The Organochlorine Pesticide Residues of Mesona Chinensis Benth by Near Infrared (NIR) Spectroscopy and Chemometrics

Yao Wanqing*, Zhang Shuhui, Guo Zhihui, Gu Bingming

School of Chemistry and Environment, Jiaying University, Meizhou, Guangdong, 514015

Abstract: Pesticide residue is a major problem in food safety. It is particularly important to detect pesticides in fruits and vegetables quickly and effectively. The near infrared spectroscopy (NIR) technique was explored as a technique to predict organochlorine pesticides in Mesona chinensis Benth. Fourty-three samples were sacaned powder in a Antaris II FT-NIR. Samples were split into two sets, one set used as calibration (n=35) where the remaining samples (n=8) were used as validation set. The hexachlorocyclohexane (BHC), dichlorodiphenyltrichloroethane (DDT) and organochlorine residues were determined by gas chromatography as the reference ratio. The prediction model of total BHC, DDT and organochlorine of mesona chinensis benth was established by partial least square (PLS) method, which were optimized by the optimal spectral band range, spectral pretreatment and regression. Moreover, the prediction ability of the model was verified by validation set. The models gave the results that RMSEC, R_C, RMSEP and R_P of BHC, DDT and organochlorine residues respectively were 0.996, 0.9384, 0.151 and 0.9964; 1.21, 0.9889, 0.409, 0.9852; 2.21, 0.9423, 0.481, 0.9953. The relative errors of the predicted values are within 0.68% ~ 0.38% and - 0.34% ~ 6.88% and - 0.46% ~ 0.58% of the total validation set. It can be seen that the three models have good prediction effect, high precision and high stability, that is, NIR can be used as a simple, non-destructive, reliable and rapid method for the determination of organochlorine pesticide residues in mesona chinensis benth.

1 Introduction

Mesona chinensis Benth (Chinese Mesona), also known as *xiān cǎo (仙草), xiānrén cǎo (仙人草),* is an annual herb of the genus Senna in the Lamiaceae family, which is widely distributed in Guangdong, Fujian and Yunnan province in China[1]. Mesona chinensis Benth mainly contains polysaccharides, phenolic acids and flavonoids[2]. According to Traditional Chinese Medicine, Mesona chinensis Benth is cool and cold, tastes sweet and light and astringent, and has the effect of clearing heat and dampness, eliminating summer heat, quenching thirst and water, and removing heat toxicity. Mesona chinensis Benth is a natural high-grade and dual-use plant resource for medicine and food, with an extremely high nutritional value and potential health care effects[3]. However, its growth is greatly influenced by environmental factors. In order to preserve yield and increase income, growers choose pesticides that are highly efficient and low-cost, or even banned. The frequent application of pesticides resulted in exceeding standard rate of pesticide residues in Mesona chinensis Benth[4]. Although high-residue organochlorine pesticides have been banned from production and use for up to 30 years, its satble chemical properties, long half-life, strong lipid solubility, and lack of easy degradation have resulted in serious residues in the soil, causing widespread contamination and ultimately endangering human health and trade exports [5]. According to the data from the food safety and quality supervision department, pesticide residues in vegetables and leaves in China have seriously exceeded the national standard, which has seriously harmed people's health[6]. The traditional pesticide content determination method requires a tedious and complex pretreatment process for the sample, which may damage the characteristic information of the sample. Furthermore, organochlorine pesticides commonly used in Mesona chinensis Benth mainly include benzene hexachloride (BHC) and dichlorodiphenyltrichloroethane (DDT). The analysis of these compounds by gas chromatography (GC), which requires sample preparation, specific analytical equipment and is time-consuming. For these reasons, it is extremely urgent to develop a pesticide residue measurement technology with high environmental protection, low cost and less detection time.

The Near Infrared Spectroscopy (NIR) technique comes forward as a rapid, non-destructive, large quantities, high environmental protection and low cost detection applications in technique rapidly in China in recent years[7]. In most of published workds so far, little attention has been given to the quantification of organochlorine pesticides in natural plant. Therefore, the aim of the present work was to examine the potential of NIR spectroscopy to determine the concentration of organochlorine pesticides in Mesona chinensis Benth

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* Corresponding author: 627744294@qq.com

from Guang Dong.

Objective of this research is to develop and evaluate new analytical methodologies for the fast authentication of the total organochlorine residues, BHC and DDT in Chinese Mesona. Several quantitative analysis models were established by combining NIR and chemometrics methods. Via comparing and analyzing the range of different modeling characteristic bands and spectral pretreatment methods, the optimal NIR spectral models of pesticide residues are established. The above methods will attempt to identify organochlorine pesticides in samples and provide a direct, fast, simple, cheap and green alternative to chromatography for general applications, while reducing solvent consumption and waste generation.

2 Materials and methods

2.1 Experimental materials

Forty-three Mesona chinensis Benth samples were dyred, removing impurities and root, leaving stems and leaves. The samples were split into two sets, 35 samples were used in calibration, where the remaining 8 samples used as external validation set. All the samples were grown at Guangdong and harvested during summer and autumn in different years (2018-2019).

2.2 Experimental reagents

Petroleum ether (60-90 ℃), sulfuric acid, sodium chloride, anhydrous sodium sulfate, dichloromethane, acetone, all were analytically pure.

2.3 Experimental apparatus

Antaris II Fourier transform infrared spectrometer (FT-NIR), Thermo Fisher Scientific (China) Co., Ltd; GC-2010 gas chromatograph, Shimadzu Enterprise Management (China) Co., Ltd; low-speed benchtop centrifuge, Jiangsu Jinyi Instrument Technology Co., Ltd; CNC ultrasonic cleaner, Kunshan Ultrasonic Instrument Co., Ltd; rotary evaporator, Shanghai Yarong Biochemical Equipment and Instrument Factory; ultrapure water machine, Sichuan Excellence Water Treatment Equipment Co.

2.4 Experimental methods

2.4.1 Gas chromatography analysis

(1) Gas chromatographic conditions

Chromatographic conditions: column: The gas chromatograph equipped with a splitl/splitless injector, an electron capture detector (63 Ni-ECD) and an quartz capillary column (HP-7,30 m×0.32 mm, 0.25 μm). The temperature of the injector and detector is set to 230 ℃ and 300℃.The oven temperature was initially held at 100 ℃ for 5 minutes, then programmed to rise to 220℃ at 10 ℃ per minute and then programmed to rise to

250 ℃ at 8 ℃ per minute. Finally maintained at 250 ℃ for 10 minutes. Carrier gas nitrogen (purity >99.999%) has an initial flow rate of 1 mL per minute and the makeup flow rate was 58.5 m Lper minute. The analyses were performed by injecting 1μL of sample in splitless mode (15 mL/min) under the constant pressure mode.

Standard curve plotting: Petroleum ether (60-90 ℃) was used as the solvent to configure the standard solutions of nine organochlorine mixed pesticide controls at a concentration of 0.01 mL/mL. According to library searching or structure elucidation, the standard working curve was drawn by the normalization method of peak area.

Determination of samples: Petroleum ether (60-90 ℃) was used as the solvent to configure the tested samples at 0.2 mL/mL and diluted 5 times. The gas chromatographic conditions were the same as those used for the standard curve, and 35 samples of Mesona chinensis Benth were determined. According to the peak area of each indicator measured, the conversion was calculated on the standard working curve to obtain the residues of nine organochlorine pesticides in the samples.

(2) Preparation of mixed solution control

0.5 mL of total hexhexane (BHC: α-BHC, β-BHC, γ-BHC, Δ-BHC), DDT (DDT: P, P '-DDE, P, P '-DDD, O, P '-DDT, P, P '-DDT) and pentachloronitrobenzene (PCNB) mixed pesticide reference were measured and placed in a 50 mL volumetric flask Dilute with petroleum ether (60 ~ 90℃) to the marking line, shake well, that is, the mixed pesticide control solution.

(3) Preparation of the test solution

Referring to the 2015 version of the *Chinese Pharmacopoeia* method[8], take test article of Mesona chinensis Benth, crushed into powder (sieve No. 3). Precise weighing 2g, put it in a 100 mL stopper conical flask, add 20 mL water and soak overnight, Precisitely add 40 mL acetone for ultrasonic treatment for 30 minutes, let it cool down, and then add about 6 g sodium chloride Afterwards, precisely add 30 mL dichloromethane, ultrasound for 15 min, and let stand to stratify The organic phase was quickly transferred into a 100 mL stopper conical flask containing an appropriate amount of anhydrous sodium sulfate and placed for 4 hours. Measure 35 mL precisely, concentrate under reduced pressure on a water bath at 40°C until nearly dry. Add a small amount of petroleum ether (60-90°C) as before repeatedly until dichloromethane and acetone are removed. Dissolve with petroleum ether (60-90°C) and transfer to a 10 mL stoppered graduated centrifuge tube. Add petroleum ether and dilute precisely to 5 mL, carefully add sulfuric acid 1 mL, shake for 1 min, and centrifuge (3000 rpm) for 10 min. The supernatant was precisely measured by 2 mL, connected to a rotary evaporator, and the solution was concentrated to an appropriate amount at 40℃ and precisely diluted to 1 mL. That is, all.

2.4.2 Establishment of NIR quantitative analysis model

Near infrared (NIR) spectra were recorded on a Antaris II Fourier transform infrared spectrometer equipped with an integrating sphere diffuse reflectance module, from 10000 to 4000 cm^{-1}. For each sample, 64 scans were made with a spectral resolution of 8 cm^{-1} and 4 times gain, then averaged. Samples were temperature equilibrated at 24^{0}C (approximately 3 min) in the instrument before scanning. Ominic software (Thermo Electron Corporation) was used for spectrometer configuration, control, and data acquisition. Three parallel samples were taken for each sample (reloading). Three NIR spectra were collected for each sample, 3 for each sample, for a total of 129 spectra. 105 spectra were used as the calibration set and 24 spectra were used as the validation set.

The quantitative models of organochlorine residue in Mesona chinensis Benth were established by TQ spectral analysis software (Thermo Electron Corporation). The precision measurement standards of the optimal quantitative analysis models mainly include the calibration correlation coefficient R$_C$, the prediction correlation coefficient (R$_P$), the cross-validation correlation coefficient (R$_{CV}$) and the calibration mean square deviation (RMSEC), the prediction mean square deviation (RMSEP) and the cross-validation mean square deviation (RMSECV) of the model, the performance index of the model, and the prediction effect of the model.

2.4.3 Application of the quantitative model

Samples No. 4, No. 17, No. 18, No. 20, No. 25, No. 26, No. 29 and No. 33 (randomly selected), which were not involved in the modeling, were used as the validation sample set and imported into the chemometric model built in 4.2 for external detection. The residues predicted by the model were compared with the actual residues determined by GC to test and evaluate the prediction performance of the built model.

3 Results and Discussion

3.1 Gas chromatography analysis

On the basis of the current Green Industry Standard for Import and Export of Medicinal Plants and Preparations and 2015 edition of Chinese Pharmacopoeia limit indexes, the residues of total BHC, total DDT and PCNB shall not exceed 100 mg/g[8-9].

The residues of total BHC (α-BHC, β-BHC, γ-BHC, δ-BHC), total DDT (p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT) and PCNB of nine organochlorine pesticides in 35 samples were determined and calculated by gas chromatography combined with reference methodology. The results in table1 showed that the total residues of organochlorine pesticides in the samples were within the standard range, which the total amount is ranged from 0 to 34.81 mg/g. Besides, for the studied compounds, the residues of total BHC, total DDT and PCNB were ranged from 1.28 mg/g to 22.93 mg/g, from 0.14 mg/g to 24.74 mg/g and from 0.50 mg/g to 2.15 mg/g, respectively. The residual amount of total BHC and total DDT in samples based on GC analysis were high, while the residual amount of PCNB residues was low In this reason, that is why PCNB residues were out of discussion here.

3.2 Near-infrared spectra

Fig. 1 shows the NIR spectra of 43 Chinese Mesona samples without any data pre-treatment in the range between 10000 to 4000 cm^{-1}. The absorption band at 4660 cm^{-1} corresponds to the C-C and C-H stretching vibration, while strong bands at 5270 cm^{-1} corresponds to the C-Cl absorption of organochlorine compound. Absorption bands at 5790 cm^{-1} are related with first order octavo absorption due to C-H stretching vibrations of methyl groups attached to an aromatic, 6950 cm^{-1} related with synchronous frequency absorption of cyclohexane C-H stretching vibration[10]. The measurement results are affected by external factors such as the density of the samples in the sample cup, the shape and size of the sample during the collection of the spectra. Given the results, this comes as no surprise that reproducibility of the spectra was mediocre.

Table 1 The Organochlorine Pesticide Residues of Mesona Chinensis Benth

sample	Amount of residue μg/kg				sample	Amount of residue μg/kg			
	Total BHC	Total DDT	PCNB	total organochlorine pesticides		Total BHC	Total DDT	PCNB	total organochlorine pesticides
1	5.83	1.14	—	6.97	19	5.08	0.14	—	5.22
2	3.39	11.82	—	15.21	20	7.59	1.67	—	9.26
3	1.28	—	—	1.28	21	2.26	0.39	—	2.65
4	8.77	1.46	—	10.23	22	9.57	24.74	0.50	34.81
5	6.45	—	—	6.45	23	22.93	1.45	—	24.38
6	1.91	—	0.95	2.86	24	17.98	14.86	—	32.84
7	4.03	—	2.26	6.29	25	13.75	4.75	—	18.50
8	7.19	0.41	—	7.60	26	9.23	1.30	—	10.53
9	—	0.41	—	0.41	27	—	8.96	—	8.96
10	3.02	0.30	—	3.32	28	—	6.80	—	6.80

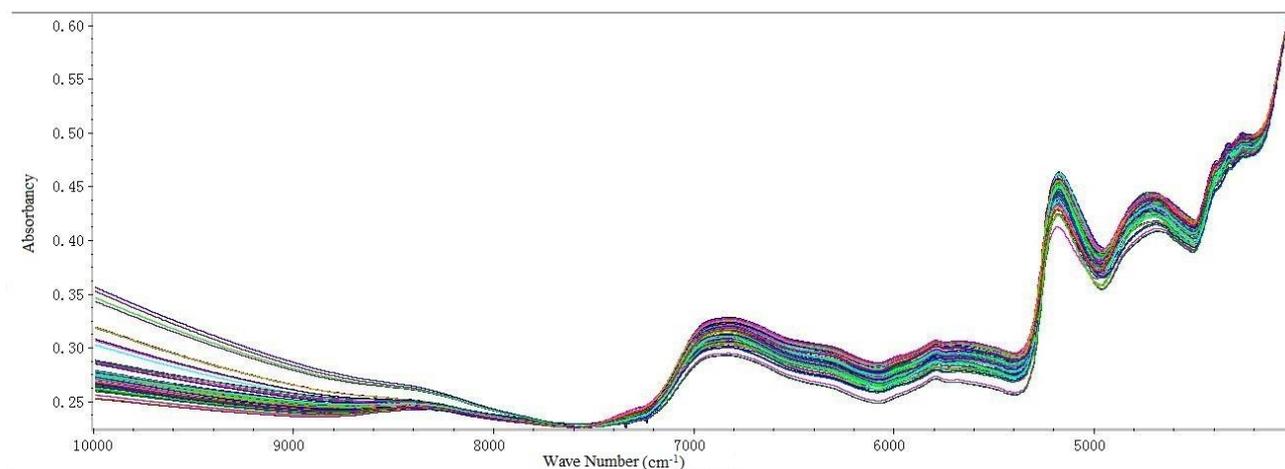| 11 | 3.40 | 1.04 | — | 4.44 | 29 | 2.24 | 3.99 | — | 6.23 |
12	—	0.73	—	0.73	30	1.48	5.50	—	6.98
13	285.9	0.73	—	29.32	31	4.92	7.22	—	12.14
14	—	—	—	—	32	7.09	7.05	0.89	15.03
15	1.86	—	—	1.86	33	9.62	3.38	—	13.00
16	—	4.16	—	4.16	34	16.09	0.45	2.15	18.69
17	1.91	29.21	—	31.12	35	8.97	1.87	1.11	11.95
18	4.88	0.52	—	5.40					

annotation："—" not detected

Fig. 1 Near infrared spectra of all samples

3.3. Selection of the best model for Chinese Mesona authentication

Several chemometric modes were assayed employing different spectral regions and data pre-treatments, including multiplicative scatter correction (MSC), Savitaky-Golay filter (SG) first (FD) and second (SD) derivative, and Norris-derivative filter (ND), standard normal variation (SNV) mean centering (MC) and autoscale, together with combinations between them.

The optimal modeling characteristic bands were selected, combined with the optimal spectral pretreatment method, and the partial least square method (PLS) was adopted to establish the quantitative analysis models of total organic chlorine (Figure 2), DDT (Figure 3) and BHC (Figure 4).

The relationship between the model predicted values of total organochlorine residues, total DDT and BHC and the measured values of GC is shown in Figure 2, Figure 3 and Figure 4, respectively. Table 2 shows the descriptive statistics for the organochlorine pesticide residue parameters used for either the calibration or the validation set. The best coefficient of determination in calibration (R_c) and the lowest standard error in cross validation (SECV.) were obtained after applying the Savitzky Golay (SG) and second (SD) derivative and using the optimal band range by PLS method. Regarding data shown, for total organic chlorine the R_c was 0.9423 (RMSECV: 0.481); for total DDT the R_c was 0.9887 (RMSECV: 0.426); for total BHC the R_c was 0.9384(RMSECV: 0.151).
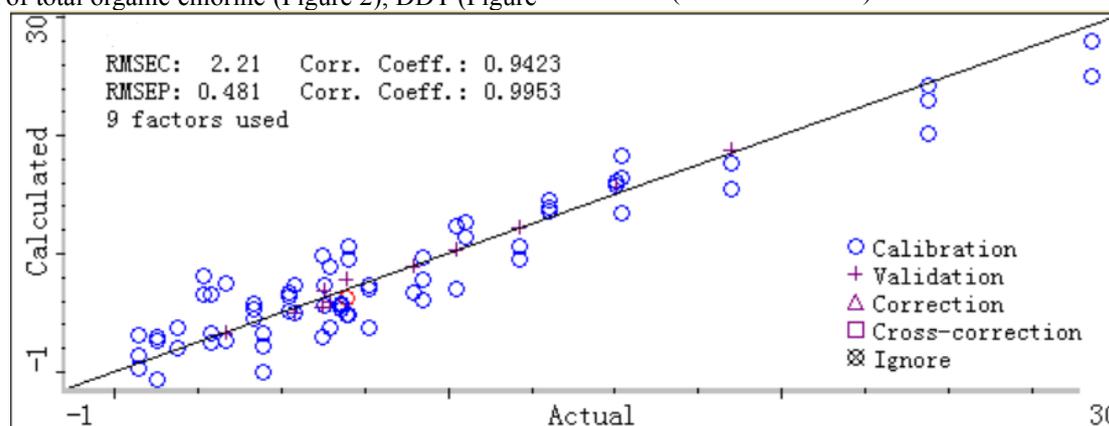

Figure 2 NIR model of total organochlorine residues
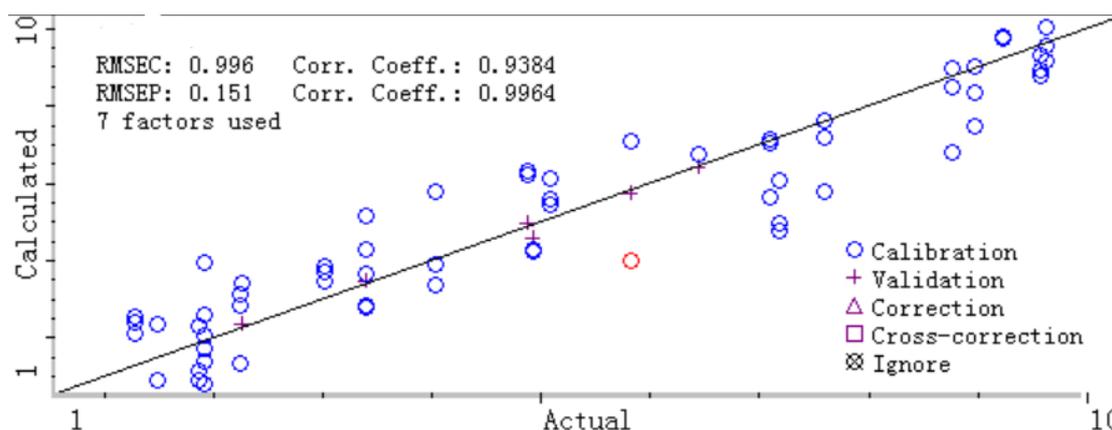
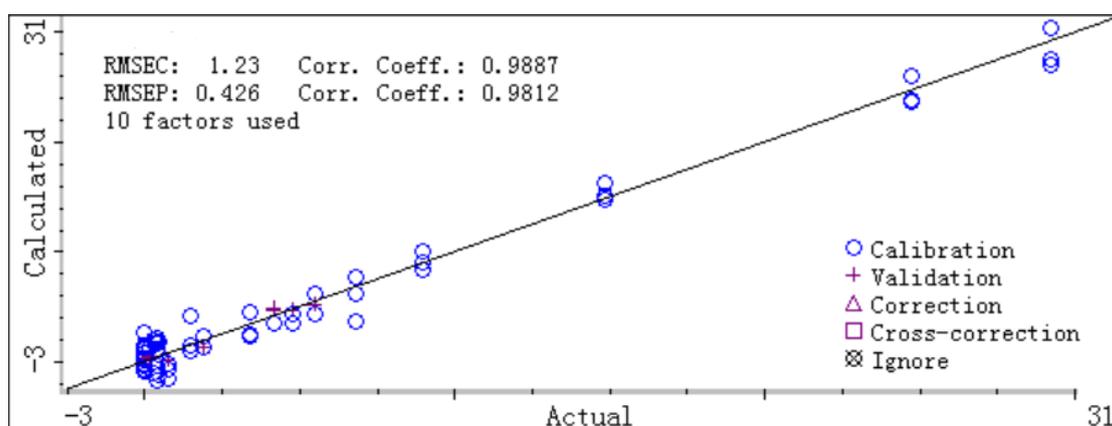**Figure 3** NIR model of total BHC residues

Figure 4 NIR model of total DDT residues

Table 2 Calibration and cross validation statistics for organochlorine pesticide residues on chinese mesona herb samples

Parameter	Preprocessing method	Modeling band（cm⁻¹）	RMSEC	R_C	RMSEP	R_P	RMSECV	R_{CV}	Performance index
Total BHC	Second order derivative (SG filtered dry)	5289.57 - 4735.18 5635.13 - 5329.63 6356.23 - 5823.54 7467.70 - 6984.22	0.996	0.9384	0.151	0.9964	2.58	0.5296	98.0
Total DDT	Second order derivative (SG filtered dry)	5199.95 - 4086.02	1.23	0.9887	0.426	0.9852	4.64	0.8292	94.9
Total organochlorines	Second order derivative (SG filtered dry)	4961.73 - 4449.88 5401.71 - 5262.43 6095.53 - 5879.11	2.21	0.9423	0.481	0.9953	5.43	0.6645	94.9

3.4. Prediction capability of NIR models

To evaluate the predictive capability of the PLS models, a set of validation samples (n=8) were introduced into the developed PLS models. According to the results obtained by GC, table 3 summarizes the parameter range of variation for reference values, predicted values and predicted values. As it can be seen, compare to relative deviation for total DDT, better prediction for DHC and total organochlorines in prediction, with a precision range spanning values between -0.68% and 0.38%, -0.46 and 0.58%, respectively. In short, it can be concluded that the developed PLS models provides a good prediction capability for total DDT, DHC and total organochlorines.

Table 3 Prediction results of NIRS models

Parameter	Range of variation of reference values (mg/g)	Range of variation of predicted values (mg/g)	Range of deviation values (mg/g)	Relative deviation (%)
Total DHC	1.91～13.75	1.36～10.06	-9.82～1.46	-0.68～0.38
Total DDT	1.30～29.21	2.10～30.63	-2.42～5.47	-0.34～6.88
Total organochlorines	5.40～31.12	2.94～20.95	-14.43～3.61	-0.46～0.58

4 Conclusions

The development and evaluation of chemometric Near Infrared spectroscopy based models has evidenced the possibility of prediction of organochlorine pesticides in Mesona chinensis Benth through the use of a PLS model established from well characterized samples by a reference methodology and the appropriate chemometric treatment. As shown and from the Green Analytical point of view, the use of infrared spectroscopy together with chemometric treatment of data reduces the use of reagents and waste generation when compared to the reference methodology, thus these methods can be considered as environmentally friendly alternative methodologies for determination of the residues of total DHC, total DDT and total organochlorines in mesona chinensis benth sample.

Acknowledgement

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