

Relative Determination of Micronutrients of Different Species of *Teff* (*Eragrestis*) Seeds of Ethiopia Origin by Calibration Free Laser Induced Breakdown Spectroscopy Techniques

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Abstract: The laser-induced breakdown spectroscopy techniques has been used to analysis the multi-component of three different species of *Teff* seeds (Red, White and *Sirgegna*) of Ethiopia origin using a second harmonic (532 nm) of a nanosecond Q-switched Nd: YAG laser focused on the surface of the pelletized powder of *Teff* seeds. Based on the idea of the plasma is homogeneous. The seven essential micronutrients in three species of *Teff* seeds are identified carbon as a matrix element. Electron density and plasma temperature are calculated applying Saha-Boltzmann equation and Boltzmann plot method. And making use of the semi-quantitative method the three species relative concentrations of (Ca, Mg, Al, Si, Mn, Fe and K) are obtained using Calibration Free Laser Induced Breakdown Spectroscopy (CF-LIBS) technique. The result demonstrated that the relative concentrations of the some elements in the species are different. In Red *Teff* species Ca is more, but Mg is least. On the contrary Mg is high in *Sirgegna* and White *Teff* as compared to Red *Teff*. And High content of Calcium, Magnesium and Iron micronutrients are found in the three species.

Keywords: CF-LIBS, plasma, relative quantitative analysis, three species of *Teff* seeds

INTRODUCTION

The growing awareness of the importance of analysis of trace, minor and major elements of food for human health has generated interest in different food preparation practice and in consequence, the need for their quantitative and qualitative determination. As a response, over the past two decades, a number of analytical methods measuring the qualitative and quantitative activity have been developed.

One of the emerging techniques is LIBS. The simplicity and versatility of the LIBS technique has allowed it to be applied to a wide variety of materials and substances (David and Rosemarie, 2009). However, because of the new technique and other robust micro analytical methods for elemental analysis for cereal crops, there has been only limited interest in cereal crops applications of the LIBS technique.

Important attributes of a LIBS sensor system for food analysis include:

- Real-time response
- In-situ analysis with no sample preparation required
- A high sensitivity to low atomic weight elements which are often difficult to determine by other techniques (Celio *et al.*, 2007)

LIBS technology is now reached its maturity and inexpensive to offer a capability for both laboratory and field-deployable analysis. Because of these merits of LIBS techniques, among available elemental analysis techniques, the Laser-Induced Breakdown Spectroscopy (LIBS) technique has shown in recent years its great potential for rapidly qualitative and quantitative analysis of micronutrients of food yields (Jez *et al.*, 2010). Quantitative analysis can be done by calibration method or calibration free-techniques. Due to difficulty of to get standard sample, calibration free-techniques is preferable and also effective method for semi-quantitative determination (Tognoni *et al.*, 2009).

People in the world use different kind of cereal crop as the main item of their food due to their diverse ecological, environmental and preparation custom difference. *Teff* (*Eragrestis*) is a fine stemmed, tufted annual grass and it is categorized in a cereal crop, preferred over other cereal crops in Ethiopia, the leading producer of *Teff*. *Teff* is estimated to feed millions of people and is extremely important for at least 60 million people comprising rural producers, processors and consumers in Ethiopia (El-Alfy *et al.*, 2012). Apart from serving as food, *Teff* has a lot of potential industrial uses but unfortunately has not been commercially processed to any significant extent. Red

Teff is one of the three *Teff* species in Ethiopia. It is less utilized for making Ethiopia bread (*Injera*) as a result of less soft, porous or sponge and traditional bias which fails to recognize the unique quality characteristics. The species has high yield, high multiplication ratio and better storability, than the preferred indigenous White and *Sirgegna teff* (El-Alfy *et al.*, 2012). Studying about the elemental and nutritional content of these cereal crops species is essential for both producers as well as for the consumers.

The aim of this study was to study the micronutrients and nutritional contents of the three species of *Teff* of Ethiopia origin and relative comparison of the preferred variety of *Teff* to bring out the differences between three species and to contribute to knowledge base of the species for product diversification and improvement on the consumption. To achieve these, three species of *Teff* of selected varieties were processed to flour and analyzed micronutrient content by CF-LIBS techniques.

EXPERIMENTAL CONDITION

Experimental setup: The LIBS experiment setup shown in Fig. 1 consists of second harmonic of (532 nm) a nanosecond Q-switched Nd: YAG laser and 10 Hz repetition rate and a 4 ns pulse width were focused onto the samples. The ocean optics LIBS 2000 + spectrometer equipped with CCD detector, fiber optic bundle, lens, translation stage and computer were used. Laser light is focused by a 12 cm diameter UV grade fused silica lens of 30 cm focal length onto sample surface. The experiments have been performed under normal atmospheric pressure and room temperature. The light from the micro-plasma is collected with an optical fiber having UV grade fused silica lens, placed at angle of 45° relative to the laser beam. The other end of the optical fiber is connected to the spectrometer of LIBS 2000 + of resolution 0.1 nm for 200-500 nm and 0.75 nm for 200-900 nm window region. The spectral signal is then stored on a computer and analyzed using OOILIBS software. Finally, the results of the quantitative analysis are graphed and analyzed using Origin software. In this optical arrangement beam waist and the calculated beam radius at the sample surface were 9 mm and 11.3 μm respectively, resulting in a maximum irradiance of 6.23×10^{11} W/cm², when operating at the laser pulse energy 10 mJ. This value of irradiance fulfils stoichiometric ablation (Andrzej *et al.*, 2006).

Sample preparation: The three species of dried *Teff* seeds Ethiopia origin were collected market place and ground in to fine powder and 50-100 ms powder was pelletized under a pressure of 30 ton/cm² for 0.5 to 1 min by Hydraulic press. This is because, ablation

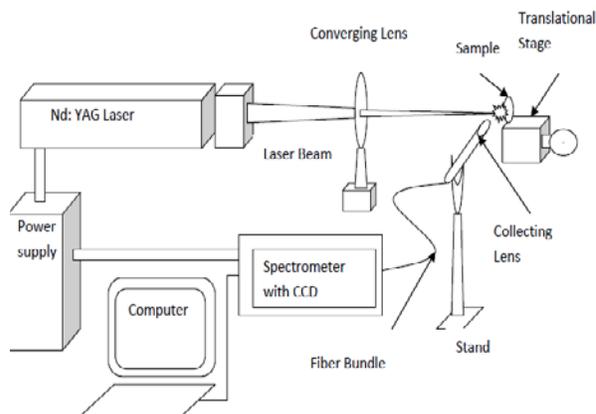


Fig. 1: Diagram of LIBS experimental setup

efficiency is better on compact sampling than the non-pressed powder and also density of the material affects the LIBS signal.

RESULTS AND DISCUSSION

Spectra of *Teff* seed samples: Emission spectra of the elements of interest were acquired full spectra by accumulating 25 consecutive laser shot as shown in Fig. 2, through four spectra window: 200-300, 300-400, 400-500 and 200-900 nm, respectively and energy of 10 mJ/pulse at an acquisition gate delay of 1.5 μs were used for recording the spectra of the pelletized *Teff* seed powder. Seven main micronutrients are found in the three species of *Teff* seed samples (mainly, Ca, Mg, Fe, Si, Mn, Al and K).

CF-LIBS techniques: For the calibration-free approach to be successful, a thorough optimization of the ablation conditions is necessary to discriminate the ejection of molecules, clusters and larger particles and to provide stoichiometric ablation. Thus, the characterization of the resulting plasma will be very helpful for determining the optimal operating conditions. To characterize the plasma, some reasonable assumptions concerning both the experimental method of operation and the theoretical representation of the plasma condition should be made. Primarily, the plasma composition is assumed that representative of the real material composition prior to the ablation and the plasma is in Local Thermal Equilibrium (LTE) conditions and the plasma is homogenous. These assumptions are consistent with Ciucci's model explaining in his study (Ciucci *et al.*, 1999).

Selection of spectral line: To determine the plasma temperature and electron density, the spectra line

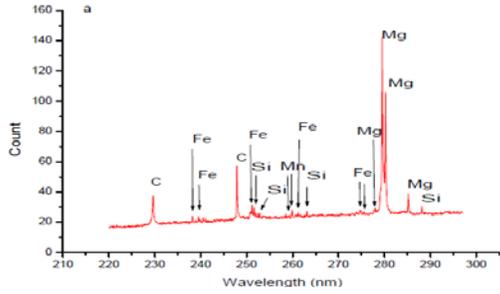


Fig. 2a: Spectral signal collected during the analysis of red *Teff* seed sample from 200-300 nm window of resolution 0.1 nm

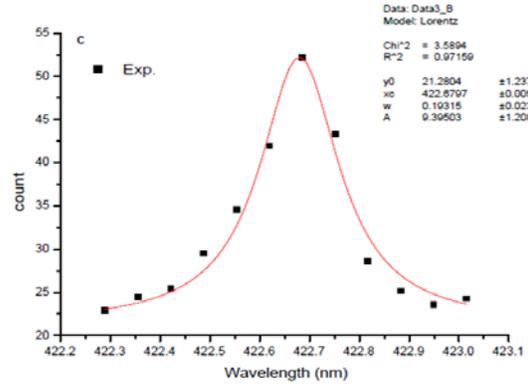


Fig. 3: Lorentzian fit to the observed data (dot) to Ca I line of 422.67 nm

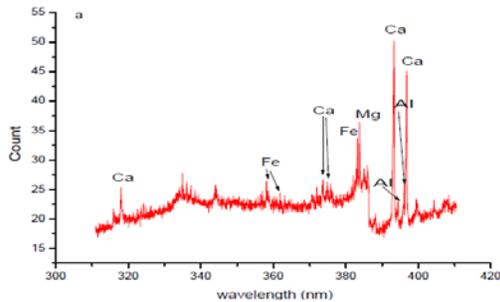


Fig. 2b: Spectral signal collected during the analysis of red *Teff* seed sample from 300-400 nm window of resolution 0.1 nm

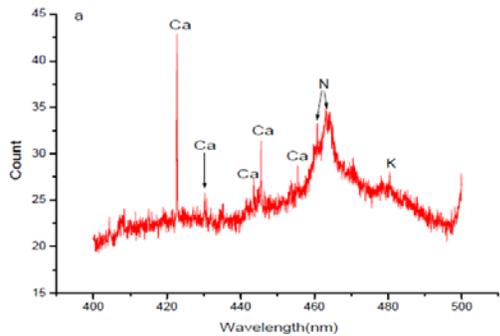


Fig. 2c: Spectral signal collected during the analysis of red *Teff* seed sample from 400-500 nm window of resolution 0.1 nm

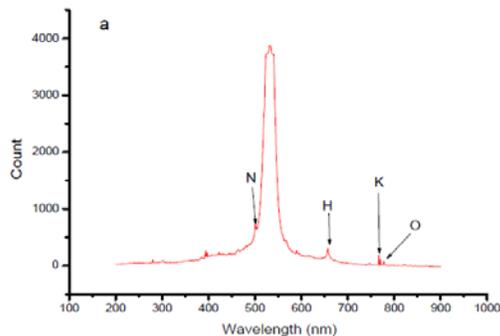


Fig. 2d: Spectral signal collected during the analysis of red *Teff* seed sample from 200-900 nm window of resolution 0.75 nm

selected from the spectra must be isolated and with a good profile. Because, these parameters are depend on the integral intensity of spectral lines. The integral intensity of spectral lines highly misinterpreted by the strong interference, strong self-absorption and resonance lines (Duixiong *et al.*, 2010); due to these factors non-resonance and line with good profile were selected. Moreover, due to variety of broadening mechanism spectral lines from plasma are broadened. Such as nature, resonance, Stark, Vander Waals, Doppler broadening and apparatus broadening of spectrometer (Andrzej *et al.*, 2006). As a result, the spectral lines detected by spectrometer are the Lorentz profile. In this study, Lorentz profile is used to fit the spectral line as shown in the Fig. 3. In the analysis of the temperature, electron density from Stark broadening and concentration, spectroscopic data are taken from NIST (National Institute of Standards and Technology) spectral database (Atomic spectra database) (<http://www.nist.gov/srd/index.htm>). The neutral and single ionization ions are the main source of the identified element lines.

Boltzmann plot method for plasma Temperature (T):

If the plasma is in LTE, then the population density of atomic and ionic states is described by a Boltzmann distribution. By measuring the relative line intensities, it is possible to estimate the electron temperature through the slope of a straight line in the Boltzmann plot. The plasma temperature was calculated according to the relationship (Andrzej *et al.*, 2006):

$$I_{ij}^{\lambda} = \frac{F N_s A_{ij} g_i}{Z(T)} \exp\left(-\frac{E_i}{kT}\right) \quad (1)$$

where,

I_{ij} = The intensity of a particular spectral line

$Z(T)$ = The partition function

N_s = The number density of relevant species

F = A constant depending on experimental conditions

g_i = The statistical weight of the upper energy level

A_{ij} = The transition probability

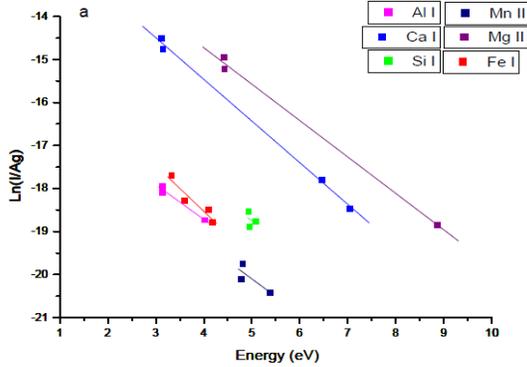


Fig. 4a: Boltzmann plot of each element in the red *Teff* sample

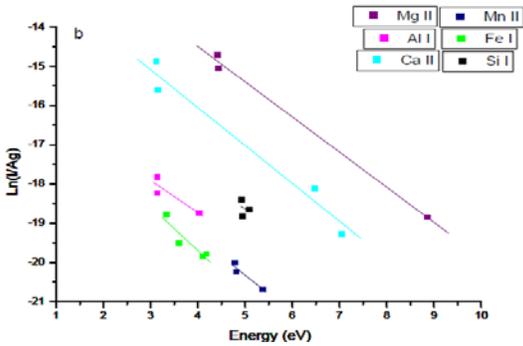


Fig. 4b: Boltzmann plot of each element in the white *Teff* sample

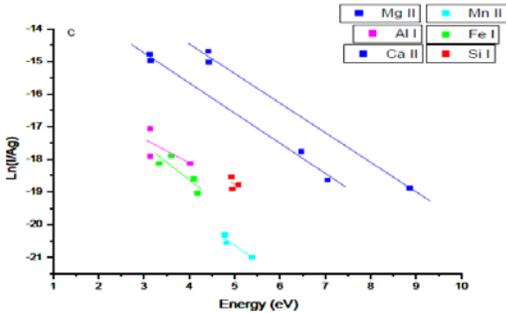


Fig. 4c: Boltzmann plot of each element in the *Sirgegna teff* sample

- E_i = The energy of the upper energy level
- k = The Boltzmann constant
- T = The electron temperature of the plasma

Putting Eq. (1) in a logarithmic form, the equation of a line is obtained. i.e.:

$$\ln\left(\frac{I_{ij}^{\lambda}}{A_{ij} g_i}\right) = \frac{-E_i}{kT} + \ln\left(\frac{F N_s}{Z(T)}\right) \quad (2)$$

This method, commonly referred to as the Boltzmann plot method, enables the determination of the excitation temperature from the lines slope and from

Table 1: Electron density calculated using a measured width of Stark Broadening of Ca I (422.6 nm) line of three *Teff* samples

Sample	Plasma temperature (K)	Electron density ($/\text{cm}^3$)
Red <i>Teff</i>	$10541.13 \pm 10\%$	9.23×10^{17}
White <i>Teff</i>	$10700.58 \pm 11\%$	9.48×10^{17}
<i>Sirgegna teff</i>	$10461.58 \pm 8\%$	9.56×10^{17}

the intercept the concentration of one species. NIST date (Atomic spectra database) ([http:// www.nist.gov/srd/index.htm.](http://www.nist.gov/srd/index.htm)) and the integrated intensity of the species of all elements lines were used to obtain the Boltzmann plot of each Species of *Teff* sample. According to Fig. 4, the plasma temperature of each sample of Red, White and *Sirgegna teff* is obtained from Boltzmann plot, which are shown in the Table 1. Temperature may vary during the acquisition time. This could be understood as stated that any change in the sample matrix produces a change in the physical and chemical properties of the target and, in turn, affects the energy distribution during the plasma generation and results in a change in the plasma parameters (T, N_e) (Tawfik and Mohamed, 2006).

Electron density: The elemental composition analysis from the line intensities in a LIBS experiment becomes simple if the plasma is optically thin and is also in LTE. Electron densities of the plasma can be obtained by using two methods that is by measuring the broadening of the emission line of the spectrum and by Saha-Boltzmann equation. In this study both methods were used to confirm the existence of the LTE.

The width of Stark broadening of well isolated spectra line of Calcium used to calculate the electron density of the plasma. The Stark line width $\Delta\lambda_{FWHM}$ was extracted from the measured line width $\Delta\lambda_{observed}$ by subtracting the instrumental line broadening $\Delta\lambda_{instrumental} = 0.05$ nm. The same element was taken into consideration for electron density measurement of the three samples at wavelength 422.67 nm of Ca I line. The three candidates' lines data points were fitted with Lorentzian fitting function using the origin software to determine Full Width Half Maximum $\lambda_{1/2}$ (FWHM) as shown in Fig. 3. The Stark broadening of impact parameter (w) value is taken from NIST at electron temperature of 10000 K (Atomic spectra database). Substituting the value in the Eq. (3), the electron densities for Ca were determined for each sample and listed in the Table 1:

$$N_e = \left(\frac{\Delta\lambda_1(nm)}{2w} \times 10^{16} \right) \quad (3)$$

Saha-boltzmann equation method for N_e : Electron density of the plasma can be calculated from Saha-Boltzmann equation. It is formulated as: (Andrzej *et al.*, 2006):

$$N_e = 2 \left(\frac{2\pi m_e k}{h^2} \right)^{\frac{3}{2}} T^{\frac{3}{2}} \left(\frac{I\lambda}{A_{ij} g_i} \right)_{atom} \left(\frac{A_{ij} g_i}{I\lambda} \right)_{ion}$$

Table 2: The electron density calculated using Saha-Boltzmann equation for the three species of *Teff* samples

Species	λ (nm)	g	A ($\times 10^8$ /s)	Red <i>Teff</i> N_e ($\times 10^{18}/\text{cm}^3$)	White <i>Teff</i> N_e ($\times 10^{18}/\text{cm}^3$)	<i>Sirgegna teff</i> N_e ($\times 10^{17}/\text{cm}^3$)
Ca I	443.40	5	0.67			
Ca II	373.67	2	1.70	1.34	2.68	5.44
Ca I	422.60	3	2.20			
Ca II	396.79	2	1.32	1.13	1.72	7.08

$$\exp\left(\frac{-E_{ion} + E_{atom} - V}{kT}\right) \quad (4)$$

where,

I = Intensity from the experiment

V = Ionization potential of the atomic species

λ = Wavelength

Table 2 shows the electron density calculated from Eq. (4) based on the emission lines of calcium element.

Table 1 and 2 noticeably shows that the results from both methods gave approximately equal value. The small deviation is due to the uncertainty of the measured value from the experiment and NIST data.

Finally the result from both methods clearly shows that LTE assumption is valid by comparing the result with criterion given by McWhiter of the lower limit of electron density for plasma will be in LTE (Cremers and Radziemski, 2006):

$$N_e \geq 1.6 \times 10^{12} T^{1/2} (\Delta E)^{3/2} \quad (5)$$

where,

ΔE = The highest transitional energy under a certain experimental condition

T = The plasma temperature

From Table 1 and 2 and plasma temperature in the above result, it has been confirmed that the LTE condition is held in this experiment.

Optically thin plasma: To confirm that the plasma is thin, the usual methods are obtaining the intensity ratio of two interference free emission lines from a species having the same upper energy level and the ratio of the corresponding product of transition probabilities, degeneracy and inverse wavelength. The uniformity between the intensity ratio and the ratio of the corresponding product of transition probabilities, degeneracy and inverse wavelength clearly prove the existence of thin plasma. The intensity ratio of the Ca II (370.6/373.6 nm) and Fe II (239.5/238.2 nm) are equal to 0.5, 0.78; 0.59, 0.75; and 0.48, 0.86 for Red, White and *Sirgegna*, respectively while the ratio of their product transition probability, degeneracy and inverse wavelength are 0.52 and 0.85, respectively, Which tangibly fulfill thin plasma condition.

Concentration of elements: In order to obtain the relative proportion of the elements in a sort of

composition, under the assumption of homogenous plasma, the Saha equilibrium equation can be applied to spatially-integrated spectra to calculate the concentration of the other ionization stages, which are all neutral and all ionic. In this case the line integral intensities from the experiment were used to calculate the concentration of one species of a given element from the intercept of Boltzmann plot i.e.:

$$N_s = \frac{Z_s(T)}{F} e^{q_s} \quad (6)$$

where,

q_s = The intercept from the Boltzmann plot

N_s = The population of the relevant species

$Z_s(T)$ = The partition function

F = Experimental parameter

And the other species of a given element concentration was deduced using Eq. (7) (Andrzej *et al.*, 2006):

$$\frac{N_e N_i}{N_A} = 2 \left(\frac{2\pi m_e k}{h^2} \right)^{3/2} T^{3/2} \frac{Z_i(T)}{Z_A(T)} \exp\left(\frac{-V}{kT}\right) \quad (7)$$

where,

m_e : The mass of the electron

N_i : The population of single ionized species

N_A : The population of single neutral species

N_e : Electron density in (e/cm^3)

$Z_i(T)$: The partition function corresponding to ionic species

$Z_A(T)$: The partition function corresponding to atomic species

v : The ionization potential of atomic species in the ground state

Therefore, the total concentration of the given element is given by:

$$C_{tot} = C_A + C_I \quad (8)$$

where,

C_A : The concentration of atomic

C_I : The concentration of ionic

To remove the unknown experimental factor F, one can use the normalization relation on the species concentration of the samples (Pandhija *et al.*, 2009) i.e.:

$$\sum_s C_s = 1 \quad (9)$$

where,

C_s = The concentration of the species

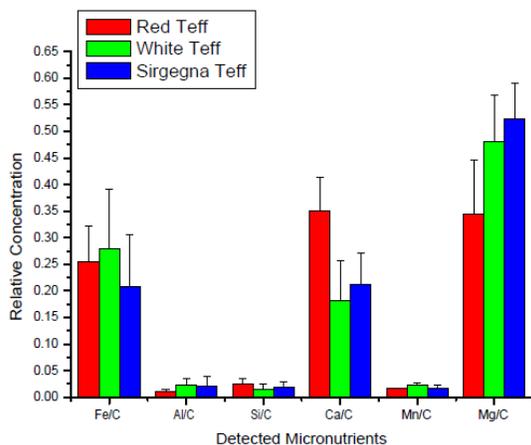


Fig. 5: Relative quantitative analytical result of the three species of *Teff* seed samples

In this relative quantitative analysis to control the huge relative error affecting the minor components because of the small uncertainty affecting the major components, organic carbon was used as internal reference. The matrix element of the samples is organic Carbon. The elemental concentration is relative. Figure 5, shows that the relative concentration of Ca, Al, Si, Mn, Fe and Mg in the three species of *Teff* seeds. Four micronutrients are dominant in all species of *Teff*. Highest concentration of calcium and less concentration of Magnesium are found in Red *Teff* as compare to White and *Sirgegna teff*. These two elements play the main role in the difference of the species. Iron in the three species is almost same. They are the minor elements in the *Teff* Samples. The Potassium is not included in relative concentration of the micronutrients due to the more peaks of potassium where found in the second region of the spectrometer which has different resolution.

CONCLUSION

The CF-LIBS technique was applied for the first time to a cereal crop of the three species of *Teff* seeds for the determination of the qualitative as well as the semi-quantitative micronutrients of the species. The major, minor and trace elements (Ca, Mg, Fe, Al, Si, K and Mn) in a Carbon matrix were identified and calculated. A procedure based on multi-elemental Saha-Boltzmann plot was used, allowing the determination of the local temperature. And the Saha equation was applied to calculate the concentration of each species from the measured intensity of one species of spectral line in the same element. Based on our study, the concentration of Calcium in the Red *Teff* seed species is highest than the other two species. On the contrary it has less Magnesium. The other micronutrients are

almost same in all species. Due to the high concentration of Ca in the Red *Teff* seeds, the Ethiopia bread (*Injera*) made from the Red *Teff* is less soft and pours or sponge than the other two Species of *Teff* Seeds. But its nutritional content is better than the other. And these cereal crops can be a good source of Iron micronutrient. It can be seen that LIBS technique's is fast and attractive tool of micronutrient's determination in the cereal crops.

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