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Quantitative analysis of cadmium in rice roots based on LIBS and chemometrics methods

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Abstract

Background: Excessive cadmium can damage cell structure, inhibit enzyme activity, and affect metabolic process, thus, leading to decline of rice yield and quality. Root is an important organ of crops, the detection of cadmium in root is essential for limitation of cadmium in rice grains.

Results: In this study, laser-induced breakdown spectroscopy (LIBS) was applied for cadmium quantitative analysis. Pretreatment methods, including median absolute deviation, wavelet transform, area normalization, were used to improve spectral stability. Scanning electron microscope and energy-dispersive X-ray spectrometer (SEM/EDS) was first used to analyze ablation pit surface characteristics and the results showed significant positive correlation with spectral lines of Cd II 214.44, Cd II 226.50 and Cd I 228.80 nm. Univariable models of spectral lines showed that three Cd spectral lines have good prediction for cadmium. Fitting methods including linear, logarithmic, and polynomial were used to propose characteristic input variables, and univariable models based on variable of polynomial fitting of $I_{214.44\text{ nm}}$ have achieved the best effect ($R_p = 0.9821$ and RMSEP = 31.1 mg/kg). Besides, partial least squares regression (PLSR), least squares support vector machine (LS-SVM) and extreme learning machine (ELM) were used for multivariate analysis. Compared with univariate analysis, ELM model based on the full spectrum ($R_p = 0.9896$ and RMSEP = 26.0 mg/kg) had more advantages for cadmium detection.

Conclusion: Compared with traditional methods (150 min), the quantitative detection method based on LIBS technology (less than 5 min) greatly reduces the detection time of heavy metals. The results showed that LIBS has proved to be a reliable method for quantitative detection of cadmium in rice roots. The research can provide theoretical support for timely detection of heavy metals in crop and food production.

Keywords: Rice root, Cadmium, Laser-induced breakdown spectroscopy, Fitting methods, Extreme learning machine

Background

Rice is the main grain crop in China. Compared with other crops, rice has strong absorption of cadmium. Excessive cadmium can hinder cell division, damage cell structure, inhibit enzyme activity, and affect metabolic process, thus, leading to decline of rice yield and quality [1–3]. Cadmium is a non-essential element of human body [1, 4, 5]. Consumption of cadmium-contaminated

rice for a long time seriously harms human health [6]. Cadmium can damage human kidney function, hinder bone metabolism, and cause various bone diseases [7, 8]. World Health Organization (WHO) recommends no more than 68 µg of cadmium per person per day. Due to the consideration of protecting human body from cadmium, countries have established some mandatory standards to limit cadmium content in food. In accordance with the National Food Safety Standards for Contaminants in Foods (GB 2762-2012), the cadmium content in food ranges from 0.005 to 2 mg/kg, and its content in rice grains should not exceed 0.2 mg/kg. Clemens et al. [9] found that there were three processes from root absorption to cadmium accumulation in grain: root

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absorption, xylem transport, and phloem migration to grain. Li et al. [10] found that cadmium content in grain was largely restricted by root absorption capacity. The transfer coefficient of cadmium in root–stem, stem–leaf, leaf–seed and stem–seed decreased successively. As front part, root is an important organ that affects accumulation and transportation of cadmium in rice grains [11, 12]. Therefore, the detection of cadmium in rice roots is of great significance to limit cadmium content in rice.

At present, atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectrometer (ICP-OES) and inductively coupled plasma with mass spectrometer (ICP-MS) are widely used for heavy metal detection [13–16]. Traditional multi-element analysis methods mean lengthy sample preparation and complex chemical experiments. They have disadvantages such as large sample loss, complicated operation, high cost, and time-consuming. It cannot meet requirements of low cost, fast response and accurate detection in precision agriculture [17, 18]. As an element analysis method, laser-induced breakdown spectroscopy (LIBS) technology was proposed by the David Cremers research group of Los Alamos National Laboratory in the United States in 1962 [13, 19]. Compared with commonly used chemical detection approaches, LIBS has the characteristics of rapidity, batch, microdamage, multi-element simultaneous analysis and no complex pretreatment [20–22]. Recently, LIBS has been used for rapid detection of elements in crops, which has significance for food security and environmental pollution. Yang et al. [23] prepared samples by adding heavy metal reagents to the standard rice and used LIBS coupled with ultrasound-assisted extraction to achieve the rapid detection of toxic elements (Cd and Pb) in rice. Liu et al. [24] soaked rice in the copper solution to prepare copper-contaminated rice samples and applied LIBS for quantitative analysis of copper in three kinds of rice, the results showed that LIBS could be considered as a useful tool for copper detection. Jabbar et al. [25] detected the composition of nutrient elements in different parts (root, stem and seed) of the rice plant using LIBS, and determined their weighted concentration. Peng et al. [26] investigated the influence of parameters in dual-pulse LIBS method to predict chromium content in rice leaves and provided a fast approach for toxic elements prediction in rice leaves. However, due to the difficulty of obtaining the real plant root samples contaminated with heavy metals and the complexity of the plant matrix effects, few studies have focused on the application of LIBS technology in the high-precision quantitative detection of heavy metals in roots. In agricultural production, cadmium in the environment is absorbed by rice roots from the soil and then transferred to the above-ground parts, resulting in the accumulation

of cadmium in rice grains. As a bridge between external cadmium pollution and above-ground rice grains, the cadmium in rice roots directly affects the accumulation of cadmium in grains. Based on the advantages of LIBS technology that can achieve batch processing and rapid detection, we used real rice roots contaminated with cadmium as samples to research rapid quantitative detection of cadmium in rice roots based on LIBS and chemometrics methods.

This study preliminarily introduces a method for quantitative detection of cadmium in rice roots using LIBS. The specific content includes: (1) to pretreat raw spectra with MAD, WT and area normalization and analyze LIBS spectra to determine Cd spectral lines; (2) to apply SEM/EDS to reflect the surface characteristics of ablation pits, then analyze the correlation between Cd spectral lines and SEM/EDS; (3) to establish univariate models based on Cd spectral lines; (4) to propose characteristic input variables about Cd and establish univariate models based on variables; (5) to establish multivariate models and compare them with univariate models to determine the optimal quantitative model for cadmium in rice roots.

Materials and methods

Sample preparation

The rice variety selected in the experiment was Xiushui 134, which was a single-season conventional late japonica rice widely cultivated in Zhejiang Province, China. Rice seeds with full granule and consistent size were selected and sterilized with 1% NaClO solution for 20 min, washed several times with distilled water, soaked in dark environment for 48 h. The soaked seeds were placed in a seedling tray and placed in an incubator (temperature: 35 °C, humidity: 85%), sprouting for 2–3 days. After the rice seeds were germinated, they were transplanted to the sponge in the seedling tray and placed in an artificial climate chamber (duration of day mode: 14 h, temperature: 30 °C, humidity: 85%, light intensity: 225 $\mu\text{mol m}^{-2} \text{s}^{-1}$; duration of night mode: 10 h, temperature: 30 °C, humidity: 85%). During cultivation, nutrient solution of rice was prepared according to international rice nutrient solution formula, with pH value of 5.3–5.6. As rice enters tillering stage of growing season, it begins to add a certain concentration of heavy metals to the nutrient solution. CdCl_2 reagent was used to prepare cadmium solution. Referring to the existing literature, Cd^{2+} concentration gradient was 0 μM (CK), 5 μM , 25 μM , 50 μM and 100 μM [27, 28].

After 10 days, 20 days and 30 days, rice roots at different concentrations were collected as samples. In total, 15 rice root samples with different concentrations were obtained, namely Day10-CK, Day10-5, Day10-25, Day10-50, Day10-100, Day20-CK, Day20-5, Day20-25,

Day20-50, Day20-100, Day30-CK, Day30-5, Day30-25, Day30-50 and Day30-100. First rice roots were washed with tap water and distilled water, respectively, to remove impurities on the surface of sample, then immersed them in EDTA-2Na solution for 60 min to remove heavy metals that may remain on samples, and finally washed again with distilled water as the experimental sample. To obtain dry root samples, the fresh rice roots were dried to a constant weight in an oven (temperature: 60 °C, time: 72 h). The dried roots were ground into powder using grinding instrument (JXFSTPRP-48, Shanghaijingxin, Shanghai, China). 100 mg of powder was weighed from each gradient of the sample. The tablet machine (YLJ-20T, Guoyan, Hebi, China) was used to compress the powder into tablets of 10 mm × 10 mm × 1 mm at a pressure of 60,000 N for 20 s, which were used as samples to be tested. Four tablets were prepared for each gradient of the sample. In total, 60 root tablets were obtained for collecting LIBS spectral data.

LIBS instrumentation

The structural diagram of the LIBS system is shown in Fig. 1. As a laser source, Q-switched Nd:YAG nano-second pulsed solid-state laser (Vlite-200, Beamtech Optronics, Beijing, China) is used to generate laser pulses, with a repetition frequency of 1–10 Hz, a spot diameter of 7 mm, and a pulse width of 8 ns. In the experiment, monochromator and ICCD camera are combined to obtain high-resolution spectral information in a specified short-wave segment. The monochromator with the wavelength range of 180–850 nm (SR-500i-A-R, Andor, Belfast, UK) is used as a spectrometer to separate the signals generated by the plasma transition. The central wavelength is adjusted

to limit the range of collected spectra. In the experiment, the central wavelength was set at 220.53 nm, and the spectra were collected in the range of 210.01–231.00 nm. The ICCD detector of IStar 340T series (time gate width: 5 ns, wavelength range: 180–850 nm) is adopted to convert the optical signal into electric signal. In addition, a digital delay pulse generator with a time resolution of 5 ps and a controllable time range of 0–2000 ns (DG645, Stanford Research Systems, California, USA) can control the timing of lasers and ICCD detectors in real time. The X–Y–Z stage with a repeatability of 5 µm and a maximum stroke of 50 mm (Zolix, Beijing, China) can realize real-time movement of samples. The working mode of different pulse outputs is realized by the optical path system built by our own experimental team. Experimental parameters of the LIBS system such as laser pulse wavelength and energy, spectrometer delay time, ICCD gate width and gain of LIBS instrument are all key factors that affect the accuracy of the collected spectral data. For acquisition of a better signal-to-noise ratio (SNR), the parameters used in the experiment are as follows: $\lambda = 532$ nm, energy = 60 mJ, delay = 1 µs, gate width = 10 µs, and gain = 1500. With the help of X–Y–Z stage, 4 × 4 array was set as the ablation path, where the distance between each row and each column is 2 mm. Then, 16 positions on the sample were collected for spectrum, which are evenly distributed on each sample. To reduce point-to-point fluctuations of laser, each position was cumulatively ablated 5 times by laser with repetition rate of 1 Hz. A single spectrum was obtained at each position, in which the spectrum was the accumulation of 5 ablations. As a result, 16 ablation pits were generated on each sample, and the average spectra of 16

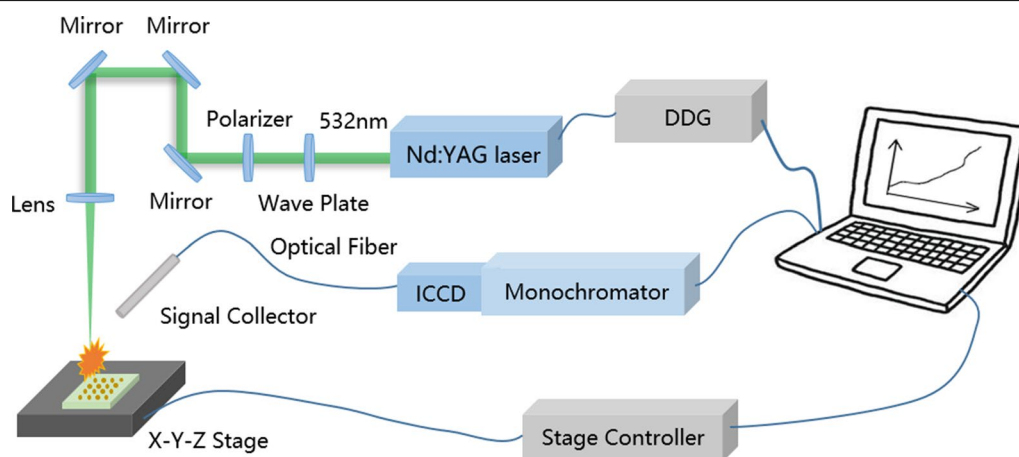


Fig. 1 Structural diagram of the LIBS system

ablation pits obtained was taken as the LIBS data of the sample.

Reference method

ICP-OES instrumentation

In the experiment, the reference cadmium content in rice roots which was used to achieve quantitative analysis of heavy metal was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Before detection, samples need to be pretreated with microwave digestion method. The specific experimental steps are as follows. The root tablets from which spectra have been collected were reground into powder. Rice root samples of a certain mass were weighed from each sample and put into Teflon digestion tube, and its mass was recorded. Then, add 5 mL of 65% nitric acid and 1 mL of 30% hydrogen peroxide to the digestion tube, and put it in the microwave digestion instrument (MARS 6, CEM, USA) for digestion. After digestion process, the digestion tubes were placed on electric heating board to remove acid from the sample. When the volume of residual liquid in digestion tube was less than 0.5 mL, the acid removal was completed. Transfer the remaining liquid to a volumetric flask and dilute with deionized water to a 50 mL scale. Then, the solution is used for the determination of Cd content. After detection of ICP-OES (Optima 8000, PerkinElmer, Waltham, USA), we need to calculate the measured value according to the mass and volume of the sample, and finally get the Cd content in the root samples. In addition, the standard sample of citrus leaves (GBW10020, Beijing, China) was used as the control group to verify whether the experimental procedures met the standard operation. In the experiment, it took about 150 min to detect the Cd content in a root sample using ICP-OES.

SEM/EDS technology

SEM/EDS used in the experiment was Phenom XL system (Phenom XL, Phenom-World BV, Eindhoven, Netherlands), which is an integrated scanning electron microscope (SEM) platform equipped with an energy-dispersive X-ray spectrometer (EDS). The low-vacuum technology in SEM of the system can effectively suppress charging effect, so the system can realize direct observation of rice root samples without spraying gold or carbon. The EDS uses a high-brightness CeB₆ filament that excites a large number of X-rays, enhances the signal, and improves the accuracy of elemental analysis. Phenom XL system enables surface microscopic observation of nano- and submicron samples with diameters less than 100 nm and magnifications up to 100,000 times. The EDS software can assess the relative contribution of each detected

element in the region of interest, expressed as a percentage [29]. In addition, the software can achieve a visual distribution of detected elements in the selected area.

Data treatment

During the LIBS ablation excitation process, complex matrix effect of the plant sample and parameters of the experimental instrument will directly affect the stability of the LIBS signal. This will cause the collected spectra to have problems such as abnormal values, background noise, point-to-point fluctuations, and baseline drift, which will lead to deviations in the results. For rice roots, it is difficult to completely eliminate these adverse effects by sample processing and instrument parameter adjustment. In this study, pretreatment methods, including abnormal elimination, wavelet transform and data normalization, were used to improve spectral stability without increasing complexity of operation.

Due to the randomness of plasma transition, there are usually some abnormal values in a series of spectra obtained at the same time, which will greatly affect accuracy. For obtained spectra, it cannot guarantee that each spectrum obtained has the characteristics of the sample. Median absolute deviation (MAD) was applied to remove outliers in this study [30]. Cd 226.50 nm was selected as the variable for outlier elimination, because the signal of Cd 226.50 nm had relatively high stability in the collected spectra. First, MAD and median of peak intensity at 226.50 nm were calculated. Then, the deviation between the peak value and the median value of each spectrum at 226.50 nm was calculated, respectively. If the difference was greater than 2.5 times of MAD, the spectrum would be regarded as an abnormal spectrum. When no abnormal spectrum is detected or less than 75% of the total spectrum is left, the program stops the loop [31]. As an effective denoising method, the wavelet transform (WT) using Daubechies 8 with decomposition scale 3 was applied to denoise spectra. For the spectra of the same sample, there are point-to-point fluctuations between single spectrum. These fluctuations have certain relationship with characteristics of some spectral lines. Spectral normalization can effectively reduce the fluctuation of point and point and improve the stability of spectra. Area normalization was used for pretreatment in this study.

To quickly and accurately detect the cadmium content in rice roots, LIBS technology was combined with chemometrics method to perform univariate and multivariate analyses of cadmium. The univariate analysis method is also called the calibration curve method. In this study, the cadmium content measured by ICP-OES was used as the reference value of model. The LIBS spectra and the reference value were fitted to establish univariate models. Different from univariate analysis, multivariate analysis

is a commonly used method for information mining on a large amount of data, which can effectively deal with multicollinearity problem and establish linear regression model [32]. To compare the different results of model and determine the optimal effect of quantitative detection, a variety of modeling methods were used for comparison in the paper. For multivariate analysis, partial least squares regression (PLSR), least squares support vector machine (LS-SVM) and extreme learning machine (ELM) were used to establish quantitative models of Cd content in rice roots. To optimize the model performance, five-fold cross-validation is used to determine the optimal model parameters.

Before modeling, the rice root samples were divided into calibration set and prediction set at a ratio of 2:1. Arrange the samples of the cadmium concentrations. For every three samples, the second one is used for prediction, and the others for calibration. Therefore, 40 samples were selected as the calibration set and 20 samples as the prediction set.

Performance evaluation

In the process of LIBS modeling, some evaluation indexes can be used to evaluate the prediction effect of quantitative model. The correlation coefficient (R) is used to evaluate the fitting effect of the model. Generally, the value of R ranges from 0 to 1. The value of R is proportional to the fitting effect. That is, the larger the value of R , the better the fitting effect. R_c and R_p , respectively, represent the calibration and prediction set correlation coefficients.

The root mean square error (RMSE) can represent the deviation between the chemical measurement value and the model prediction value, and can be used as a standard for measuring the model prediction result. RMSE includes root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). The RMSE value is proportional to the prediction error of the model. The smaller the RMSE value, the better the model modeling effect obtained.

Results and discussion

Reference analysis of ICP-OES

The reference content of cadmium in rice roots was shown in Fig. 2. Cadmium concentrations in 15 root samples ranged from 0 to 537.79 mg/kg. The cadmium content in CK groups (Day10-CK, DAY20-CK, and Day30-CK) was 0. For cadmium content, the samples of the 13 cadmium stress groups increased in the following order: Day10-5 → Day30-25 → Day30-5 → Day20-5 → Day10-25 → Day10-50 → Day20-25 → Day10-100 → Day30-50 → Day20-50 → Day20-100 → Day30-100.

LIBS spectral analysis

Referring to the National Institute of Standards and Technology (NIST), in the range of 210.01–231.00 nm, there are three cadmium emission lines with good strength, namely, ionic emission line Cd II 214.44 nm, ionic emission line Cd II 226.50 nm, and atomic emission line Cd I 228.80 nm, as shown in Table 1. The spectra of rice roots with different cadmium concentrations, including

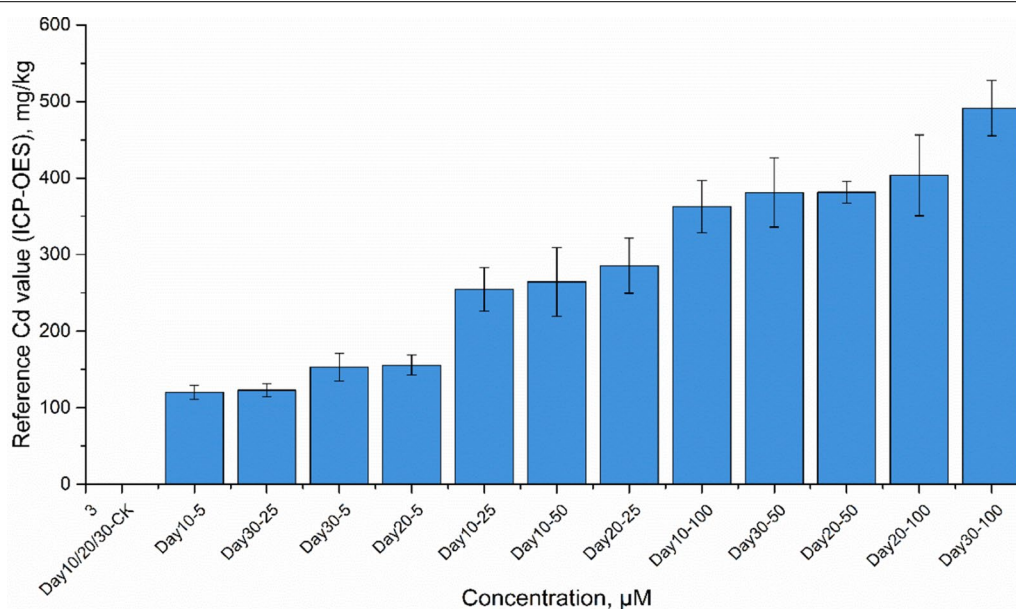


Fig. 2 Reference cadmium content in rice roots

Table 1 Spectral properties of cadmium emission lines

Ion	Wavelength (nm)	Rel. Int. ^a	A_{ki} (s ⁻¹) ^b	E_i-E_k (eV) ^c
Cd II	214.43	1000	3.778×10^8	0–5.80
Cd II	226.50	1000	3.177×10^8	0–5.48
Cd I	228.80	1500	5.300×10^8	0–5.43

^a Rel. Int.: relative intensity of emission lines^b A_{ki} : transition probabilities^c E_i-E_k : lower level and upper level energies of the transition

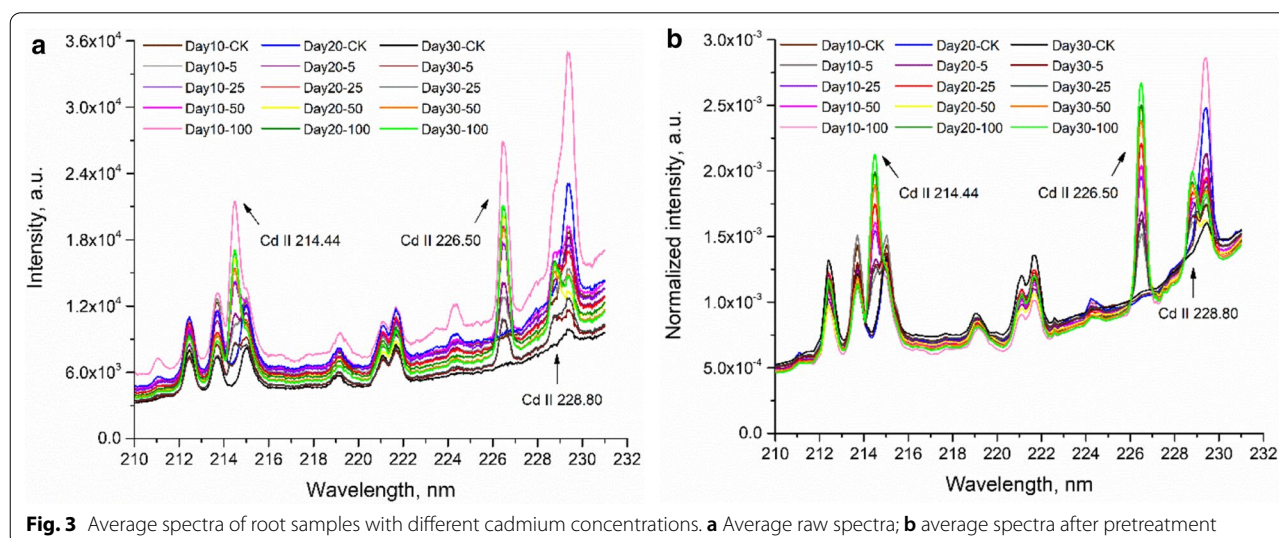
Day10-CK, Day10-5, Day10-25, Day10-50, Day10-100, Day20-CK, Day20-5, Day20-25, Day20-50, Day20-100, Day30-CK, Day30-5, Day30-25, Day30-50 and Day30-100, were collected by monochromator. In Fig. 3, the strongest intensities were observed at Cd II 214.44 nm, Cd II 226.50 nm, and Cd I 228.80 nm, which are associated with cadmium. The average raw spectra of rice roots are shown in Fig. 3a. X-axis is wavelength, where the range is 210.01–231.00 nm, and Y-axis is spectral intensity. Each spectral line is average spectrum of rice root samples in the same group. In this study, median absolute deviation (MAD), wavelet transform (WT), and area normalization were used to preprocess raw spectra. The average spectra after pretreatment are shown in Fig. 3b, where Y-axis is the spectral intensity after normalization. Compared with raw spectra, the background noise is reduced and the spectra are smoother after pretreatment.

The comparison of rice root spectral intensities with different cadmium concentrations at Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm was shown in Fig. 4. Figure 4a, c, e, respectively, shows the spectral intensities of all samples in the range of 213.00–217.00 nm, 224.00–227.00 nm and 227.00–230.00 nm. No significant Cd lines were detected in the CK group

(Day10-CK, Day20-CK and Day30-CK) at 214.44 nm, 226.50 nm and 228.80 nm. The reason was that there was no cadmium in the CK group, the cadmium content failed to reach the detection limit of instrument. According to the specific concentration sequence (Day10-5 → Day30-25 → Day30-5 → Day20-5 → Day10-25 → Day10-50 → Day20-25 → Day10-100 → Day30-50 → Day20-50 → Day20-100 → Day30-100), the spectral intensity of rice roots showed regularity at Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm. In the non-CK group, obvious spectral peaks appeared at the spectral lines of Cd, and the intensity of spectral lines showed a trend of gradually increasing according to the specific concentration sequence. It was shown that the LIBS spectral lines were consistent with the cadmium concentration sequence analyzed by ICP-OES. The comparison of spectral intensities at Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm for different cadmium concentration samples is shown in Fig. 4b, d, f. Under the same time conditions, with the increase of cadmium concentration, the line intensity of rice roots showed a gradually increasing trend. The trend was applicable to Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm. The results indicates that the accumulation of cadmium in roots is strongly related to the spectral values of the three Cd lines (Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm). The three Cd lines can be used as characteristic spectral lines for cadmium detection in rice roots.

SEM/EDS analysis

When spectrum was collected, each position in samples was continuously ablated 5 times with a laser pulse at a frequency of 1 Hz, an ablation pit was finally formed. To obtain the microscopic morphology, SEM/EDS was



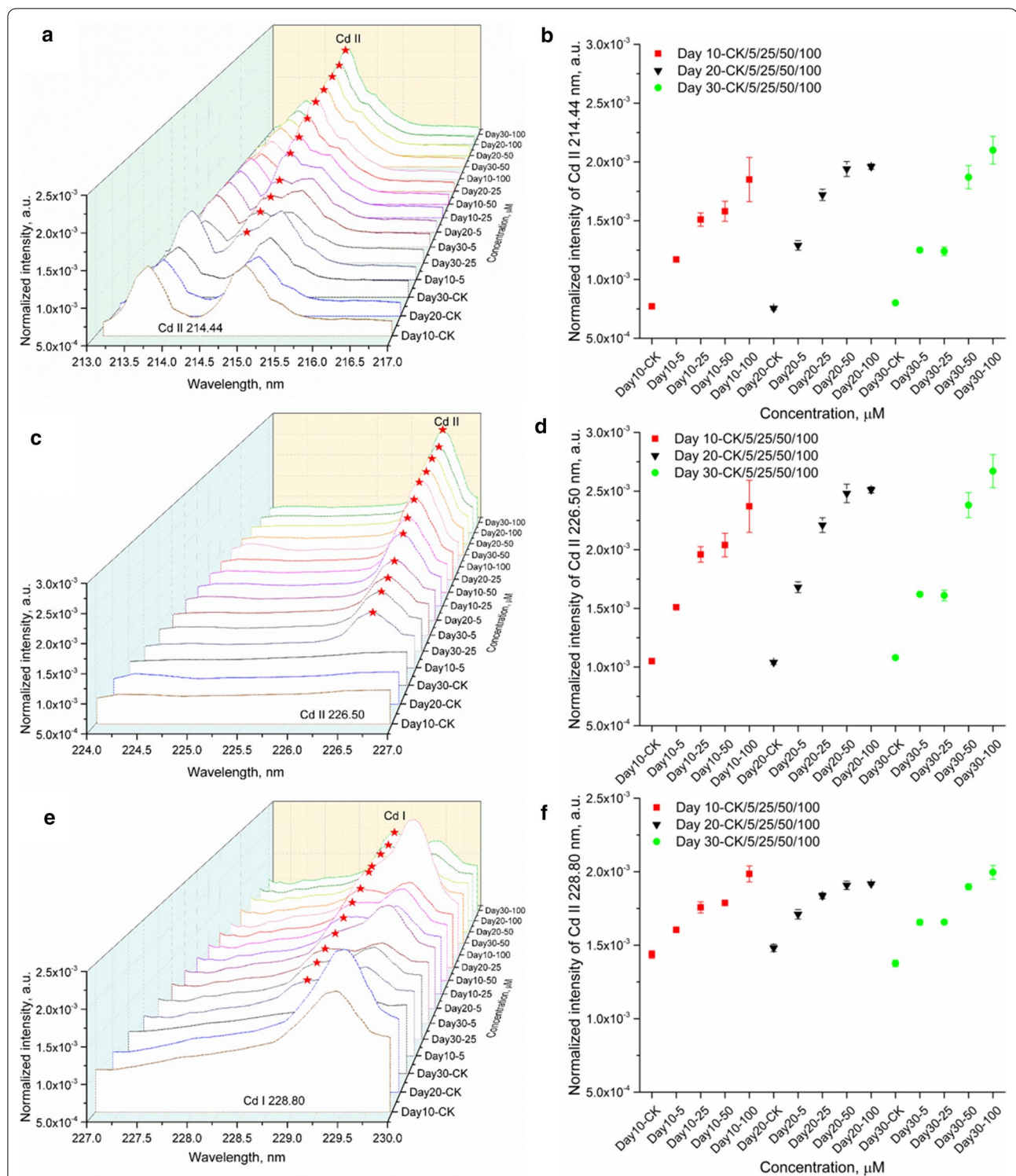


Fig. 4 Average spectra after pretreatment of root samples with different cadmium concentrations. Comparison of rice root spectral intensities with different cadmium concentrations at Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm. **a** Spectral intensities in the range of 213.00–217.00 nm; **b** comparison of spectral intensities at Cd II 214.44 nm; **c** spectral intensities in the range of 224.00–227.00 nm; **d** comparison of spectral intensities at Cd II 226.50 nm; **e** spectral intensities in the range of 227.00–230.00 nm; **f** comparison of spectral intensities at Cd I 228.80 nm

applied to analyze surface morphology and element distribution information of ablation pit. A rectangular frame that encloses the ablation pit was selected as the region of interest. 15 rice root tablets with different cadmium concentrations were used for SEM/EDS analysis in the experiment. In this paper, the sample of Day30-100 is taken as an example to describe surface morphology and cadmium distribution of an ablation pit (Fig. 5). The ablation pit had a contour that is approximately circular (about 700 μm in diameter) and was concave downward in the middle. The formation of the morphology was due to the small loss of samples when the sample was continuously ablated by laser pulse. Due to laser ablation, the surface of ablation pit area was uneven and cracked, and has attached sample fragments. Compared with the ablation pit, the surface of the surrounding area is relatively flat and firm. SEM/EDS shows the distribution of cadmium in the region of interest in red, where the shade of the color represents the amount. It can be found that for ablation pits, the red at the edge is darker than the red at the middle position. We suspected that the difference in cadmium distribution was due to more samples at the center of the ablative pit being ablated by laser.

SEM/EDS scan results of the root tablets showed the elemental composition of the sample and the relative content of each element, which was expressed in weight percent. The main components of the sample were O, C and N, accounting for about 90%, followed by elements such as Na, Cl, Si and K, accounting for about 10%, and the remaining elements Mg, Ca and Cd accounted for a very small proportion, about 1%, which are shown in Table 2. Under the same time conditions, the Cd content in roots showed an increasing trend with the increase of

cadmium concentration. No cadmium was found in the CK group (Day10-CK, Day20-CK, Day30-CK). The sample of Day30-100 had the highest content of Cd, which was 0.32%.

The correlation analysis was conducted on LIBS spectral data (Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm) and the cadmium weight percentage obtained by SEM/EDS, which were used to obtain the correlation between LIBS and SEM/EDS methods. According to the Shapiro–Wilk test, cadmium weight percentage and LIBS intensity data were in a normal distribution ($P > 0.05$). Pearson correlation analysis was used to evaluate the relationship between EDS and LIBS line intensity. A significant positive correlation was found between SEM/EDS and LIBS intensity, $r_{214.44} = 0.880$, $r_{226.50} = 0.880$, $r_{228.80} = 0.895$, $P < 0.001$.

SEM/EDS was first used as a complementary technology to the LIBS method to provide microscopic information about ablation pits. SEM/EDS analyses of root tablets successfully reflected surface morphology, elemental composition and element distribution of cadmium in the ablation pit.

Quantitative analysis of cadmium

Univariate analysis of Cd spectral lines

LIBS spectrum can reflect the difference of cadmium concentration in different samples. To achieve accurate detection of heavy metals in rice roots, it is necessary to combine LIBS spectrum with quantitative analysis methods. Univariate analysis is a common quantitative analysis approach. It correlates the elemental content with the detection signal through a calibration curve, and then makes prediction. Univariate analysis was used to explore

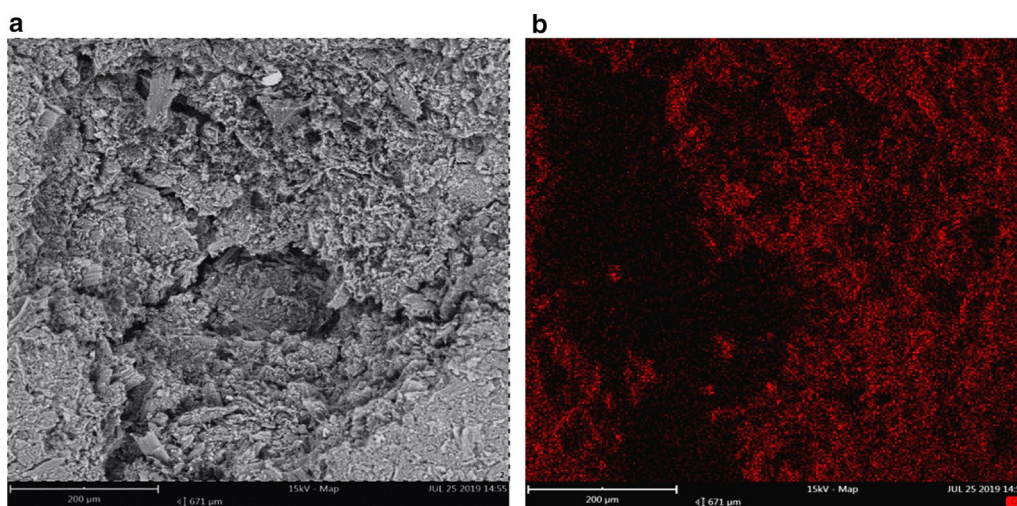


Fig. 5 SEM/EDS analysis of ablation pit in the sample of Day30-100. **a** Surface morphology; **b** distribution of cadmium

Table 2 Element weight percentage obtained by EDS

Group		O (%)	C (%)	N (%)	Na (%)	Cl (%)	Si (%)	K (%)	Mg (%)	Ca (%)	Cd (%)
Day10	CK	72.79	14.46	3.84	2.76	2.67	1.53	1.42	0.31	0.22	0
	5	71.54	13.82	3.98	3.44	2.63	2.42	1.58	0.28	0.20	0.10
	25	72.20	14.67	3.63	2.95	2.53	1.80	1.51	0.33	0.25	0.15
	50	70.53	14.43	4.15	3.35	2.44	1.91	1.84	0.60	0.45	0.29
	100	70.23	14.35	3.57	3.45	2.39	2.35	2.22	0.63	0.53	0.28
Day20	CK	73.14	15.74	2.97	2.32	2.23	1.93	1.28	0.24	0.14	0
	5	73.98	14.89	2.88	2.64	2.31	1.60	1.20	0.28	0.12	0.09
	25	73.59	15.24	2.59	2.27	2.22	2.13	1.30	0.24	0.21	0.20
	50	75.30	14.80	2.58	2.23	2.02	1.42	0.95	0.27	0.22	0.20
	100	73.97	14.86	2.62	2.41	2.30	1.78	1.32	0.27	0.26	0.20
Day30	CK	75.52	15.53	2.42	2.03	1.83	1.24	0.83	0.29	0.29	0
	5	74.80	14.61	2.65	2.40	2.11	1.54	1.34	0.25	0.17	0.12
	25	75.05	15.83	2.48	1.85	1.68	1.44	0.86	0.39	0.22	0.20
	50	76.05	14.83	2.08	1.81	1.79	1.54	1.08	0.33	0.30	0.19
	100	74.38	14.48	2.36	2.08	1.96	1.80	1.47	0.64	0.51	0.32

the correlation between Cd spectral lines and cadmium content in rice roots.

With Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm as input and reference cadmium content obtained by ICP-OES as output, univariate models of cadmium content in rice roots were established, respectively, to compare fitting effects of different Cd spectral lines. Table 3 shows the univariable analysis results based on Cd spectral lines under the conditions of raw spectra and pretreatment. As for raw spectra, compared with other Cd spectral lines, Cd I 228.80 nm has poor correlation with Cd content due to baseline drift. From the results, it can be seen that the modeling effect after pretreatment are better than that of raw spectra, especially for Cd I 228.80 nm, R_p is increased from 0.6529 to 0.9626. Conclusively, the spectral lines of Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm have good correlation with cadmium in rice roots. We can see from the results that, Cd II 226.50 nm after pretreatment has the best prediction

effect on cadmium with highest R and lowest RMSE ($R_c = 0.9840$, $RMSEC = 28.1$ mg/kg, $R_p = 0.9794$, $RMSEP = 33.2$ mg/kg).

Univariate analysis of characteristic input variables

We also used a variety of fitting methods including linear, logarithmic, and polynomial to mine the relationship between Cd spectral lines (Cd II 214.44 nm, Cd II 226.50 nm, and Cd I 228.80 nm) and Cd content in rice roots, and proposed characteristic input variables about Cd. We performed logarithmic and polynomial fitting based on the intensity of different Cd spectral lines ($I_{214.44\text{ nm}}$, $I_{226.50\text{ nm}}$ and $I_{228.80\text{ nm}}$), and obtained 6 feature input variables. In addition, the combination of different Cd spectral line intensities ($I_{214.44\text{ nm}} + I_{226.50\text{ nm}}$, $I_{226.50\text{ nm}} + I_{228.80\text{ nm}}$, $I_{214.44\text{ nm}} + I_{228.80\text{ nm}}$, $I_{214.44\text{ nm}} + I_{226.50\text{ nm}} + I_{228.80\text{ nm}}$) was used as input to perform multiple linear regression, and 4 characteristic input variables were obtained.

Table 3 Univariable analysis results based on Cd spectral lines

Pretreatment methods	Wavelength (nm)	Calibration set		Prediction set	
		R_c	RMSEC (mg/kg)	R_p	RMSEP (mg/kg)
Raw	214.44	0.8812	74.6	0.9295	61.5
	226.50	0.8703	77.8	0.9222	65.0
	228.80	0.5495	133.0	0.6529	128.0
After pretreatment ^a	214.44	0.9822	29.5	0.9786	33.7
	226.50	0.9840	28.1	0.9794	33.2
	228.80	0.9548	46.8	0.9626	46.4

Bold emphasis: the optimal model for given conditions

^a Pretreatment methods: MAD, WT and area normalization

Table 4 Univariable analysis results based on characteristic input variables

Pretreatment methods	Wavelength (nm)	Model	Determination coefficient	Calibration set		Prediction set	
				R_c	RMSEC (mg/kg)	R_p	RMSEP (mg/kg)
Raw	214.44	Logarithmic	0.9101	0.8995	68.9	0.9140	64.7
		Polynomial	0.9222	0.9116	64.8	0.9248	60.7
	226.50	Logarithmic	0.9044	0.8931	70.9	0.9081	66.9
		Polynomial	0.9168	0.8996	68.8	0.9160	64.3
	228.80	Logarithmic	0.6473	0.6336	122.0	0.6336	124.0
		Polynomial	0.6833	0.6800	116.0	0.6290	124.0
	214.44 + 226.50	Linear	0.9406	0.9329	56.7	0.9479	54.8
	226.50 + 228.80	Linear	0.9712	0.9738	35.8	0.9610	45.9
	214.44 + 228.80	Linear	0.9709	0.9734	36.0	0.9614	46.0
	214.44 + 226.50 + 228.80	Linear	0.9712	0.9738	35.8	0.9612	45.9
After pretreatment	214.44	Logarithmic	0.9805	0.9622	42.9	0.9566	46.7
		Polynomial	0.9922	0.9833	28.7	0.9821	31.1
	226.50	Logarithmic	0.9834	0.9683	39.3	0.962	43.9
		Polynomial	0.9924	0.9844	27.7	0.9818	31.4
	228.80	Logarithmic	0.9759	0.9485	49.9	0.9521	50.8
		Polynomial	0.9815	0.9563	46.1	0.9734	42.6
	214.44 + 226.50	Linear	0.9922	0.9856	26.7	0.9796	33.2
	226.50 + 228.80	Linear	0.9926	0.9868	25.5	0.9793	32.4
	214.44 + 228.80	Linear	0.9922	0.9851	27.1	0.9779	33.1
	214.44 + 226.50 + 228.80	Linear	0.9929	0.9878	24.5	0.9793	32.6

Bold emphasis: the optimal model for given conditions

Table 4 shows the univariate analysis results with above characteristic input variables as input. Analyzing the results of raw spectra, we can get the following conclusions. For a single Cd spectral line, the effect of polynomial-based characteristic input variables is better than logarithmic. Compared with a single Cd spectral line, the combination of multiple Cd spectral lines has a better determination coefficient, and the univariate model based on the corresponding characteristic input variables has a better prediction effect. For raw spectra, the univariable analysis based on the characteristic input variable of multiple linear fitting of $I_{214.44 \text{ nm}} + I_{228.80 \text{ nm}}$ has achieved the best prediction effect, where R_p reached 0.9614 and RMSEP was 46.0 mg/kg. Compared with raw spectra, it can be found that the model established after pretreatment has better results, with higher R and lower RMSE, which is consistent with the results of univariate analysis based on Cd spectral lines. For the pretreatment spectra, the univariate analysis based on the characteristic input variable of polynomial fitting of $I_{214.44 \text{ nm}}$ has achieved the best modeling effect, where R_p reached 0.9821 and RMSEP was 31.1 mg/kg. Based on the above results, we can find that compared with univariable analysis based on Cd spectral lines, the univariable analysis based on characteristic input variables has better modeling effect.

Multivariate analysis

The monochromator collected spectral information in the range of 210.01–231.99 nm and obtained 1024 variables. Compared with univariate analysis, multivariate analysis usually contains more variables. It can extract more useful information from a large amount of data for modeling and prediction. The multivariate analysis method is beneficial to eliminate sample matrix effects, background influences, spectral line interference and point-to-point fluctuations, which can effectively improve the modeling effect. Three Cd spectral lines (Cd II 214.44 nm, Cd II 226.50 nm, and Cd I 228.80) and full spectrum were used as inputs. PLSR, LS-SVM and ELM algorithms were, respectively, used to establish the cadmium quantitative detection model based on the raw spectra and the pretreatment spectra. Multivariate analysis results based on different variables are shown in Table 5. For raw spectra, PLSR and LS-SVM models have better effects, while ELM model has poor effects. After the pretreatment, the effects of the three models have been improved, of which ELM model has the most obvious improvement and becomes the model with the best effect. For Cd spectral lines after pretreatment, ELM model works best, R_c reached 0.9921 and RMSEC was 19.70 mg/kg, R_p reached 0.9869 and

Table 5 Multivariate analysis results based on different variables

Pretreatment methods	Variable (nm)	Model	Parameter ^a	Calibration set		Prediction set	
				R_c	RMSEC (mg/kg)	R_p	RMSEP (mg/kg)
Raw	214.44 + 226.50 + 228.80	PLSR	2	0.9708	37.7	0.9612	46.1
		LS-SVM	(588,390.13, 86.94)	0.9869	25.3	0.9743	38.6
		ELM	36	0.9511	48.6	0.9429	54.3
	Full	PLSR	3	0.9778	33.0	0.9659	42.1
		LS-SVM	(1974.88, 16,315.65)	0.9923	19.5	0.9806	31.6
		ELM	28	0.9573	45.5	0.9557	49.4
After pretreatment	214.44 + 226.50 + 228.80	PLSR	2	0.9859	26.3	0.9791	32.4
		LS-SVM	(2026.25, 151.09)	0.9911	21.0	0.9828	29.4
		ELM	31	0.9921	19.7	0.9869	25.6
	Full	PLSR	3	0.9863	26.0	0.9808	31.1
		LS-SVM	(767.71, 35,346.51)	0.9935	17.9	0.9837	28.7
		ELM	15	0.9943	16.8	0.9896	26.0

Bold emphasis: the optimal model for given conditions

^a Parameters of different models: the optimal number of latent variables (LVs) for PLSR, the bandwidth of kernel function (sig2) and the trade-off between the minimum model complexity and the minimum training error (gam) for LS-SVM, the number of hidden nodes for ELM.

RMSEP was 25.6 mg/kg. For full spectrum after pretreatment, ELM model also achieved the best results, R_c reached 0.9943 and RMSEC was 16.80 mg/kg, R_p reached 0.9896 and RMSEP was 26.0 mg/kg. It is obvious that the multivariate analysis based on full spectrum is better than that based on Cd spectral lines.

Univariate analysis vs multivariate analysis

After univariate analysis and multivariate analysis, we obtained the optimal models under corresponding conditions. We compared the results of the above best models, and the relationship between the reference Cd content and prediction Cd content of different model is shown in Fig. 6. For univariable analysis, compared with Cd spectral lines, the model based on characteristic input variables has better prediction effect, in which the model based on the characteristic input variable of polynomial fitting of $I_{214.44}$ nm obtained the best effect ($R_c=0.9833$, RMSEC=28.7 mg/kg, $R_p=0.9821$, RMSEP=31.1 mg/kg). For multivariate analysis, compared with the local spectral lines, the model based on the full spectrum has better prediction effect, in which ELM model has the best effect ($R_c=0.9943$, RMSEC=16.8 mg/kg, $R_p=0.9896$, RMSEP=26.0 mg/kg). The multivariate analysis results of the full spectrum based on ELM model are superior to the univariate analysis based on the characteristic input variable of polynomial fitting of $I_{214.44}$ nm. The results showed that compared with the univariate analysis, the multivariate analysis based on full spectrum had more advantages in the rapid detection of the heavy metal cadmium in roots. This is because the full spectrum contains all sensitive spectral lines and effective

matrix information of cadmium, and the combination of spectral lines increases the prediction modeling information of cadmium. In addition, traditional methods take more than 150 min to achieve heavy metal detection for a sample, while the combination of LIBS technology and chemometrics takes less than 5 min. LIBS technology greatly reduces the time required to detect heavy metals in samples.

Conclusions

In this study, a method for quantitative detection of cadmium in rice roots using LIBS technology was introduced. The results indicated that cadmium accumulation of roots was strongly related to the LIBS spectral lines of Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm. SEM/EDS was successfully used for the first time to reflect the surface morphology, element composition and spatial elemental distribution of ablative pits. What is more, it has been proved that Cd spectral lines showed significant positive correlation with SEM/EDS analysis results, that is, $r_{214.44}=0.880$, $r_{226.50}=0.880$, $r_{228.80}=0.895$, $P<0.001$. The univariable analysis results based on Cd spectral lines showed that Cd II 214.44 nm, Cd II 226.50 nm and Cd I 228.80 nm have good correlation with cadmium in rice roots. Cd II 226.50 nm after pretreatment has achieved the best prediction on cadmium, with R_p of 0.9794 and RMSEP of 33.2 mg/kg. A variety of fitting methods including linear, logarithmic, and polynomial were used to propose characteristic input variables about Cd. We can see from the results that the univariate analysis based on the characteristic input variable of polynomial fitting of $I_{214.44}$ nm has achieved the

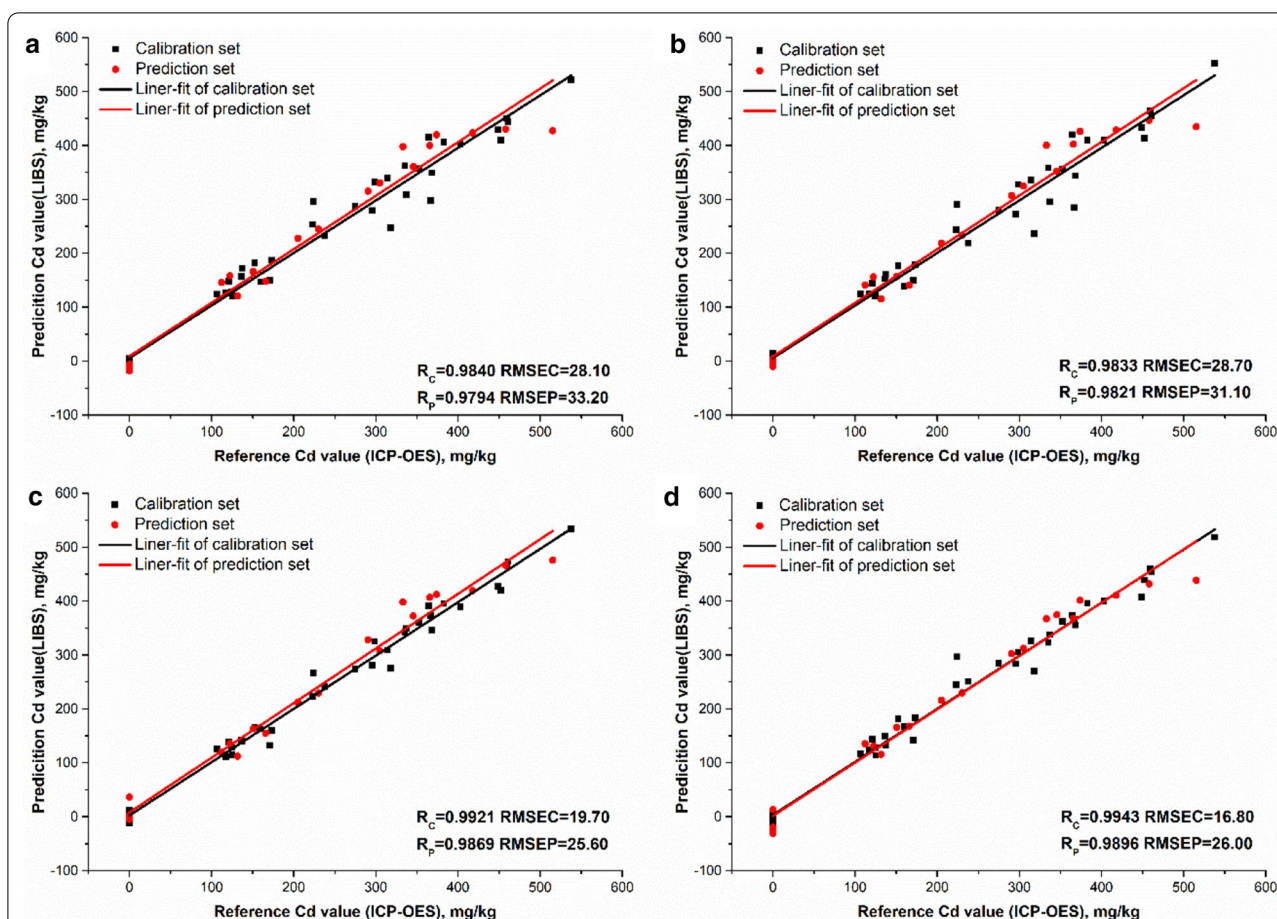


Fig. 6 Relationship between the reference Cd content and prediction Cd content of different models: **a** univariate analysis of Cd II 226.50 nm after pretreatment; **b** univariate analysis based on the characteristic input variable of polynomial fitting of $I_{214.44\text{ nm}}$ after pretreatment; **c** ELM model of three Cd spectral lines after pretreatment; **d** ELM model of full spectrum after pretreatment

best effect, where R_p reached 0.9821 and RMSEP was 31.1 mg/kg. Besides univariate models, PLSR, LS-SVM and ELM were, respectively, used to establish quantitative detection model. The ELM model based on the full spectrum achieved the best results, with R_c reaching 0.9943, RMSEC of 16.80 mg/kg, R_p of 0.9896, and RMSEP of 26.0 mg/kg. It can be found that the multivariate analysis based on full spectrum is better than that based on Cd spectral lines. Compared with univariate analysis, the multivariate analysis based on full spectrum had more advantages for the rapid detection of cadmium content in rice roots. Compared with traditional methods (150 min), the quantitative detection method based on LIBS technology (less than 5 min) greatly reduces the detection time of heavy metals. The present work indicated that LIBS has significant advantages in rapid and accurate detection of cadmium in rice roots. The research can help solve the problem that heavy metals in contaminated food is difficult to detect quickly and accurately,

and provides theoretical support for timely detection of heavy metals in crop and food production.

Abbreviations

LIBS: Laser-induced breakdown spectroscopy; SEM/EDS: Scanning electron microscope/energy-dispersive X-ray spectrometer; PLSR: Partial least squares regression; LS-SVM: Least squares support vector machine; ELM: Extreme learning machine; AAS: Atomic absorption spectroscopy; ICP-OES: Inductively coupled plasma optical emission spectrometer; ICP-MS: Inductively coupled plasma with mass spectrometer; MAD: Median absolute deviation; WT: Wavelet transform; R : Correlation coefficient; R_c : Calibration set correlation coefficients; R_p : Prediction set correlation coefficients; RMSE: Root mean square error; RMSEC: Root mean square error of calibration; RMSEP: Root mean square error of prediction.

Acknowledgements

We thank Jiming Xu in College of Life Science, Zhejiang University for providing ICP-OES assay. We also thank Yuechao Liang, Chuanjie Zhang and Bo Wei of Phenom-Scientific for providing SEM/EDS assay.

Authors' contributions

FL and YH provided experimental equipment and test funds. WW designed experimental scheme, and was a major contributor in writing the manuscript. ZM collected the LIBS spectra. TS performed the experiments of ICP-OES.

WK and WZ analyzed the data. All the authors read and approved the final manuscript.

Funding

This research was funded by Natural Science Foundation of China, grant number 61975174, Synergistic Innovation Center of Jiangsu Modern Agricultural Equipment and Technology, grant number 4091600021 and the Key Laboratory of Spectroscopy Sensing, Ministry of Agriculture and Rural Affairs, P.R. China, grant number 2018ZJUGP004.

Availability of data and materials

The datasets obtained and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 24 January 2021 Accepted: 16 March 2021

Published online: 25 March 2021

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