Microanalysis of Multi-Element in *Juncus effusus* L. by LIBS Technique*

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Abstract Laser-induced breakdown spectroscopy (LIBS) was used to decipher the unique multi-elemental characteristics of *Juncus effusus* L. The spectral fingerprints of *Juncus effusus* L. were established based on elemental microanalysis via LIBS. Microanalysis and multimode sampling methodologies were designed in this study. The relative standard deviation (RSD) approach was performed to optimize the multi-shot measurements. Taking advantage of the capability with no or minimal sample pre-treatment of LIBS, a thermodynamic chart of four elements (Mg, Ca, Ba, and Na) was created from twelve collection regions. The diagram of elemental distribution on a micro-scale was generated to explore the nature of *Juncus effusus* L. by LIBS. The results demonstrated that LIBS is a promising technique for rapid elemental microanalysis of heterogeneous samples.

Keywords: LIBS, *Juncus effusus* L., RSD approach, thermodynamic chart, elemental microanalysis, micro-scale

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(Some figures may appear in colour only in the online journal)

1 Introduction

The dried stem of the whole aerial part of *Juncus effusus* L. is traditionally used in China to treat fidgeting and insomnia, a collection of symptoms related to anxiety [1]. *Juncus effusus* L. is a heterogeneous medical plant primarily comprised of macromolecular cellulose, hemicelluloses, lignin, and low molecular-weight substances, i.e. extractives, and minerals [1,2].

Most fingerprint studies of Chinese Materia Medica (CMM) have focused on organic composition rather than examining the inorganic elements [3−5]. However, the mineral compositions have a great impact on the quality of the herb. Meanwhile, numerous plants can be used as bio-indicators of the environment [6]. The mineral composition of plants is related to external parameters such as soil, fertilizer, contamination, and disturbances involving human activity. *Juncus effusus* L., a wetland plant, is an ideal bio-indicator. Wu et al. reported that *Juncus effusus* L. was used as a wetland filter to treat drinking water [7]. Therefore, it is significant to rapidly and accurately monitor the mineral element of *Juncus effusus* L.

Advances in chemical science have led to an increasing demand of microanalytical techniques that allow a spatially resolved analysis of solid samples without an extensive sample pretreatment. The conventional beam and particle techniques, such as electron probe and secondary ionization mass spectrometry, can analyze spatial features on a micro-scale with high sensitivity [8]. However, they need the necessary experimental conditions, i.e. high vacuum, laborious sample pretreatment, or rigid matrix-related interference corrections. On the other hand, fast information on spatially resolved elemental distributions on a micro-scale is required in many production processes.

Laser-induced breakdown spectroscopy (LIBS), an advanced elemental microanalysis technique, is a powerful tool to investigate structure, chemical, or mineral components on the micro-scale [9]. LIBS can detect diverse materials, including solids, liquids, gases and aerosols with no or minimal sample pretreatment [10]. It relies on plasma microanalysis [11,12]. Plasma is induced by a high energetic laser pulse. Sufficient irradiance enables the generation of ionized plasma including atoms and ions. Subsequent detection of the specific spectral emission reveals the information about elemental compositions of samples LIBS provides fast, in situ, and even remote multi-element analysis that has triggered some additional interest in the application of

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LIBS [13–15].

Recently, LIBS has drawn interest for application of plant materials, notably for a chemical sensor technique to analyze both macro- and micronutrients in plants [16,17]. Santos et al. reported the LIBS technique in the application of plant materials [18]. Krizkova et al. studied the response of sunflower plants to stress induced by silver (I) ions as well as the basic physiological parameters by LIBS [6]. Galiová et al. used LIBS to investigate heavy-metal accumulation in selected plant samples [19]. Liu et al. explored the rapid elemental analysis and provenance study of a medicinal herb (*Blumea balsamifera* DC) using LIBS [20].

LIBS also provides information about organic elements, i.e. carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) together with molecular bands (C-N and C-C), which are difficult to detect by other conventional techniques. Based on multivariate analysis, the LIBS technique has shown good classification performance. Wang et al. studied the physical mechanism of laser-induced plasma of an organic explosive and demonstrated that LIBS coupled with the chemometric techniques had the capacity to discriminate an organic explosive from plastics [21].

The aim of this study is focused on the microanalysis of *Juncus effusus* L. by LIBS. Robust instrumentation and user-friendly methodologies were designed to study the nature of heterogeneous samples on a micro-scale. Specifically, the relative standard deviation (RSD) approach and elemental thermodynamic chart were applied to process the spectral data and visualize the distribution of the main elements.

2 Materials and methods

2.1 Experimental setup

A commercial LIBS system (TSI, ChemReveal™-3764, USA) equipped with a Q-switched Nd:YAG laser was employed to collect spectra. Experiments were performed at ambient air with a laser energy about 340 mJ per pulse, repetition rate of 2 Hz, and pulse duration of 3-5 ns at 1064 nm. The spectrum covers a continuous wavelength from 170 nm to 985 nm. A detector delay of 1 μs and a fixed spectrometer integration time of 1 ms were used. These values resulted from an optimization study carried out for the detection of 21 standard reference materials (SRM), including food, clay, sludge, steelmaking alloy, and geochemical and agricultural materials [22]. The optimization study was also used to analyze carbon in coal [23]. The laser beam passed through a pierced parabolic mirror and was focused vertically onto the sample. The plasma was collected by a 50 mm focal length lens and optical fibers. It was then fed into the spectrometer and recorded by 7-channel-charge-coupled device (CCD) camera. A three-dimensional (3D) translation stage with stepper motors was used to ensure accurate movement of the sample. The LIBS data system was supported by ChemReveal LIBS software.

2.2 Materials

Plant materials were collected from different regions of China (shown in Table 1). The samples were either the dried stem or medulla of *Juncus effuses* L., which were confirmed to be *Juncus effuses* L. by Dr. Jian-mei Huang (a principle investigator of the Institute of Chinese medicine, Beijing University of Chinese Medicine). The samples were designated S1-S12. The voucher specimens (No. GT110 - No. GT121) were kept at the Herbarium of Beijing University of Chinese Medicine.

Table 1. A summary of the tested samples

Sample codes	Collection regions
S1	Guizhou
S2	Anhui
S3	Bozhou, Anhui
S4	Bozhou, Anhui
S5	Shizhen large pharmacy
S6	Jiangxi
S7	Heilongjiang
S8	Xi'an, Shanxi
S9	Guangzhou
S10	Laifeng xian, Hubei
S11	Fuzhou, Fujian
S12	Hubei

2.3 Data acquisition and microanalysis

A schematic diagram of LIBS experiment is shown in Fig. 1. Spectra were obtained on the micro-scale on the samples' surface. For each sample, ten micro-locations were randomly selected along the sample and each micro-location consisted of nine laser pulses (matrix, 3×3 (100 μm × 100 μm)).

2.4 Data analysis

Data analysis was performed under the Windows 7 Professional operating system using Matlab R2014a (Mathworks, Inc., Natick, MA).

2.4.1 The relative standard deviation approach

The sampling source is one of the fundamental points of LIBS toward accurate analysis. Matrix effects are of critical importance in several analytical spectroscopy techniques, and LIBS is no exception to this [23]. An approach of LIBS spectra described in the case of a highly heterogeneous system, named the relative standard deviation (RSD) approach [12], was applied to optimize the number of laser shots during sampling. RSD approach is based on a single-shot and individual spectrum. As stated in Ref. [12], the conventional spectra could be acquired by plotting the signal versus wavelength, while the "RSD spectra" were obtained by plotting RSD versus wavelength.

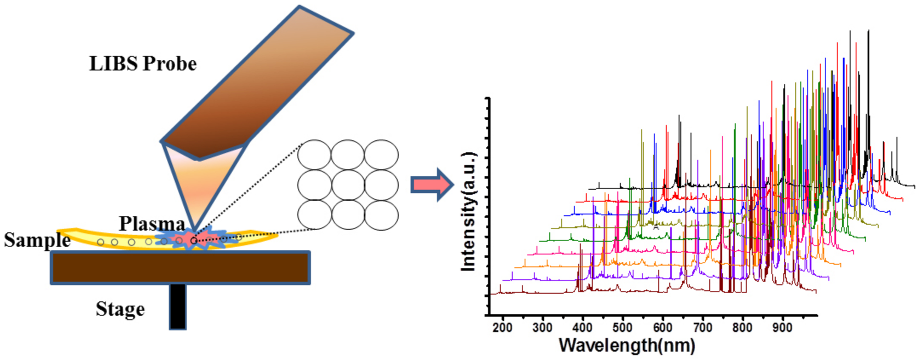

Fig.1 A schematic diagram of data acquisition on a micro-scale during LIBS experiment

The observed profile of the "RSD spectra" depends on the source of the limiting noise, the relative intensity of the signal, and their correlation. Thus, "RSD spectra" can be used to choose the optimal sampling mode. With a little difference, "RSD spectra" were used to assess the multi-shot acquisition in this set of investigations.

2.4.2 Elemental thermodynamic chart

An elemental thermodynamic chart was used to explore the distribution of different elements in plants and to further study the dominant signal in "RSD spectra". In an elemental thermodynamic chart, different colors demonstrate different intensities of spectral lines. For example, in an elemental thermodynamic chart red represents high intensity of emission lines while yellow represents low intensity of emission lines. Thus, the distribution of elements could be visualized directly.

3 Results and discussion

3.1 Elemental identification of *Juncus effuses* L.

The identification of spectral lines was performed by using the National Institute of Standards and Technology (NIST) database and relative references [24−26]. The representative LIBS spectra are shown in Fig. 2(2).

According to the results of qualitative detection by the LIBS technique, 33 emission lines of 12 elements and molecular bands were identified to establish a spectral "fingerprint". The alkali metal and alkaline earth metals (Ca, K, Ba, Na) were clearly detectable because they dominate the LIBS spectra. Magnesium (Mg) was also detected. The light organic elements such as carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) together with C-N molecular bands were also simultaneously monitored. Table 2 lists the elements observed in the spectra and their corresponding emission wavelengths.

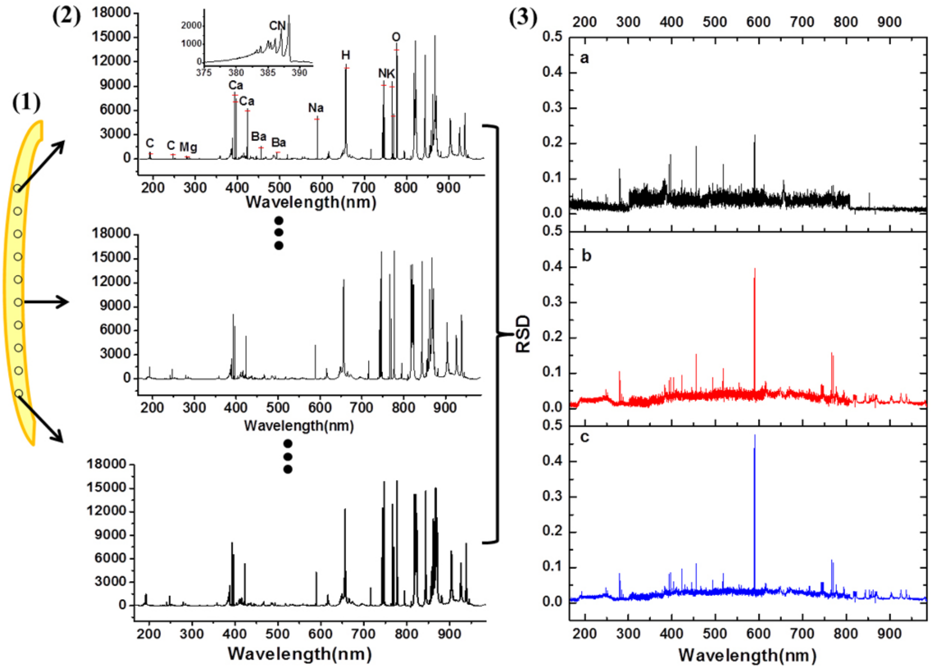

Fig.2 (1) The sampling sketch map of ten micro-locations for each sample, (2) the representative LIBS spectra of *Juncus effuses* L., (3) "RSD spectra": (a) $n = 1 \times 9$ (one micro-location), (b) $n = 5 \times 9$ (five separate micro-locations), and (c) $n = 10 \times 9$ (ten separate micro-locations)

Table 2. The observed LIBS emission lines of *Juncus effusus* L. samples

Element	Wavelength (nm)	Element	Wavelength (nm)
C	192.77, 247.725	Na	588.952, 589.554
Mg	279.418, 280.123	H	656.315, 777.492
Ca	393.375, 396.816	Li	670.754
C-N	383.522, 386.105, 387.08, 388.296	K	766.52, 769.96
Al	394.417, 396.097	O	777.212, 777.492
Ba	455.358, 493.388, 553.52	N	715.709, 744.306, 746.918, 843.762, 869.367, 870.256, 870.947
Mg	517.245, 518.316		

3.2 "RSD spectra" of *Juncus effuses* L.

Fig. 2(1) shows the sampling characteristic (such as one micro-location, five and ten micro-locations) and representative LIBS spectra of *Juncus effuses* L. "RSD-spectra" at one micro-location (nine pulses measurement) and separate micro-locations (five and ten) per sample are shown in Fig. 2(2).

In the "RSD spectra", the dominant noise would be the background, which indicates the measurement precision during the plasma evolution. Moreover, the dominant signal demonstrates the mineralogical variability among heterogeneous samples. In "RSD spectra" of *Juncus effuses* L., the dominant signals were Mg 279.418 nm, Ca 393.375 nm, Ba 455.358 nm, and Na 588.952 nm, 589.554 nm, etc. Further analysis will be given in the following section.

Fig. 3 is a detailed graph of Fig. 2(3) in a wavelength of 660-740 nm. In general, the values of RSD decreased with the increasing number of laser pulses. Firstly, the values of RSD at one micro-location (n=9) were less than 0.08. Secondly, the values of RSD at five micro-locations (n=9×5) were less than 0.06. Finally, at ten separate micro-locations (n=9×10) the values of RSD spectra were less than 0.05. Therefore, a total of 90 shot measurements per sample at ten separate micro-locations were better for the spectroscopic analysis.

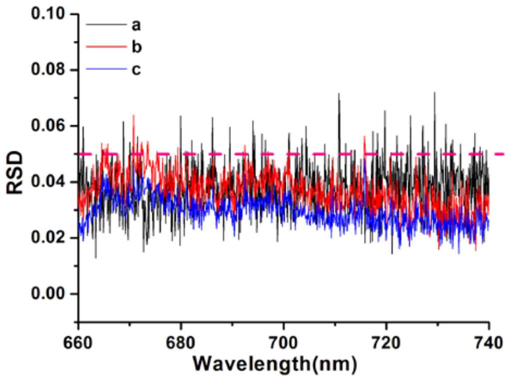

Fig.3 The detailed graph of Fig. 2(3) in wavelength of 660-740 nm

3.3 Elemental distribution of *Juncus effuses* L.

The thermodynamic chart of four elements (Mg, Ca, Ba, and Na) obtained at different micro-locations across the medulla of *Juncus effuses* L. was used to explore the elemental distribution (as shown in Fig. 4).

For the 12 samples, the color of four elements was different. Moreover, the color of four elements at different micro-locations was distinct, especially for Ca and Ba. In Fig. 4(a), the image of Mg was dark red or light red

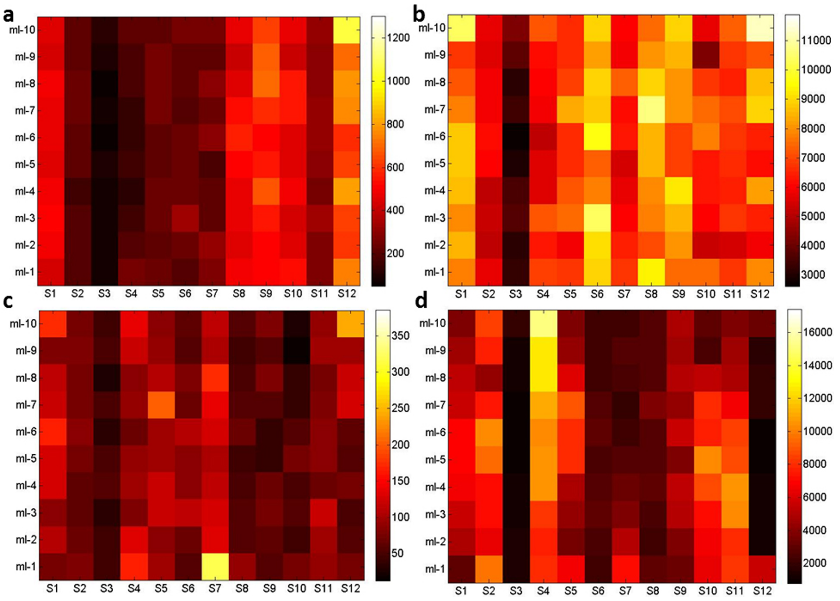

Fig.4 Intensity dependent thermodynamic chart of four elements at ten different micro-locations ("ml") on the micro-surface of stem samples from twelve collection regions. (a) Mg 279.418 nm, (b) Ca 393.375 nm, (c) Ba 455.358 nm, and (d) Na 588.952 nm. The color legend (bar on the right) refers to the intensity of spectral lines

overall, whereas the color was alternating between red and yellow in S9 and S12 at different micro-locations. This meant that the distribution of Mg was uneven in S9 and S12.

In Fig. 4(b), the red color was alternating in the chart, which demonstrated that the distribution of Ca was heterogeneous in numerous samples. Moreover, the color of S3 was wine in the diagram illustrated the homogenous distribution of Ca. Clearly, the intensity of Ba spectral line was homogeneous in all of the samples except S7 (as shown in Fig. 4(c)) because the image of Ba spectral line was light yellow at “ml-1” of S7.

Additionally, the intensity of Na spectral line changed greatly in numerous samples, especially in S4 (as shown in Fig. 4(d)). The diagram of Na showed light yellow at the “ml-8”, “ml-9” and “ml-10” of S4, which meant that the intensity of Na spectral line was higher than that of other micro-locations. Generally, the distribution of Na was heterogeneous at different micro-locations due to the large variation of color. The intensity of the four elements was low and relatively steady in S3 because the diagram of S3 was red in color.

These results of intensity dependent thermodynamic chart of four elements elucidated the mineralogical variability among the samples. The intensity (or the quantity) of elements was distinct in the heterogeneous samples. Therefore, the multimode sampling methodology of microanalysis was significant for the heterogeneous samples.

4 Conclusions

In this investigation, LIBS was found to be a valuable tool for the analysis of *Juncus effusus* L. samples. Specifically, microanalysis and RSD approach were used to explore a multimode sampling method for heterogeneous samples. Furthermore, the thermodynamic chart of four elements (Mg, Ca, Ba and Na) successfully characterized the mineralogical distribution of *Juncus effusus* L. samples on a micro-scale.

Due to a popular Chinese herb and an ideal bioindicator, the microanalysis of *Juncus effusus* L. research on the spectral “fingerprint” is significant. The similar algorithms can be triggered in the microanalysis of LIBS within plant materials. CMM has its own characteristic and it is the treasure of China. The Chinese LIBS community is one of the most dynamically developing communities in the world [27]. LIBS has potential in a CMM based on microanalysis.

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