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Full Length Research Paper

Estimation of heavy metal concentration in rice leaves of farmland by hyperspectral leaf reflectance and partial least square regression

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ABSTRACT

Protecting people from heavy metal contamination is an important public-health concern and a major national environmental issue in China. The objective of this study was to quantitatively estimate the heavy metal concentration in rice leaves using leaf hyperspectral data and partial least squares regression (PLSR) models. 21 rice leaf samples and spectrum were gathered from farmlands in Zhangjiagang area, China. Copper (Cu), Cadmium (Cd) concentrations of rice leaves were measured within the lab. Firstly, the spectral data were treated by some methods, including, original reflectance (OS), First Derivative (FD) and Second Derivative (SD). Secondly, in order to select input variables for PLSR models, the correlation analysis between heavy metal concentration and spectral bands (OS, FD and SD), spectral indices were performed. Finally, we constructed the PLSR models between heavy metal concentration and spectrum. The results showed that correlation coefficients between Cu concentration and spectral data were higher than Cd. And that the bands significant correlation ($P<0.05$) with Cu concentration were far more than Cd. Ultimately, we selected 453 variables (442 bands and 11 spectral indices) and 19 variables (18 bands and 1 spectral index) as input variables of PLSR model for Cu and Cd, respectively. Moreover, we found that the Cu and Cd concentrations significantly correlated with spectral variables for (R^2=0.41, RMSE=1.93) and (R^2=0.38, RMSE=0.018) of PLSR models, respectively. These results indicated that they were good predicting models for estimating heavy metal concentration in rice leaves.

Keywords: Hyperspectral remote sensing, Rice leaves, Heavy metal concentration, PLSR models, Quantitatively estimation

INTRODUCTION

With the rapid development of society and economy, discharge of industrial wastes, using pesticide and fertilizer and waste water irrigation, the heavy metal contamination of soil and crops was becoming deteriorative (Zhou et al., 2015). In China, the concentration of Cu, Hg Zn, Cd significantly exceeded their background level in Jiangsu province. Among all the heavy metals, Hg and Cd brought the dominating potential ecological hazard (Zhong et al., 2007). The crops also had been affected inevitably, which would be harmful to human health. Some researchers found that the Yangtze River Delta region was polluted by Cd, Pb, Cr, Cu and Zn. And among them, Cd pollution was the most serious and had the highest conversion coefficients (Xiao et al., 2010). Moreover, these heavy metals were absorbed into roots, stems, leaves and grains of rice. Furthermore, some researchers found that the average concentrations of Cd, Pb and Zn were 20.1, 1234.9 and 305.2 mg kg-1 respectively in Zamfara State, Nigeria, which greatly exceeded the soil background value (Abdu and Yusuf, 2012). In the suburban areas of Varanasi, India, some researchers found that the content of Cd, Pb and Ni exceeded the background values because of waste water irrigation (Sharma et al., 2007). Usually, heavy metals consist of many characteristics (Nagajyoti et al., 2010), such as concealment, lag, irreversibility and long cycle period. At present, the main problem of humanity faced is that we are difficult to monitor heavy metal contamination from large area. However, hyperspectral remote sensing has the ability to do this job (Li et al., 2010), so it is promising in monitoring the heavy metal pollution in large scales.

The conventional methods of measuring heavy metal concentration have the advantages of high precision and low detection limit. However, these methods also have disadvantages, such as trival steps, destroying soil and wasting resources, et al (Rao et al., 2014). Instead, hyperspectral remote sensing has the superiorities of convenience, rapidity and no damage. Application of portable spectrometer, aerial remote sensing and space remote sensing would monitor heavy metal pollution from points to surfaces and qualitative analysis to quantitative analysis (Jing et al., 2015).

Partial Least Squares Regression (PLSR) is a common method for spectral analysis. And it combined the Principal Component Regression (PCR), Multiple Linear Regressions (MLR) and correlation analysis (Martens, 2001). Although PCR can prevent overfitting by internal inspection, yet it is unsatisfied in accuracy and stability. Moreover, its components are created solely by the spectral data (Ergon, 2014); however, the components in PLSR are created by the spectral data and response variables jointly. Moreover, PLS superior to PCR and MLR in many cases. It can be used in these situations (Axelsson et al., 2013): 1) the two groups of variables are large, 2) there is multicollinearity among variables, 3) and the number of observations are less than variables. Additionally, comparing with artificial neural network (ANN) of regarded as ‘‘black boxes’’, regression coefficients of PLSR can show important bands for prediction, which is capable of improving the accuracy of predicting heavy metal concentration (Farifteh et al., 2007).

This study aimed to analyze the relationship between hyperspectral reflectance, spectral indices and heavy metal concentration. We committed to select the optimal variables showed sensitivity to heavy metal concentration (P<0.05). Furthermore, we constructed PLSR models to predict Cu and Cd content of rice leaves using hyperspectral data.

MATERIALS AND METHODS

Introduction of Study Area

The study area is located in the Zhangjiagang city, Jiangsu province of China (Figure 1). It is part of Yangtze River Delta Economic Zone and flat terrain (31°43'-32°02'N, 120°21'-120°52'E). The average annual temperature is 15.2°C and the average annual precipitation is 1039 mm, respectively. The city soil mainly consists of two types, moisture soil and paddy soil. The main crops include rice and cotton. Due to the rapid development of chemical industries and waste-water irrigation, heavy metal pollution of soil is becoming more and more serious (Shao et al., 2006), and which would pose a great threat to crops security and human health.

Collection of Rice Leaf Samples and Spectral Data

The research was carried out in rice paddy field in September, 2017. Sampling sites were randomly distributed in Figure 1. In total, 21 rice leaf samples were gathered in farmland over Zhangjiagang city. Five random samples on each plot were taken and bulked together as one composite sample. Then five spectral measurements of three fully-expanded

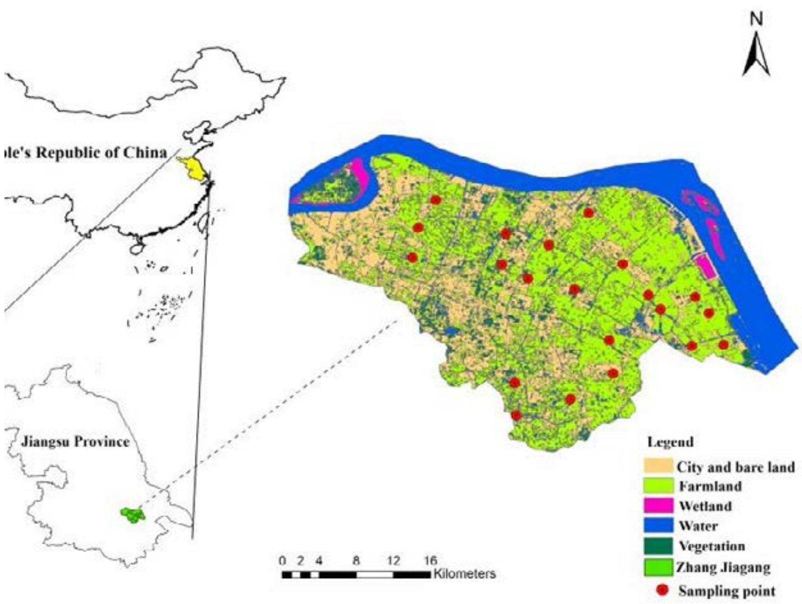

Figure 1. Location of sample points in Zhangjiagang city, Jiangsu province, China.

leaves near top of each bundle were made. Additionally, the spectrum was measured by field portable spectrometer (UniSpec, PP systems, Haverhill, MA, USA.), which measured from 310 nm to 1130 nm. The spectral resolution is 10 nm and sampling interval is 1 nm. In each sample site, the exact coordinates of each composite sample were registered using a GPS. Eventually, rice leaf samples were put into polyethylene bags and were taken to lab for measuring heavy metal concentration.

Measurement of Heavy Metal Concentration in Rice Leaves

Firstly, rice leaf samples were washed by water for three times. Then the rice leaf samples were dried at 60°C for 12 h, and they were sieved through a 0.25 mm nylon mesh to remove big debris. The content of Cu and Cd in rice leaves was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, X2, Thermo Electron Corporation) (Yang et al., 2014). For pretreated samples, 0.2 g was added to dissolve tank to digestion for 20 minutes, simultaneously added 5 ml HNO_3 and 2 ml H_2O_2. Finally, the solution was diluted to 50 ml and the heavy metal concentration of rice leaves were measured when solution became clarified.

Contamination caused by single heavy metal was assessed by enrichment coefficient (P), according to the following formula (Sun and Hou, 2005).

Pi=G/S (1)

Where G is the measured value of metal i and S is the criteria for metal i authorized by national standards (GB2715-2005 and GB15199-1994). Pi with lower value than 1 indicates that rice leaves are not polluted by metal "i" and safe for public health. Otherwise, it denotes that rice leaves are contaminated.

Selection of spectral indices

We selected 16 spectral indices of commonly used. They were listed and calculated as shown in Table 1. Some studies found that the primary effect of Cu and Cd on rice is the corresponding reduction in chlorophyll (Liu et al., 2010), so exception of the VARI and WI, the remaining 14 spectral indices are all related to chlorophyll or pigments. Spectral indices of λ_r, λ_b and λ_y were derived from first derivative reflectance and the others were derived from original reflectance. To improve the accuracy in estimating heavy metal concentration in rice leaves, the correlation analysis between spectral indices and heavy metal concentration would be carried out. Finally, the spectral indices sensitive (P<0.05) to heavy metal concentration were selected as input variables of PLSR models according to previous studies (Liu et al., 2011).

Construction and Validation of PLSR Models

We selected PLS method to construct mathematical models between spectral data and heavy metal concentration. PLS model is based on latent variable decomposition of two blocks of variables, matrices X and Y, which contain spectral data and heavy metal concentration, respectively. And the purpose of the method is to find a small number of latent factors that are predictive for Y and use X efficiently (Mevik, 2007).

Due to the number of available samples were limited, so we used leave-one-out validation procedure to verify the prediction accuracy of the PLSR model. From all n samples within the dataset, n-1 was utilized to build the regression model. This procedure was repeated for all n samples, resulting in predictions for all samples (Kooistra et al., 2001). The parameter used to evaluate the quality of the results was the root mean square error of cross-validation (RMSECV) (Kooistra et al., 2001) given by

$$RMSECV=\sqrt{\frac{\sum(C_m-C_p)^2}{N_c}} \quad (2)$$

Table 1. Summary of spectral indices used in the sensitivity analysis.

Spectral Indices Name	Abbreviation	Formulation	Reference
Red edge position	λ_r	$\lambda_r=\lambda_i$(R'(λ)=MAX(R'(λ∈670-780))	(Chang and Collins, 1983)
Blue edge position	λ_b	$\lambda_b=\lambda_i$(R'(λ)=MAX(R'(λ∈450-550)	(Chang and Collins, 1983)
Yellow edge position	λ_y	λb=λi(R'(λ)=MIN(R'(λ∈550-650)	(Chang and Collins, 1983)
Green peak position	λ_g	λg=λi(R(λi)=Rg)	(Gamon et al., 1992)
Green Normalized Difference Vegetation Index	GNDVI	(R800-R550)/(R800+R550)	(Daughtry et al., 2000)
Normalized Difference Vegetation Index	NDVI	(R800-R670)/(R800+R670)	(Tucker, 1979)
Ratio Vegetation Index	RVI	R810/R560	(Schuerger et al., 2003)
Plant Senescence Reflectance Index	PSRI	(R680-R500)/R750	(Merzlyak et al., 1999)
Optimized Soil-Adjusted Vegetation Index	OSAVI	1.16×(R800-R670)/(R800+R670+0.16)	(Daughtry, et al., 2000)
Photochemical Reflectance Index	PRI	(R570-R531)/(R570+R531)	(Gamon et al., 1992)
Structure-insensitive Pigment Index	SIPI	(R800-R445)/(R800-R680)	(Thomas et al., 1971)
Visible Atmospherically Resistant Index	VARI	(R555-R680)/(R555+R680-R480)	(Gitelson et al., 2002)
Modified Chlorophyll Absorption Reflectance Index	MCARI	(R700-R670)-0.2(R700-R550)×(R700/R670)	(Daughtry et al., 2000)
Water Index	WI	R900/R970	(Thomas et al., 1971)
Vogelmann Red Edge Index	VOGI	R740/R720	(Vogelmann et al., 1993)
MERIS Terrestrial Chlorophyll Index	MTCI	(R750-R710)/(R710-R680)	(Dash and Curran, 2007)

R' means the first derivative reflectance where C_m is the measured value for a rice leaf parameter and C_p is the value predicted by the PLSR model. N_c is the number of samples.

This study selected various pre-treatments, concluding original spectra (OS), First Derivative (FD) and Second Derivative (SD). Then the correlation analysis of processed reflectance (OS, FD and SD), spectral indices and heavy metal concentration were determined. Then the bands and spectral indices with significant correlation ($P<0.05$) indicated that they were sensitive to heavy metal concentration. So that they would be selected as input variables of PLSR models. Then the performance of PLSR models were assessed with coefficient of determination (R^2) and RMSE values (Wu et al., 2005). The construction of PLSR models and cross-validation were achieved in the TQ Analyst (8.3.125, Thermo Fisher Scientific Inc.). The spectral pre-treatments (OS, FD and SD) and all of graphs were done in OriginPro 8. And the correlation analysis of pre-processed reflectance (OS, FD and SD), spectral indices with heavy metal concentration were performed in IBM SPSS Statistics 22 using bivariate related analysis.

RESULTS AND ANALYSIS

Heavy Metal Concentration in Rice Leaves

Cu is an essential element, but high doses can adversely affect plant growth. Excess Cu can inhibit the synthesis of chlorophyll or cause chlorophyll damage, leading to the peroxidation of lipids in photosynthesis biofilms, which affects photosynthesis of plants and leads to a decline in biological yield. Moreover, high amounts of Cu in the soil can affect the normal metabolic function of plant roots and cause plant growth disorders. And it will pose a serious threat to human health when the Cu migrates into brown rice. While Cd is a non-essential element that can be easily absorbed by plants (Pahlsson, 1989). In order to compare the prediction accuracy of these two elements, Cu and Cd were selected as the research objects. As shown in Table 2, average of Cu and Cd concentration in rice leaves was 7.23 mg kg^{-1} and 0.04 mg kg^{-1}, respectively. What's more, average of Cu and Cd concentration in rice leaves were all lower than authorized by national standards (GB2715-2005 and GB15199-1994). And according to enrichment coefficients in Table 1, there were no obvious Cu contamination (Pi=0.72) and Cd contamination (Pi=0.20) in this region.

Table 2. Statistical information of heavy metal concentration in rice leaves for 21 samples.

	Cu	Cd
Min	4.08	0.02
Max	12.2	0.1
Mean	7.23	0.04
Std	2.28	0.02
CV (%)	31.5	40.8
P_i	0.72	0.2

Std: Standard Deviation; CV: Coefficient Variation (unit: mg kg^{-1}); Pi: Enrichment coefficients

Reflectance Spectra of Rice Leaves

There were similar trend among different 21 rice leaf samples in Figure 2(A). As referred above, the spectra were measured from 310 nm to 1130 nm. However, the range of 310-400 nm and 1001-1130 nm were improper to be used to construct models, because they were unstable and had excessive noises (Gomez et al., 2008). Therefore, the bands of 401-1000 nm were used to build PLSR models.

As displayed in Figure 2(B), there was obvious absorption at the wavebands of 490, 570, 610 and 670 nm in the visible regions. And peak values of reflectance spectra were located at the wavebands around 520, 710 nm. Moreover, the reflectance spectra were low in the range of 401~730 nm mainly on account of the absorption effect of chlorophyll (Benhaddya et al., 2016). Then a reflectance spectrum in the whole visible region was obviously lower than the near infrared region because of chlorophyll absorption (Liu et al., 2010). With the influence of cellular structure and water of

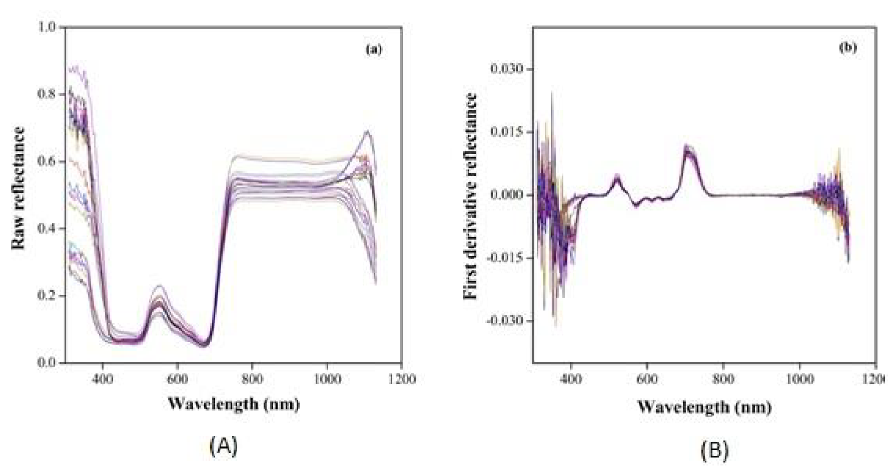

Figure 2. (A) Original reflectance of 21 rice leaf samples; (B) First derivative reflectance of 21 rice leaf samples.

leaves, the spectral reflectance was increased sharply in the range of 730~1130 nm (Algora, 2000).

Correlation Analysis between Heavy Metal Concentration and Hyperspectral Data

The Person's correlation coefficients between pre-processed reflectance (OS, FD, SD) and heavy metal concentrations in rice leaves were shown in Figure 3 and Table 3. The optimal bands were selected as input variables to construct PLSR models according to the significant correlation (P<0.05). Among three pre-processed methods, the original reflectance (OS) had the most wavelengths with significant correlation as shown in Figure 3(A). The significant correlation between Cu concentration and spectral data were all distributed in visible spectroscopy with 272 bands in the OS correlogram, and main in visible spectroscopy with 94 bands in the FD correlogram and 76 bands in the SD correlogram.

Obviously, correlation coefficients between Cd concentration and pre-processed reflectance (OS, FD, SD) were lower than Cu and no bands reach the extremely significance level (P<0.01) as shown in Figure 3(B) and Table 3. And that there were no bands reach significance level (P<0.05) in original reflectance form Table 3. The correlation coefficients between Cd concentration and spectral data with P<0.05 were main in visible spectroscopy with 8 bands in the FD correlogram and 10 bands in the SD correlogram.

As displayed in Table 3, the maximum correlation coefficients of pre-processed reflectance (FD and SD) were larger than the original reflectance (OS), and the minimum correlation coefficients of pre-processed reflectance (FD and SD) were lower than the original reflectance (OS). These indicated that the pre-processing techniques (FD and SD) could remove redundancy information and made some subtle information clear in the spectral to improve the accuracy of PLSR models (Wang and Ding, 2010).

Correlation coefficients between spectral indices and heavy metal concentrations were shown in Table 4. Spectral indices demonstrated a wide range of correlations with Cu (-0.47--0.60) and Cd (-0.46--0.30). However, the correlation coefficients between spectral indices and Cd concentration were all low and only 1 spectral index reached significant correlation level. While among 16 spectral indices, the correlation coefficients of 11 spectral indices reached 0.05 levels for Cu concentration. Heavy metal toxicity in rice leaves was assessed by the decrease in chlorophyll and protein contents (Hsu and Kao, 2004), so exception of WI and VARI, the others are related to chlorophyll or pigments.

PLSR Models for Predicting Cu and Cd Concentration

Eventually, we selected 453 variables (442 bands and 11 spectral indices) and 19 (18 bands and 1 spectral index) variables as input variables of PLSR models for Cu and Cd,

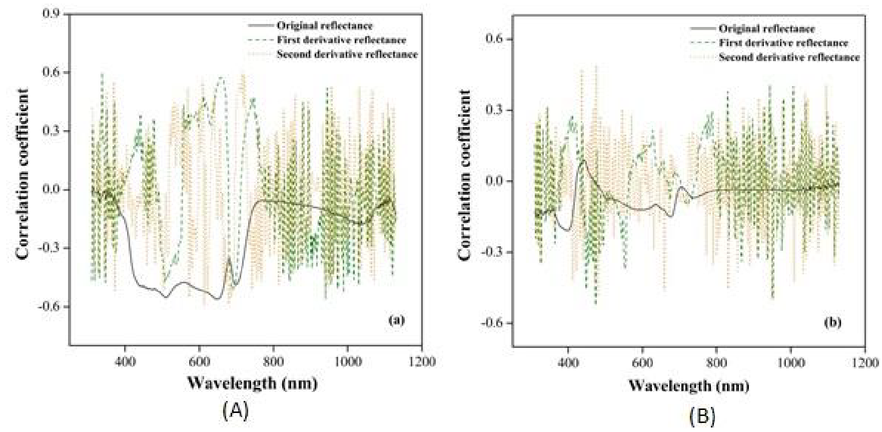

Figure 3. Correlation coefficient between pre-processed reflectance (OS, FD, SD) and heavy metal concentrations of Cu (A) and Cd (B) in rice leaves.

Table 3. Correlation analysis of pre-processed reflectance and heavy metal concentration.

Heavy metals	Spectral pre-processed	Maximum correlation band (nm)	Correlation coefficient	Minimum correlation band (nm)	Correlation coefficient
Cu	OS	315	0.018	646, 647, 648	-0.562**
	FD	338	0.602**	940	-0.560**
	SD	715	0.604**	612	-0.589**
Cd	OS	439	0.093	399	-0.208
	FD	943	0.408	474	-0.525*
	SD	477	0.489*	948	-0.501*

*means correlation is significant at the 0.05 level (P<0.05); **means correlation is significant at the 0.01 level (P<0.01).

Table 4. Correlation coefficients between spectral indices and heavy metal concentration.

	λ_r	λ_b	λ_y	λ_g	GNDVI	NDVI	RVI	PSRI	OSAVI	PRI	SIPI	VARI	MCARI	WI	VOGI	MTCI
Cu	0.60**	0.45*	0.04	-0.13	0.53*	0.48*	0.52*	0.52*	0.3	-0.24	0.32	-0.46*	-0.47*	0.45*	0.53*	0.52*
Cd	0.02	0.26	-0.18	-0.46*	0.08	0.14	0.11	-0.07	0.08	-0.3	-0.21	0.1	-0.05	0.03	-0.05	-0.07

*Correlation is significant at the 0.05 level (P<0.05);**Correlation is significant at the 0.01 level (P<0.01).

respectively. Comparison of measured concentrations against the predicted concentrations of Cu and Cd using PLSR models were shown in Figure 4(A) and 4(B), respectively. The criteria of optimal PLSR models were higher R2 closed to 1 and lower RMSE closed to 0 (Wu et al., 2005). We can see from Figure 4, the Cu and Cd concentrations significantly correlated with spectral variables for (R^2=0.41, RMSE=1.93) and (R^2=0.38, RMSE=0.018), respectively, which were all indicative of good predicting models. The Cu concentration had higher prediction accuracy than Cd, maybe because the Cu is essential element and Cd is unessential element of crops and the crops absorbed more Cu concentration. The interaction between Cu and Cd is complex and has an effect on their individual functions (Pahlsson, 1989). We can use the PLS models with high prediction accuracy to predict the Cu and Cd concentrations of rice leaves if we are sure about that the rice leaves are stressed by a certain heavy metal concentration. To some extent, prediction accuracy of Cu and Cd concentrations still would be improved in the future, some appropriate methods including gathering more rice leaf samples, grinding and sieving rice leaves by more aperture meshes, and covering many more types of rice (Ihedioha et al., 2016), could be taken to improve the stability and accuracy of prediction models.

DISCUSSION

Existed Tissues

The quantitatively prediction of rice leaves contamination used hyperspectral data still face many other problems, such as leaf roughness and moisture, sun zenith, low signal noise ratio (SNR), atmospheric attenuation, pixel mixing, (Mo et al., 2005). And PLS models for quantitatively estimating the heavy metal concentration of rice leaves still exist problems, such as low prediction accuracy. Moreover, it should be noted that the results of this study were only valid for the rice leaves types represented in the investigated region. The Further studies are required to examine the usefulness of the reflectance spectra on other regions and devote to enhancing the prediction accuracy.

In order to protect the crops from polluting by heavy metals, the following studies should pay more attention to predict the heavy metal concentration of rice grains and build the warning systems of heavy metal contamination from source. Moreover, effects on the prediction accuracy of heavy metal concentration through this method should be further studied and prediction accuracy of PLS models still should be improved.

Efforts to improve the Prediction Models

Some researchers found that selecting the optimal bands for prediction models was a good method to improve the prediction accuracy (Huang et al., 2010). For example, comparing with the full bands, the optimal bands selected by genetic algorithm could achieve better prediction result, the proposed algorithm in the study can be useful in the fast preprocessing of hyperspectral data (Zhang et al., 2009). A researcher constructed PLS models to assess contaminant metals (Ni, Cr, Cu, Hg, Pb, Zn) and As in the suburban soils of the Nanjing area, finally, he calculated the PLSR regression vector of the final model for Ni, it shows the important

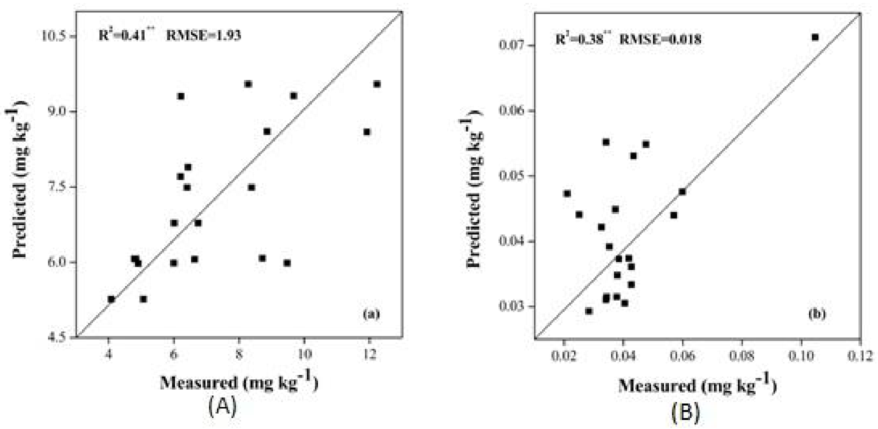

Figure 4. Plot of measured concentrations against the predicted concentration of Cu (a) and Cd (b) of rice leaves using PLSR models.

bands for predicting Ni : 0.52, 0.90, 1.42, 1.90, and 2.0–2.2 µm (Wu et al., 2005).

This study selected optimal bands and spectral indices with significant correlation between heavy metal concentration and pre-processed reflectance (OS, FD and SD), spectral indices. Comparing with full bands, the PLS models constructed by optimal bands and spectral indices had higher prediction accuracy. Exception of that, some appropriate methods, including gathering more rice leaf samples, grinding and sieving rice leaves by more aperture meshes, and covering many more types of rice (Ihedioha et al., 2016), would help to improve the stability and accuracy of prediction models.

The Focus of Following Studies

There were many studies focused on soil heavy metal contamination using hyperspectral remote sensing (Yan and Qing-Tian, 2007). However, for food security and people health, paying more attention to crops heavy metal contamination would be a more important research direction (Kalita et al., 2010). This paper verified that it's potential to use hyperspectral data to quantitatively estimate heavy metal concentration of rice leaves, but the accuracy wasn't very high. So we should make effort to improve the prediction accuracy through using more suitable methods to select optimal bands, such as PLS regression vector method and genetic algorithm method (Tomczak and Kamiński, 2012). So for these applications where we have to deal with relatively noisy spectra, the application of wavelength selection could be a promising pre-treatments method.

The threshold value of heavy metal contamination also is an important problem (Moreno et al., 2009). In order to prevent the crops contaminated by heavy metals in advance, it is vital to summarize that when the amount of heavy metal is reached, the spectrum changes. Therefore, these techniques is promising to truly achieve the target that monitoring heavy metal pollution in large area and accurately mapping the heavy metal concentration in crops.

CONCLUSION

Based on the field hyperspectral reflectance data and heavy metal concentration of rice leaves, this study established the PLS models to quantitatively estimate heavy metal concentration in rice leaves using hyperspectral data.

In Zhangjiagang city, Cu and Cd content of rice leaves were low, and the enrichment coefficients indicated that there were no obvious Cu contamination (Pi=0.72) and Cd contamination (Pi=0.20) in this region. Correlation coefficients between Cu concentration and pre-processed reflectance (OS, FD and SD) were higher than Cd, and that the bands with significant correlation ($P<0.05$) for Cu concentration were far more than Cd concentration. Among 16 spectral indices, 11 spectral indices and 1 spectral index were significantly correlated with Cu concentration and Cd concentration, respectively. 453 variables (442 bands and 11 spectral indices) and 19 variables (18 bands and 1 spectral index) were selected as input variables of PLS model for Cu and Cd, respectively. Finally, we found that the Cu and Cd concentrations significantly correlated with spectral variables for (R^2=0.41, RMSE=1.93) and (R^2=0.38, RMSE=0.018) of PLS models, respectively. These results indicated that they are good predicting models for predicting heavy metal concentration in rice leaves.

In summarize, hyperspectral data and PLSR models could be employed as an alternative solution to quickly assess heavy metal concentration in rice leaves.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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