4	27.8	0.56	2.0	25.1	110.5
Wheat 5	35.0	0.54	1.5	32.2	108.7
Wheat 6	41.1	0.84	2.1	39.5	104.0
Wheat 7	22.4	0.75	3.4	22.8	98.3
<b>P</b>					
LIBS					
	$X/g\text{ kg}^{-1}$	<i>SD</i>	<i>RSD</i> (%)	AAS/ICP-OES	Recovery (%)
Rape 1	7.14	0.34	4.7	7.21	99.1
Rape 2	5.30	0.12	2.3	5.71	93.0
Rape 3	5.62	0.06	1.1	5.79	96.9
Wheat 1	4.80	0.10	2.0	5.03	96.1
Wheat 2	3.95	0.08	2.0	4.01	98.8
Wheat 3	2.86	0.05	1.7	2.62	110.0
Wheat 4	3.25	0.01	0.2	3.09	104.8
Wheat 5	3.91	0.10	2.6	4.19	93.0
Wheat 6	4.33	0.07	1.7	3.81	113.8
Wheat 7	2.26	0.08	3.1	2.48	90.4
<b>Mg</b>					
LIBS					
	$X/g\text{ kg}^{-1}$	<i>SD</i>	<i>RSD</i> (%)	AAS/ICP-OES	Recovery (%)
Rape 1	1.84	0.05	2.5	1.91	97.0
Rape 2	1.49	0.04	2.9	1.59	93.1
Rape 3	1.54	0.07	4.6	1.70	90.3
Wheat 1	1.16	0.04	3.7	1.21	96.8
Wheat 2	1.07	0.04	3.4	1.01	107.5
Wheat 3	0.98	0.05	5.4	1.11	89.0
Wheat 4	0.81	0.06	8.0	0.90	89.8
Wheat 5	0.81	0.04	5.4	0.91	90.1
Wheat 6	1.64	0.04	2.5	1.49	109.3
Wheat 7	0.64	0.02	3.2	0.78	80.0
<b>Ca</b>					
LIBS					
	$X/g\text{ kg}^{-1}$	<i>SD</i>	<i>RSD</i> (%)	AAS/ICP-OES	Recovery (%)
Rape 1	8.88	0.22	2.5	8.93	99.5
Rape 2	12.82	0.54	4.2	12.71	100.9
Rape 3	14.10	1.22	8.7	14.59	96.6
Wheat 1	5.73	0.35	6.1	5.71	100.3
Wheat 2	4.30	0.19	4.5	3.90	110.2
Wheat 3	3.38	0.15	4.3	3.62	93.8
Wheat 4	4.59	0.13	2.9	4.31	106.7
Wheat 5	4.10	0.08	1.9	4.32	95.3
Wheat 6	4.58	0.22	4.8	4.88	93.6
Wheat 7	4.34	0.14	3.2	4.12	105.3

**Table 4** Parameters and statistical characteristic of regression equation comparing AAS or ICP-OES and LIBS results

Element	Equation	95% interval of reliability		$R^2$ (%)
		$\beta_0$ (intercept)	$\beta_1$ (slope)	
K	$C_{K-LIBS} = -0.126 + 0.984C_{K-AAS/ICP-OES}$	-0.496 to 0.748	0.81 to 1.16	95.33
P	$C_{P-LIBS} = 0.031 + 0.919C_{P-AAS/ICP-OES}$	-0.021 to 0.083	0.80 to 1.04	97.28
Mg	$C_{Mg-LIBS} = -0.009 + 1.020C_{Mg-AAS/ICP-OES}$	-0.036 to 0.019	0.81 to 1.23	94.12
Ca	$C_{Ca-LIBS} = -0.028 + 0.961C_{Ca-AAS/ICP-OES}$	-0.012 to 0.068	0.91 to 1.02	99.64

*SD*, from the measurement of three pellets prepared from each sample.

The parameters of the following regression equation were calculated to evaluate the parity between the AAS/ICP-OES and the LIBS results:

$$C_{LIBS} = \beta_0 + \beta_1 C_{AAS/ICP-OES}$$

where  $C_{LIBS}$  represents the concentration of a particular element in the crop plant sample ( $g\ kg^{-1}$ ) measured by LIBS and  $C_{AAS/ICP-OES}$  is the average value of the element concentration determined by the accredited laboratories. The statistical parameters of regression for measured macronutrients are summarised in Table 4.

The 95% reliability intervals for the slope include a value of 1 and the 95% reliability intervals for the intercept include 0. Consequently, it can be stated that there is no statistically significant difference between results obtained by LIBS and by AAS/ICP-OES for any measured element.

#### Analysis of certified reference materials

Six certified reference materials of plant tissues (GBW 07605—tea leaves, GBW 07603—bush branches, NIST 1575—pine

needles, NCS DC 73550—poplar leaves, QCM 3—strawberry leaves and 2Theta—ryegrass) were analysed by the above mentioned LIBS method. Results of the LIBS summarised in Table 5 were obtained by measurement of three pellets prepared from each CRM.

Satisfactory results were obtained for K and P when the certified concentration of the element in the CRM was inside the concentration range of the used calibration standards. In other cases, worse recovery values were observed. On the basis of a strong linear dependence between LIBS and the certified values, an essential improvement of the results after a calibration range extension using appropriate standards is assumed.

For Mg and Ca, non-conformance recovery values can be noted for all CRMs. Any strong interference connected with the mechanical feature of samples weren't supposed because of the substantial uniformity of the particle size distribution in all pellets prepared from cryogenically ground material. The explanation could be any matrix effect related to the different chemical composition of the CRMs and the calibration standards.

The most evident composition difference between the LIBS calibration standards and the analysed CRMs seems to be the content of K. The concentration of this element in the CRMs varied from 4 to 30  $g\ kg^{-1}$  whereas the K concentration range for

**Table 5** Results of LIBS analysis of plant CRMs

Sample	K			P		
	$C_{LIBS}/g\ kg^{-1}$	$C_{cert}/g\ kg^{-1}$	Recovery (%)	$C_{LIBS}/g\ kg^{-1}$	$C_{cert}/g\ kg^{-1}$	Recovery (%)
GBW 07605	16.9 ± 0.9	16.6 ± 0.6	101.8	2.82 ± 0.08	2.84 ± 0.06	99.3
GBW 07603	10.0 ± 1.1	9.2 ± 0.6	108.7	0.93 ± 0.09	1.0 ± 0.03	93.3
NIST 1575	2.12 ± 0.3	4.17 ± 0.07	50.8	1.28 ± 0.15	1.07 ± 0.08	119.6
NCS DC 73350	15.2 ± 0.4	13.8 ± 0.7	110.3	1.37 ± 0.11	1.68 ± 0.06	81.7
QCM3	23.2 ± 3.1	21.2 ± 2.1	109.5	—	—	—
Ryegrass	30.4 ± 1.6	29.99 ± 2.92	101.4	3.88 ± 0.4	4.04 ± 0.25	95.8
Sample	Mg			Ca		
	$C_{LIBS}/g\ kg^{-1}$	$C_{cert}/g\ kg^{-1}$	Recovery (%)	$C_{LIBS}/g\ kg^{-1}$	$C_{cert}/g\ kg^{-1}$	Recovery (%)
GBW 07605	1.40 ± 0.04	1.7 ± 0.1	82.4	4.25 ± 0.1	4.3 ± 0.2	98.8
GBW 07603	3.34 ± 0.04	4.8 ± 0.3	69.6	9.7 ± 0.1	16.8 ± 0.6	57.7
NIST 1575	0.82 ± 0.01	1.06 ± 0.17	77.5	2.6 ± 0.1	2.5 ± 0.1	104.4
NCS DC 73350	4.93 ± 0.16	6.5 ± 0.5	75.8	11.8 ± 0.4	18.1 ± 1.3	65.2
QCM3	3.78 ± 0.05	4.21 ± 0.42	89.7	13.5 ± 0.1	15.5 ± 0.15	87.1
Ryegrass	1.7 ± 0.04	1.78 ± 0.11	95.5	6.85 ± 0.3	6.68 ± 0.35	97.5

**Table 6** Ratios of declared concentrations Mg, Ca and K in measured CRMs

	GBW 07605	GBW 07603	NIST 1575	NCS DC 73350	QCM3	Ryegrass
Mg/K	0.10	0.52	0.25	0.47	0.20	0.06
Ca/K	0.26	1.83	0.60	1.31	0.73	0.18

the calibration standards is 30–60 g kg<sup>-1</sup>. The best results of Ca and Mg recovery were obtained for the CRMs ryegrass, QCM-3 and GBW 07605, which contain 30, 21 and 17 g kg<sup>-1</sup> of K, respectively. Results for other CRMs with markedly lower K contents were substantially worse.

Generally, there is no evident linear dependence between the Ca and Mg recovery values and the K content in corresponding CRMs. Different concentration ranges of Ca and Mg in LIBS calibration standards and CRMs could be the reason. To describe the combined influence of the corresponding analyte and the K content on the recovery results, Mg/K and Ca/K ratios were calculated and are summarised in Table 6. As it can be seen, the Mg/K rate in the used calibration standards varied from 0.022 to 0.044 and the rate of Ca/K varied from 0.14 to 0.36. The best recovery values were obtained for CRMs where the ratios Mg/K and Ca/K corresponded to the rates in the calibration standards

## Conclusion

A procedure for the analysis of K, P, Mg and Ca in crop plant samples using a commercially available LIBS spectrometer was developed. Real plant samples previously analysed by ICP-OES or AAS after microwave digestion in three accredited laboratories were used as calibration standards. A satisfactory agreement between LIBS and AAS/ICP-OES results was achieved when real crop plant samples with similar chemical composition were

measured. The results for CRMs with different ratios of the main matrix elements in comparison to the used calibration standards were unsatisfactory. K was identified as a main interfering element.

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