Minireview Article

Laser induced breakdown spectroscopy: a rapid analytical technique for soil and plant

Abstract

Laser induced breakdown spectroscopy (LIBS) has become one of the most prominent analytical technique with great potential for real time and large-scale soil and plant analyses. In this technology, an intense pulse of laser radiation is focused onto sample where it ablates the material from surface and creates a microplasma. The plasma, excites atoms and atomic ions which emits radiation specific to the elemental composition of sample. In a characteristic LIBS spectrum, wavelength corresponds to the type of element and relative strength of spectrum signifies concentration of the element. A well calibrated LIBS instrument performs diverse soil and plant analysis. Its application in detection of soil texture, micro, macro and heavy metals in soil was well documented with high calibration and prediction accuracy. A method for rapid, high resolution (100 μm) and multi-element imaging of root-rhizoshere interface was prepared with LIBS and imaging software to map switch grass (*Panicum virgatum*) rhizosphere with good limit of detection for soil matrix components and nutrients. LIBS combined with chemometrics was used to detect the cadmium content in rice using extreme learning machine (ELM) model and obtained a prediction accuracy of 93.33 per cent. Laser induced breakdown spectroscopy requires minimal sample preparation for analysis without production of polluting waste and incurs relatively low cost compared to traditional techniques. On the other hand, matrix dependency of measurement, relatively high limit of detection for some elements, unstable signal intensity, and low reproducibility of results due to variations in laser spark are the major limitations.

1. Introduction

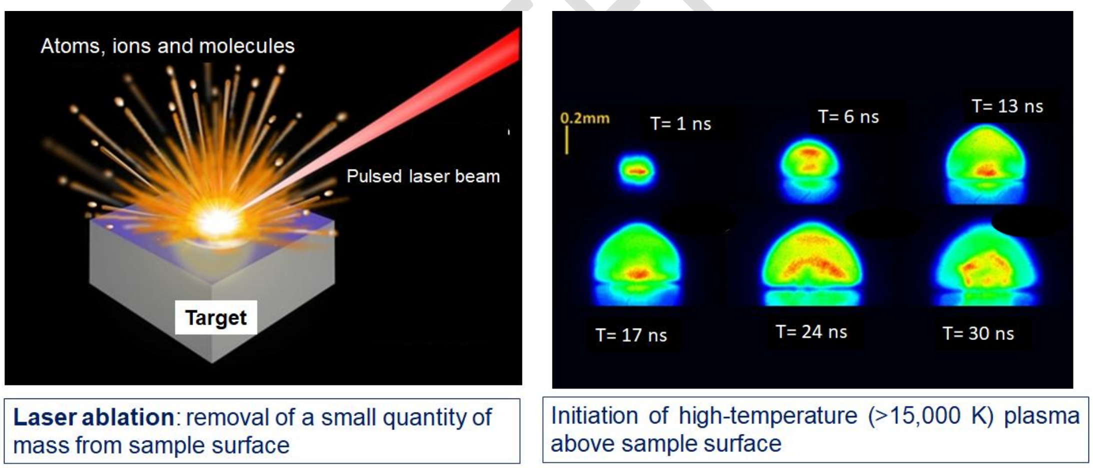
Rapid determination of elemental composition of soil and plant is important for improving agricultural production and reducing environmental impacts. There are several conventional techniques for analyzing soil and plant, but they involve laborious extraction and estimation procedures. Moreover, data processing after analyses is a cumbersome task until and unless suitable software's are available. This situation has led to invention of different rapid analytical techniques among which laser induced breakdown spectroscopy is a real time analytical technique which can support or co-exist with conventional technique (Yu et al., 2020).

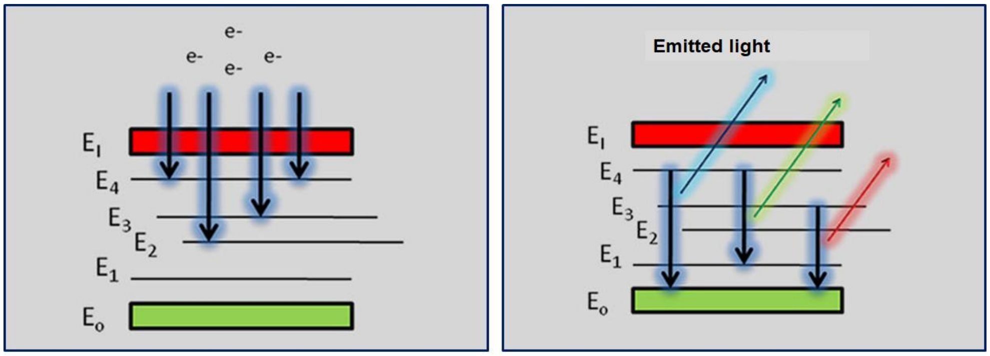
Laser induced breakdown spectroscopy is a multi-elemental analytical method based on measurement of atomic emission lines from laser plasma generated at the sample surface (Noll, 2012). LIBS can analyse solid, liquid and gaseous materials. Because of its simple setup, LIBS can be used in a wide range of environments, including hazardous environments (radioactive and explosive), difficult to access and remote areas (deep ocean and Mars), and directly in field (Lee et al, 2004).

2. Principle of operation of LIBS

In this technology, an intense pulse of laser radiation is focused onto sample where it ablates the material from surface and creates a microplasma. The plasma, in turn, excites atoms and atomic ions which emits radiation but these radiations does not contain information about elements. Later as plasma cools, electrons return back to low energy level by emitting radiations specific to the elemental composition of sample (Figure 1). In a characteristic LIBS spectrum, wavelength corresponds to the type of element and relative strength of spectrum signifies concentration of the element (Pasquini, et al., 2007). Use of chemometrics in processing LIBS data broaden the horizons of its applications in soil and plant analysis (Deming et al., 1991).

Figure 1. Principle of operation of LIBS





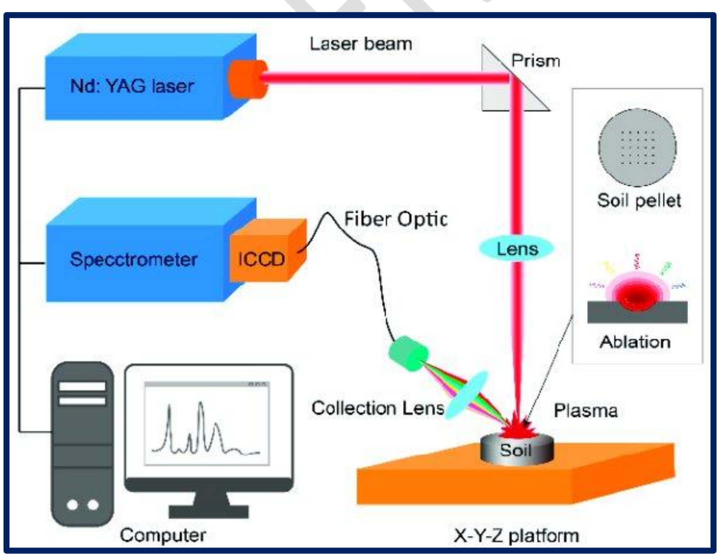
Emission of continuum light during early stage (< 200 ~300 ns) of plasma

Emission of discrete atomic lines during cooling of plasma (> 1 µsec)

3. Instrumental setup of LIBS

A conventional LIBS instrument consists of laser source, spectrometer, sample holder, lenses and optical fibers, data acquisition system, and a computer control. The most commonly used laser source is neodymium doped yttrium aluminium garnet (Nd: YAG) with a characteristic emission wavelength of 1024 nm (Miziolek, *et a.,l* 2006). Pulse duration, wavelength and energy of laser beams are the major deciders of accuracy of the instrument. Lenses or optical fibers of LIBS instrumentation assembly delivers emitted light to spectrometer which separates white light into different wavelengths. A charge coupled device (CCD) or an intensified charge coupled device (ICCD) converts optical signal to electronic signal. Finally, the computer software delivers composition of elements in sample under test with the help of various statistical models. Samples are provided as pellets for laser ablation. Sample holder is not a must for *in situ* measurement using LIBS (Villas-Boas *et al*., 2020).

Figure 2. Instrumentation assembly of LIBS



4. Applications of LIBS

The application of LIBS can be broadly classified into qualitative and quantitative analysis of soil and plant. Qualitative analysis is important for environmental monitoring especially in the case of pollutants (Barbafieri et al., 2011; Capitelli et al., 2002; Santos et al., 2009). Quantitative determination of elemental composition has applications in agriculture. This review is mainly focus on the application of laser induced breakdown spectroscopy with special reference to agriculture. This can be mainly for soil characterization and plant analysis.

4.1 Soil characterization

- Evaluation of soil texture
- Evaluation of soil pH
- Quantification of nutrients and heavy metals
- Classification of soil minerals
- Imaging of soil-root interface

4.2 Plant analysis

- Evaluation of nutrient and heavy metal content
- Pesticide residue mapping on plant tissue

4.1.1 Application of LIBS for evaluation of soil texture

Soil texture is the relative proportion of sand, silt and clay and is an important physical property that crucially affects other properties such as erosion susceptibility, drainage, water holding capacity *etc*. Villas-Boas *et al.* (2016) proposed a method for evaluating texture of soils using LIBS. Sixty Brazilian soils were selected for the study and acquired spectra from individual soils. Corrected spectra was used for identification of prominent peaks. They have used two calibration model to evaluate the spectra. First one by using entire spectra of soils and second by using prominent spectral peaks identified. Soil separates measured by LIBS with two calibration models (A and B) provided a strong correlation coefficient (0.89 and 0.90) with those measured by the international pipette method. After identifying the proportion of sand, silt and clay using two models they classified the soil using algorithms and the results projected a good classification ability of the instrument.

4.1.2 Application of LIBS for evaluation of soil pH

Soil pH is a key indicator that regulates capacity of soils to store and supply nutrients and also is the main factor determines the microbial diversity in soils. Xu *et al* (2019) investigated prediction performance of LIBS for soil pH. LIBS spectra was acquired from 200 soil samples collected from four types of farmlands in China. Morphological weighted penalized least squares (MPLS) and wavelet transformation (WT) were the denoising algorithms used. Partial least square regression (PLSR) was employed to build model containing pretreated LIBS spectra and chemical properties analysed in the laboratory. Data points in both the calibration and validation sets were located close to the 1:1 line, which indicated robust prediction accuracy of LIBS spectra for soil pH. Higher R^2, lower root mean square error (RMSE), and high values of relative percentage difference (RPD) (above 2) for prediction of soil pH were achieved, suggesting excellent prediction ability of LIBS spectra for soil pH. They also noted that emission lines of K, Ca, Mg, Al, Si, Fe, and O were the major contributor in soil pH evaluation.

4.1.3 Application of LIBS for quantification of macro and micro nutrients

Rapid determination of soil nutrient elements is beneficial to the evaluation of crop yield and is of great importance in agricultural production. He *et al* (2018) used single (SP) and double pulse (DP) LIBS for analysing soil macro and micronutrients. Single pulse LIBS has only one laser beam to ablate the samples whereas two laser pulses are used in double pulse LIBS, one for complete ablation and other for plasma reheating. Sixty three soil samples were collected for single pulse (SP) and double pulse (DP) signal acquisition. Calibration models were prepared using chemometric methods such as partial least square regression (PLSR) and least square support vector machine (LS-SVM). The calibration and prediction data points of both SP and DP LIBS signals fitted well, indicating that least square support vector machine (LS-SVM) model have reliable prediction power for quantitative analysis of soil elements. Compared to SP LIBS detection sensitivity of DP LIBS was higher with all R^2 being greater than 0.96.

Erler *et al.* (2019) explored the potential of hand held LIBS with various multivariate regression models for soil nutrient determination. Partial least square regression (PLSR), least absolute shrinkage and selection (Lasso) and Gaussion process regression (GPR) were the calibration model used to eliminate variations with respect to soil matrix. Compared to other usually used models PLSR showed best coefficient of determination for Mg and Fe. GPR yielded best results for six soil parameters which are Ca, Mg, Fe, K, Al and pH. Lasso and GPR are especially more stable in the case of soil parameters with low emission intensity and indirectly predicted parameters. In the case of lasso, N, Mn and humus are in the difficult to predict category and for GPR it includes total and available P. The results suggested that regression models like lasso and GPR gives better predictability in hand-held instruments since it overcomes matrix interference more effectively.

4.1.4 Application of LIBS for classification of soil minerals

Yang *et al* (2019) used LIBS technology and different machine learning algorithms to rapid and precise classification of 10 iron ores. The ten iron ore samples used in the experiments were magnetite, cobalt-bearing magnetite, hematite, oolitic hematite, mica hematite, maghemite, pyrite, pyrrhotine, siderite, and limonite. At first, the spectral data of 10 iron ores were obtained by LIBS. A 3x3 matrix was ablated on the surface of each ore. Each point of the matrix was ablated five times, and the data of each time was recorded. Forty-five independent spectral data were collected on each ore for subsequent analysis. The principal component analysis (PCA) was employed for the reduction of dimensionality of raw data and 10 principal components identified were used for classification. Then k- nearest neighbor (KNN), support vector machine (SVM) and neural network algorithms were used to classify the ore. Support vector machine model with linear kernel function obtained highest accuracy in iron ore classification. The discrimination accuracy of the model on training set was 98.7% and test set was 94.07%. The confusion matrix is used for evaluating performance of a classification model. The matrix compares actual target values with those predicted by machine learning model. The obtained results sufficiently demonstrated that LIBS coupled with machine learning algorithm is a practical technique for rapid and on-line analysis of categories of iron ore.

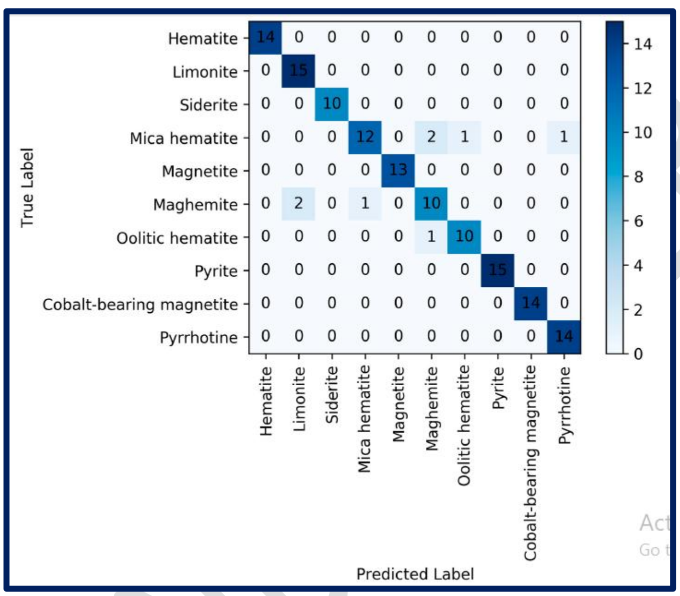


Figure 3. Confusion matrix for support vector machine model in iron ore classification

(Integers in the matrix shows number of samples. Samples in the diagonal represents correctly classified ones and color intensity of individual grid also varies with accuracy of classification).

4.1.5 Application of LIBS for imaging of soil-root interface

Understanding complex chemical nature of root-soil interface is important for suggesting long term environmental remediation strategies. Ilhardt *et al* (2019) used LIBS with two dimensional imaging software to map switch grass (*Panicum virgatum*) rhizsosphere which was grown in a rhizotron. The points for laser shots were spaced 100µm to avoid overlapping of spectra and the chemical images were prepared from single shot spectra from each location of sampling grid. Two dimensional heatmaps were prepared using Akima and field packages in R software. Individual spectral peaks were identified by using NIST database. LIBS imaging technique can be successfully applied to map sub millimeter detection scale such as root-soil-atmospheric continuum.

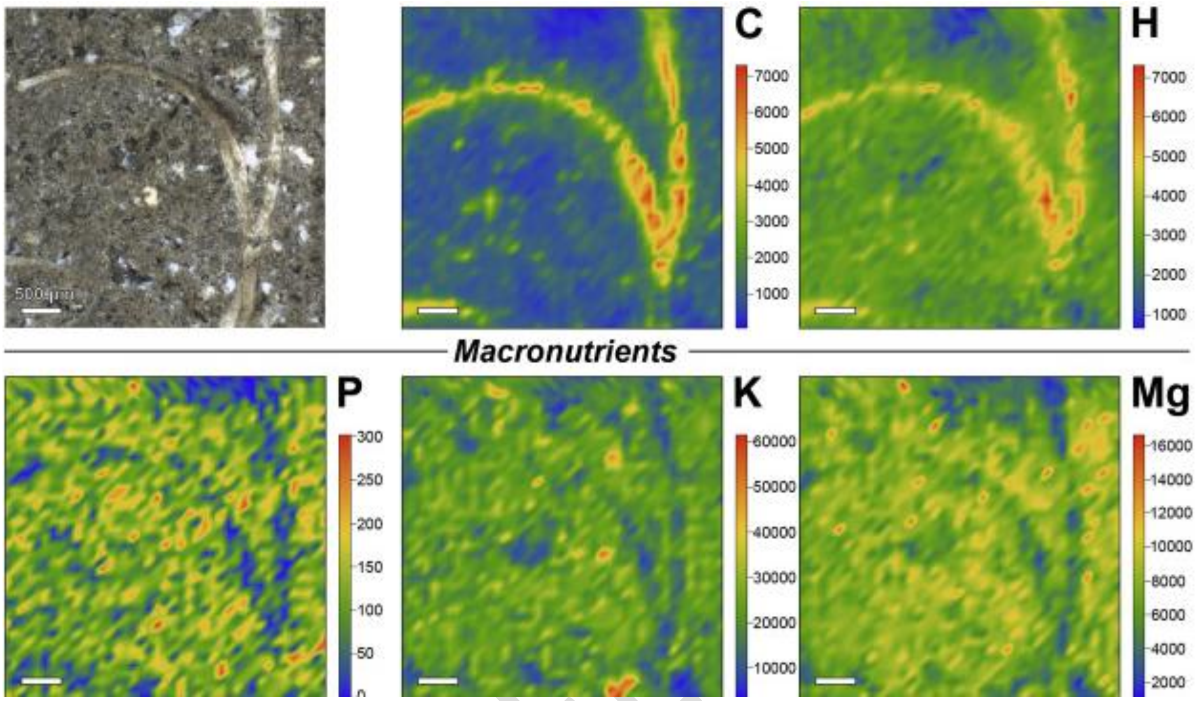


Plate 1. Distribution of organic elements and macro nutrients in root-rhizosphere-soil continuum (Heatmaps denotes relative distributions of single elements across the root-soil sample area. Colour scale bars show variation in LIBS signal intensity which indicates relative concentrations of the elements).

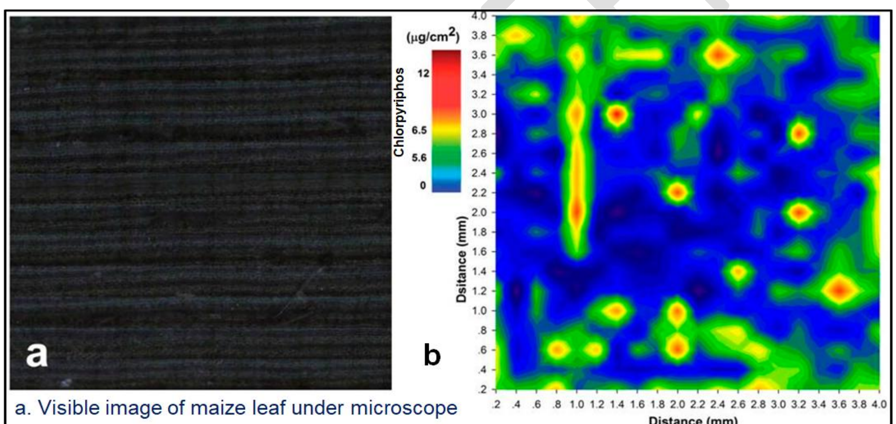
6.21. Application of LIBS for evaluation of nutrient and heavy metal content

Laser induced breakdown spectroscopy accompanied with chemometrics efficaciously used for qualitative and quantitative detection of nutrients and heavy metals from plant parts. Wang *et al* (2020) used LIBS for determining cadmium content in rice culms. They cultivated rice plants by supplying different concentrations of Cd (0, 5, 25, 50, and 100µM $CdCl_2$) to obtain rice culms with gradient of Cd. Rice plants with similar growth were selected at 10, 20 and 30 days after planting and collected culms as samples. After drying and powdering, samples were made into tablets for laser ablation. Spectra obtained from rice culms were processed using wavelet transformation and area normalization algorithms. Discrimination of Cd stress from 10, 20, and 30 days old rice culms were classified using various models and the results showed a good calibration and prediction accuracy for extreme Learning Machine (ELM) model. Similarly ELM model had the best calibration (91%) and prediction accuracy (93.33%) for quantitatively determining Cd content in rice culms. The scatterplot with all data points near to 1:1 line showed best correlation between predicted and reference value determined using ICP-OES. The results showed that LIBS spectrum with ELM model can realize fast detection of Cd in rice culm more effectively.

6.21. Application of LIBS for pesticide residue mapping on plant tissue

Mapping and quantitative observation of pesticide residues in plant parts are important aspect. Zhao *et al* (2016) developed a removable mapping system based on LIBS, known as LipsImag. It is portable device which consists of a LIBS module, a three dimensional movement platform, an observation camera and a removable hand. Chlorpyriphos is a pesticide containing elements such as P, S, H, Cl, O, N and C. The authors attempted to determine the presence and quantification of chlorpyriphos using spectral characteristics of P, S and Cl. For calibration experiment, 20µL pesticide sample was diluted 40-4000 times and dropped into maize leaf area of about 1cm^2. Thereby they obtained pesticide residues of 0-30µg cm^{-2}. They used LipsImag to measure spectral intensities from the spots and prepared calibration model. In the mapping experiment, they diluted 100ml of 40.7% chlorpyriphos to 50L and sprayed to an area of 666.7m^2 and collected spectra from different leaf samples.

Figure 4. Two dimensional mapping of chlorpyriphos residue on maize leaf



a. Visible image of maize leaf under microscope

b. Mapping of chlorpyriphos on maize leaf

After successful calibration and installation of calibration model in LipsImag, two dimensional map was prepared after 20 minutes of chlorpyriphos application (figure 4). Variation in colour intensity shows difference in concentration of chlorpyriphos in µg cm^{-2}(figure 4)

5. Advantages

- Rapid or real-time analysis analytical tool
- Simultaneous multi-element detection capability
- Little or no sample preparation are required

- Versatile to analyse all types of sample such as gases, liquids and solids
- Useful in diverse spectral regions (Villas-Boas *et al*. 2020)

6. Disadvantages

- Matrix dependency of measurement results in unstable signal intensity
- Relatively high limit of detection for some elements
- Detection limits varies with elements and type of specimen
- Measurements are subjected to variation in laser spark and resultant plasma which limits reproducibility analysis

7. Methods to enhance sensitivity in LIBS

- Use of double pulse LIBS instead of single pulse LIBS
- Multivariate models instead of univariate models can be adopted
- Self-correcting models for improving signal intensity (Fu et al, 2020)

8. Conclusion

Laser induced breakdown spectroscopy is an emerging real time analytical technique due to its high ability to detect a broad range of elements with little or no sample preparation. But there some limitations that reduce accuracy of measurement. In order to meet that soil scientists and LIBS engineers are jointly working to make the technique more stable, robust, and universal.

11. References

Anabitarte F, Cobo A, and Lopez-Higuera JM. Laser induced breakdown spectroscopy: fundamentals, applications, and challenges. *Spectroscopy* 2012: 1-10.

Barbafieri M, Pini R, Ciucci A, and Tassi E. Field assessment of Pb in contaminated soils and in leaf mustard (Brassica juncea): The LIBS technique. *Chem. Ecol.* 2011; 27: 161–169

Capitelli F, Colao F, Provenzano MR, Fantoni R, Brunetti G, and Senesi N. Determination of heavy metals in soils by laser induced breakdown spectroscopy. *Geoderma* 2002; 106: 45–62.

Deming SN, Palasota JA, and Palasota JM. Experimental design in chemometrics. Journal of Chemometrics 1991; 5:181–192.

Erler A, Riebe D, Beitz T, Löhmannsröben HG, and Gebbers R. Soil nutrient detection for precision agriculture using handheld laser-induced breakdown spectroscopy (LIBS) and multivariate regression methods (PLSR, lasso and GPR). *Sensors* 2020; 20(2): 418-435.

Fu X, Li G, and Dong D. Improving the detection sensitivity for laser-induced breakdown spectroscopy: a review. *Front. Phys.* 2020; *8*: 68.

He Y, Liu X, Lv Y, Liu F, Peng J, Shen T, Zhao Y, Tang Y, and Luo S. Quantitative analysis of nutrient elements in soil using single and double-pulse laser induced breakdown spectroscopy. *Sensors* 2018; 18(5): 1526-1541.

Ilhardt PD, Nuñez JR, Denis EH, Rosnow JJ, Krogstad EJ, Renslow RS, and Moran JJ. High-resolution elemental mapping of the root-rhizosphere-soil continuum using laser induced breakdown spectroscopy (LIBS). *Soil Biol. Biochem.* 2019; 131: 119-132.

Lee W, Wu J, Lee Y, and Sneddon J. Recent applications of laser-induced breakdown spectrometry: A review of material approaches. *Appl. Spectrosc. Rev*. 2004; 39: 27–97

Miziolek AW, Palleschi V, and Schechter I. Laser-induced breakdown spectroscopy (LIBS): Fundamentals and applications. New York, NY: Cambridge University Press. 2006; 620p.

Noll R. Laser-induced breakdown spectroscopy: Fundamentals and applications. New York, NY: Springer, 2012; 542p.

Pasquini C, Cortez J, Silva L, and Gonzaga FB Laser induced breakdown spectroscopy. *J. Braz. Chem. Soc.* 2007; 18:463-512.

Santos D, Nunes LC, Trevizan LC, Godoi Q, Leme FO, Braga JWB, and Krug FJ. Evaluation of laser induced breakdown spectroscopy for cadmium determination in soils. Spectrochimica Acta Part B 2009; 64, 1073–1078.

Villas-Boas PR, Franco MA, Martin-Neto L, Gollany HT, and Milori DM. Applications of laser induced breakdown spectroscopy for soil analysis, part I: review of fundamentals and chemical and physical properties. *Eur. J. Soil Sci.* 2020; 71(5): 789-804.

Villas-Boas PR, Romano RA, de Menezes Franco MA, Ferreira EC, Ferreira EJ, Crestana S, and Milori DMBP. Laser-induced breakdown spectroscopy to determine soil texture: A fast analytical technique. *Geoderma*, 2016; 263: 195-202.

Wang W, Kong W, Shen T, Man Z, Zhu W, He Y, Liu F, and Liu Y. Application of laser induced breakdown spectroscopy in detection of cadmium content in rice stems. *Front. Plant Sci.* 202; 11: 2073-2084.

Xu X, Du C, Ma F, Shen Y, and Zhou J. Fast and simultaneous determination of soil properties using laser induced breakdown spectroscopy (LIBS): a case study of typical farmland soils in China. *Soil Syst.* 2019; 3(4): 66-84.

Yu K, Ren J, and Zhao Y. Principles, developments and applications of laser induced breakdown spectroscopy in agriculture: A review. *Artif. Intell. Agric.* 202; 4: 127-139.

Yang Y, Hao X, Zhang L, and Ren L. Application of scikit and keras libraries for the classification of iron ore data acquired by laser-induced breakdown spectroscopy (LIBS). *Sensors* 202; 20(5): 1393.

Zhao C, Dong D, Du X, and Zheng W. In-field, *in-situ*, and *in-vivo* 3-dimensional elemental mapping for plant tissue and soil analysis using laser induced breakdown spectroscopy. *Sensors* 2016; 16(10): 1764-1778.