



Simultaneous determination and exposure assessment of six common pesticides in greenhouses through modified QuEChERS and gas chromatography–mass spectrometry

Tingting Ma^{1,2} · Shengli Li³ · Yuqi Li⁴ · Xiuhua Li² · Yongming Luo²

Published online: 3 August 2020

© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Owing to the excessive use of pesticides in greenhouses, soils and vegetables herein are more heavily contaminated than those in non-facility cultivation. Based on a developed QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, four methods of sample pretreatment were compared for the monitoring of six pesticides (bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil, and pyriproxyfen) commonly used in greenhouses of Hubei Province. Using different extraction strategies and purification methods with different solid phases and eluent mixtures, an optimised method for residual analysis in soil and plant samples via gas chromatography–mass spectrometry was determined. The detection limits of the method for the six pesticides ranged from 0.1 to 0.8 µg/kg in three spiked matrixes and average recovery rates of 80.6–118.3% were achieved for single target pesticides at spiked concentrations of 0.1, 1.0, and 10 mg/kg with standard deviations of 2.4–8.4%. Health risk assessment was conducted through the analysis of greenhouse soil and vegetable samples at Pengdun, a typical agricultural greenhouse facility in Jingmen, Hubei Province, central China. Based on the quantification of the six target compounds in four vegetable types, only pyriproxyfen in tomato exceeded the Canadian and WHO/FAO maximum residue limits. No chronic, acute, non-carcinogenic, or carcinogenic health risks to farmers working in the greenhouses or consumers of the vegetables were observed for all age groups in the study area. Residual results showed that pyridaben is the most often quantified pesticide in all greenhouses of the study area. Pesticide combinations, including bifenthrin, chlorfenapyr, and pyridaben, were similar for cucumbers and eggplants. Nevertheless, simultaneous detection of multiple contaminants and health risk assessment are required because combined contamination may lead to much higher toxicity to both workers and consumers with the emergence of pesticide resistance and the development of new pesticide products. The quantification limits of the developed method for the simultaneous analysis of the six target pesticides showed high reliability, accuracy, effectiveness, and applicability.

Keywords Carcinogenesis · Combined contamination · Greenhouse vegetables · Residual risk · Solid-phase microextraction

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00477-020-01844-7>) contains supplementary material, which is available to authorized users.

✉ Shengli Li
11709@hbuas.edu.cn

Tingting Ma
ttma@issas.ac.cn

Yuqi Li
yuqi.li@hbuas.edu.cn

1 Introduction

Pesticides are often composed of heterogeneous chemical groups containing active and inert ingredients to combat pests or diseases in order to increase crop yield and

Xiuhua Li
xhli@issas.ac.cn

Yongming Luo
ymluo@yic.ac.cn

Extended author information available on the last page of the article

productivity (Cayir et al. 2019). Biological control is a more sustainable option but the incidence of pests and diseases in greenhouses is very high under particular climatic conditions and high crop densities, making chemical control more feasible for growing high-quality agricultural products (Rincón et al. 2018). However, foliar-sprayed pesticides can adhere to the leaves of target plants or infiltrate the soil and accumulate in vegetables via soil and/or foliar absorption and therefore lead to concerns about food safety (Razzaghi et al. 2018). The current Chinese annual usage of pesticides in agriculture is 1.75 million tonnes and the quantity of pesticides used per hectare is 2.5 times the world average (Sun et al. 2016). Given the disadvantageous conventional pesticide formulation, more than 90% of pesticides are released into the environment and reside in agricultural products (Zhao et al. 2017). In 2015, new control measures of pollution from non-point agricultural sources were introduced in China to reduce the application rates of pesticides (Guo and Wang 2018). The contamination levels, distribution, and risk evaluation of organochlorine pesticides have been of concern to the public for many years (Tang et al. 2015; Fang et al. 2017), but the application strategies and risks of common pesticides used in greenhouses still need more attention. A policy banning highly toxic and restricting conventional pesticide use was implemented at the start of the year 2000; however, pesticide toxicity incidents are still reported, especially in intensive greenhouse systems (Li et al. 2018). Residual pesticides are known carcinogens and toxins, and monitoring of residual pesticide levels is important to ensure the maintenance of food safety and good agricultural practices (Saidi et al. 2017).

Based on sales data collected in Jingmen and our preliminary experiment, the following six pesticides, bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil, and pyriproxyfen, were selected as the targets for greenhouses in this area. Bifenthrin kills a broad spectrum of foliar pests in aubergine (eggplant), tomato, and other vegetables (Gupta et al. 2008). The application of bifenthrin has been supported by the Chinese government since 2002 as a substitute for more toxic compounds such as carbofuran, phorate, and isofenphos-methyl (MOA China 2007). Chlorfenapyr is a long-lasting insecticidal net that was recently recommended by the WHO Pesticide Evaluation Scheme, and it is applied to vegetables such as Chinese cabbage, pak choi, and cauliflower in China (Camara et al. 2018). Acting on the insect nervous system, lambda-cyhalothrin is especially used in tomato cultivation (Mathirajan et al. 2000). Pyridaben has been used effectively against several groups of phytophagous mites and piercing-sucking insect pests on vegetables and fruits (Ínak et al. 2019). Pyrimethanil is very efficient against resistant fungal strains and has therefore been used extensively for

pre- and post-harvest protection of crops such as apple, banana, carrot, citrus, grape, melon, onion, potato, strawberry, and tomato (Araújo et al. 2014). It was in the recommended list of highly effective and low-toxicity pesticides of the Ministry of Agriculture of the People's Republic of China in 2017. Pyriproxyfen is an insect growth regulator with juvenile hormone analogue properties against a range of arthropods, and it has been in use since its introduction to the agrochemical market in the early 1990s (Sihuincha et al. 2005). The application of these six pesticides in conjunction with each other in many large greenhouse facilities may lead to multiple sources of residues and combined contamination problems in both the environment and vegetables. However, thus far, most studies have focused on individual qualitative source identification but less on health risks (Ren et al. 2019). In this context, the method described as quick, easy, cheap, effective, rugged, and safe (QuEChERS) has the advantages of excellent capacities of recovery, enrichment and extraction of the analyte of interest compared with traditional methods (Shahrbabki et al. 2018; Zainudin and Salleh 2017). Although a few studies have focused on the determination of individual target pesticides in the soil or in vegetable plants (Sun et al. 2016; Chen et al. 2010; Lozowicka et al. 2015; Du et al. 2018), and some used the QuEChERS method combined with gas chromatography, no attempt has been made to determine the levels of these six hazardous compounds simultaneously (Chen et al. 2017; Kurz et al. 2018).

Pengdun Wisdom Agriculture Company is the largest comprehensive agricultural production unit in Jingmen city, Hubei Province, China. It has 0.27 km² of vegetable production greenhouses that are engaged mainly in off-season and for cultivating specialty vegetables such as chilis and tomatoes. Their most famous business feature is that they offer city people the chance to pick their own vegetables during sightseeing and to experience farm life. They declare that their produced vegetables do not contain any pesticides residue and that the vegetables, including cucumbers, tomatoes, and so on, can be picked and eaten directly. Aiming at proving the safety of the vegetables, they developed and achieved an accurate and reliable method for analysis, which is used in the present study focused on the optimisation of extraction, purification, and simultaneous analysis of the six target pesticides (bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil and pyriproxyfen) in soil and vegetable samples in conjunction with commonly equipped gas chromatography–mass spectrometry (GC–MS). Based on the modified QuEChERS method, the optimised method for pretreatment was screened by the comparison of four methods. The instrument test condition was also optimised before real vegetable and soil samples from the study area

were analysed. Contamination by the six target pesticides in the study area (Jingmen) was determined and health risk assessment based on the determined pesticide levels was conducted to verify the safety of working in the agricultural production areas and consuming the vegetables.

2 Materials and methods

2.1 Sampling collection

Blank soil and greenhouse soil and vegetable samples were collected on 8 March 2019. Blank soils were collected from non-planted field soil outside the greenhouses investigated that were not sprayed with pesticides. The vegetables not subjected to pesticides (hereafter termed blank vegetables), chili, the prior chosen vegetable blank due to short growth cycle and its representativeness, were planted in pots in quintuplicate on the blank soils in the laboratory without pesticides throughout the growth period. All the contaminated soil (16, 0–15 cm depth) and vegetable (16 foliar and 16 vegetable fruit) samples were from 16 greenhouses out of a total of 74 greenhouses (4/16 for chili, 4/22 for cucumber, 4/12 for eggplant and 4/24 for tomato). Each of the 48 samples was collected as a composite of five individual samples in a quincunx pattern from the 16 greenhouses screened, giving a total 240 samples for analysis.

Soils were collected using a soil corer. The vegetable leaf and fruit samples were selected and cut randomly from non-adjacent and non-marginal plants and analysed separately in triplicate. Vegetable samples were frozen for 6–8 h before freeze-drying in a Free Zone 2.5-L Freeze Dry System (Labconco Corporation, Kansas City, MO). Contaminated soil samples were freeze-dried after freezing before determination after transfer to the laboratory in linen sampling bags. The pH value, electrical conductivity (EC), organic matter content (SOM), and mechanical properties of the contaminated soils were determined following the methods of Lu (1999) and are listed in Table S1. Blank soils were used directly in pot experiments for chili cultivation after adjustment of the soil water content to ~ 60% of the maximum water-holding capacity. Chili seeds were obtained from local farmers. Before sowing, the seeds were surface sterilised by immersion in 10% (v/v) sodium hypochlorite solution for 15 min, rinsing three times with deionised water, and soaking in deionised water overnight. Forty days after sowing (28 March to 7 May, 2019) at 28 ± 0.2 °C under a light regime of 12-h days (4500 lx) with an air humidity of 80%, chili fruits and leaves were harvested and freeze-dried before use as blank.

2.2 Reagents and standard solutions

Certified standards of the six target pesticides bifenthrin (99.7%), chlorfenapyr (99.5%), lambda-cyhalothrin (99.8%), pyridaben (99.5%), pyrimethanil (99.8%) and pyriproxyfen (99.5%) and the internal standard (IS) chlorpyrifos D10 (99.5%) (Fig. 1) were purchased from Dr Ehrenstorfer (Augsburg, Germany). Acetone, acetonitrile, dichloromethane, hexane, toluene, and other solvents were of chromatographic grade and were purchased from Tedia Company (Fairfield, OH). Primary secondary amine (PSA) and graphitised carbon black (GCB) were obtained from Bonna-Agela Technologies, Inc. (Newark, DE). Anhydrous sodium sulfate, sodium chloride, anhydrous magnesium sulfate, and other reagents of analytical grade were purchased from the National Pharmaceutical Group Chemical Reagent Co., Ltd., Shanghai, China.

Standard stock solutions of the individual target pesticides and the internal standard were prepared in hexane at a concentration of 1000 µg/mL and stored at -20 °C before use. Calibration curves were constructed by diluting stock standard solutions with hexane before use. Stock standard solutions were stored at -20 °C for less than one month. A chromatogram of a mixture of the six pesticides and the internal standard solution at 1000 µg/L each, soil blank matrix, and cucumber leaf sample are shown in Fig. S1 a, b, and c, respectively.

2.3 Instruments

For sample pretreatment the following instruments were used: Free Zone 2.5-Litre Freeze Dry System (Labconco Corporation, Kansas City, MO), constant temperature culture shaker XY-ZDP-200 (Xin Yi Instruments and Meters Co., Ltd., Shanghai, China), vortex mixer VORTEX 4 (Brave Construction Development Co., Ltd., Shanghai, China), rotary evaporator R-215 with a vacuum controller V8-50 and a vacuum pump V-700 (Büchi Labortechnik AG, Flawil, Switzerland), tabletop high-speed micro centrifuge CT15RE (Hitachi Koki Co., Ltd., Tokyo, Japan), and a solid phase extraction (SPE) instrument LC-CQ-12F (Lichen Instrument Technology Co., Ltd., Shanghai, China).

2.4 Comparison of sample pretreatment methods

For the analysis of the six target pesticides in both soil and vegetable samples, extraction, purification, enrichment, and determination were performed. Procedures used in previous investigations (Goncalves et al. 2005; Mukherjee et al. 2007) were modified and four methods were

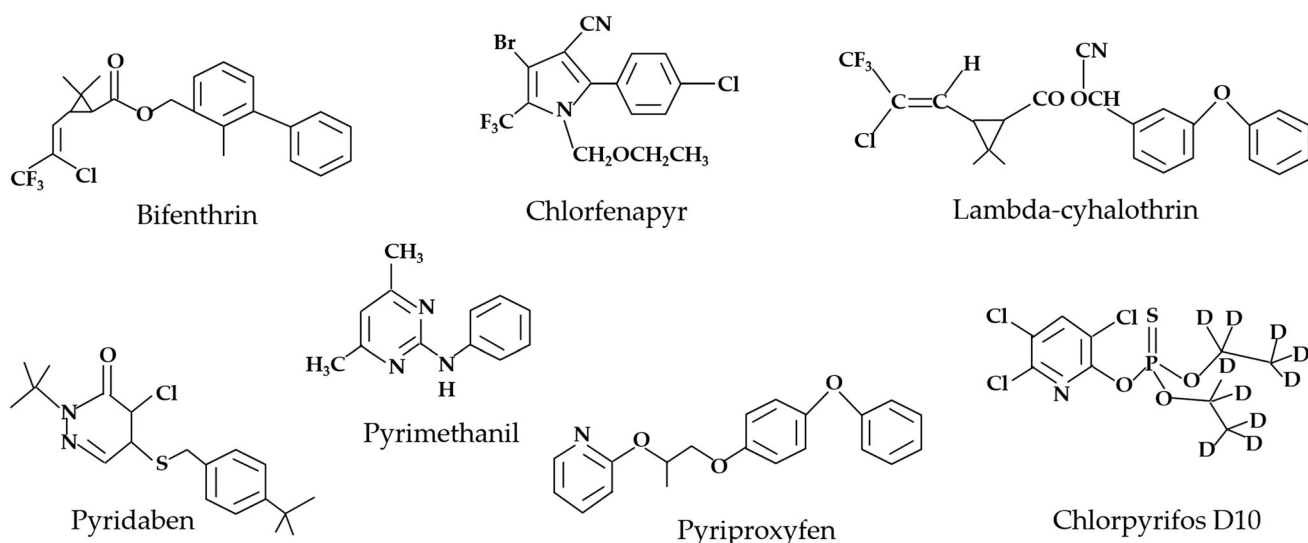


Fig. 1 Chemical structures of six target pesticides and the internal standard (chlorpyrifos D10)

compared using different extraction solvents, extraction techniques (dispersive solid-phase extraction and liquid-liquid partition extraction), purification methods with different solid phases (anhydrous magnesium sulfate, PSA, GCB and Florisil), and eluent mixtures

(acetonitrile:toluene and hexane:acetone) (Fig. 2). The spiked concentrations of the six target pesticides and the internal standard in both soil and vegetable samples were 1000 $\mu\text{g}/\text{kg}$ with blank samples, respectively for four methods. The recovery rates and the standard deviations

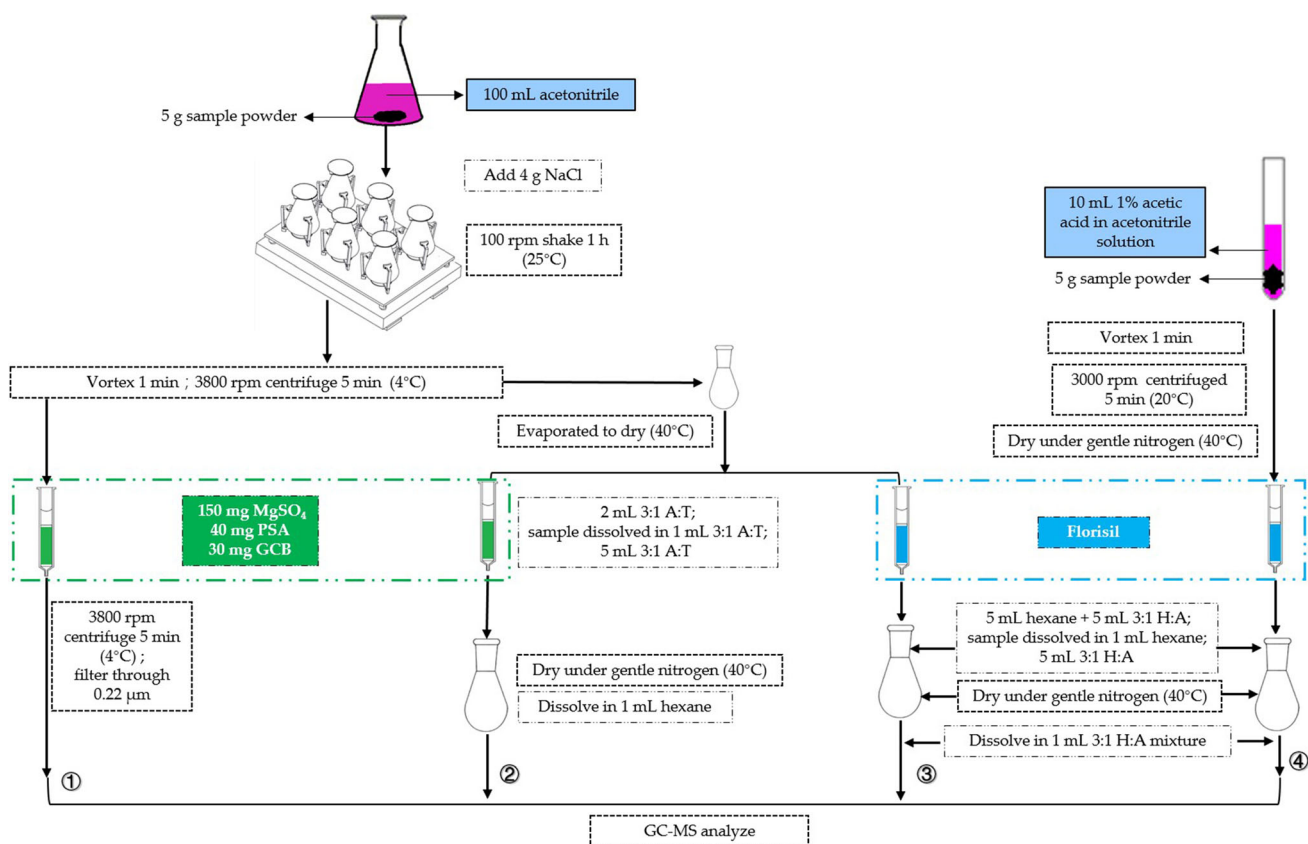


Fig. 2 Procedures of four pretreatment methods for six target pesticides. PSA, primary secondary amine; GCB, graphitised carbon black; A:T, acetonitrile: toluene mixture; H:A, hexane:acetone mixture

(SDs) of bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil, and pyriproxyfen were used to identify the best of the four methods. Real samples were treated with the developed optimised method (Method 3).

Details for four methods are presented in Fig. 2. Five grams of freeze-dried, ground and sieved (60 mesh) soil or vegetable samples were weighed into a 250-mL flask, mixed with 100 mL of acetonitrile and 4 g of sodium chloride on a constant temperature culture shaker for 1 h for extraction (25 °C) for Methods 1, 2, and 3. After vortex mixing for 1 min, the extractions were centrifuged at 3800 rpm for 5 min (25 °C). For Method 1, SPE columns filled with 150 mg anhydrous magnesium sulfate, 40 mg PSA, and 30 mg GCB were prepared before the addition of supernatant extractions (1 mL/min). Then, the elution was centrifuged at 3800 rpm for 5 min (25 °C) and filtered through a 0.22 µm microfiltration membrane before determination via GC–MS. For Methods 2 and 3, the entire supernatant was evaporated to dryness in a round-bottom flask under vacuum on a rotary evaporator in a water bath at 40 °C before different strategies were applied for SPE. The filling materials in Method 2 were the same as those in Method 1, but the column was pre-washed with 2 mL of an acetonitrile:toluene mixture (3:1 v:v), and the dried sample was dissolved in 1 mL of the mixture. Finally, 5 mL of the acetonitrile:toluene mixture (3:1 v:v) was added for elution. All the collected liquid was dried under gentle nitrogen stream in a water bath at 40 °C and then dissolved in 1 mL of hexane before determination via GC–MS. For Method 3, 1 mL of hexane was added to dissolve the residual target compounds before SPE. In the clean-up step, 1 mL of the extract was passed through a florisil column preconditioned with 5 mL hexane and 5 mL hexane:acetone (3:1 v:v). After the addition of the extract, 5 mL of hexane:acetone (3:1 v:v) was added for elution. The eluate was condensed to dryness under gentle nitrogen stream as described above. The extract was dissolved in 1 mL of hexane:acetone (3:1 v:v) and transferred to vials for GC–MS analysis. For Method 4, 5 g of grounded sample was weighted into a 25-mL glass tube and mixed with 10 mL of 1% acetic acid in an acetonitrile solution, before vortex mixing for 1 min and centrifuging for 5 min at 3000 rpm under 20 °C. The extraction was dried under gentle nitrogen stream as described earlier and then dissolved in 1 mL of hexane before SPE following the same procedure as Method 3. Each sample was prepared and analysed in triplicate.

2.5 Instrument and determination parameters

An Agilent 7890BGC-5977A MSD gas chromatograph-mass spectrometer (Agilent Technologies, Santa Clara, CA) with a Chemstation workstation was used. The MS

operating parameters were as follows: ionisation under electron impact (EI) mode at 230 °C with detector voltage at 1.012 kV and transfer line at 280 °C. A DB-5 (5% phenyl–95% polydimethylsiloxane; 30 m × 0.25 mm, 0.25 µm ID) fused silica capillary column from J&W Scientific, Inc. (Folsom, CA) with helium (purity > 99.999%) as carrier gas at 1.5 mL/min was used to separate the compounds. The injector temperature was set at 250 °C with a volume of 1 µL in the split-less mode, and non-pulse injection. The GC oven temperature was programmed as follows: initial temperature of 140 °C held for 1 min, increased at a rate of 15 °C/min to 200 °C, held for 1 min, then heated at a rate of 8 °C/min to 240 °C, then held for 1 min, then increased at a rate of 8 °C/min to 280 °C, then held for 2 min. Post-run was at 290 °C for 1 min. The selected ion monitoring (SIM) mode was used and the characteristic ions are listed in Table S2.

2.6 Quality assurance and quality control (QA/QC)

Chili cultivated in blank soil and blank soil were used after freeze-drying for QA/QC confirmation of the optimised pretreatment method and instrumental measurement procedure. Soils and vegetable samples collected from greenhouses were also analysed.

Standard mixture solutions of the six target compounds (bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil, and pyriproxyfen) at seven different concentrations (20, 50, 100, 200, 500, 1000 and 2000 µg/L) and the internal standard chlorpyrifos D10 (at 1000 µg/L) dissolved in hexane were used to build calibration curve equations (Table S2) and test for linearity. Samples with concentrations higher than 2000 µg/L were linearly diluted before analysis for accuracy. The six pesticides were quantified using the daily calculated response factor of the internal standard. The relative standard deviations (RSD) of the relative response factors (RRF) of each target pesticide based on the internal standard at different concentrations must be < 25% (Table S2).

The instrumental detection limit (IDL) is defined as the quantity corresponding to 3 times the instrument background signal generated by the substrate blank, while the instrumental quantification limit (IQL) is defined as quantity corresponding to 10 times the instrument background signal generated by the substrate blank (USEPA 2000). IDLs and IQLs for individual pesticides were calculated as follows:

$$IDL = 3Q \times N/I \quad (1)$$

$$IQL = 10Q \times N/I \quad (2)$$

where Q is the lowest concentration of standard solution to develop the calibration curve, 20 $\mu\text{g/L}$; and N/I is the noise to signal ratio of the instrument; IDLs and IQLs of the six target pesticides are listed in Table S3.

At the spiked concentrations of 5 times the calculated IQL values (Ma et al. 2013), three matrixes (blank soil, blank chili leaf and chili fruits) were spiked and analysed in septuplicate to calculate the practical detection limits of method (MDL) and the practical quantification limits of method (MQL) of the six target pesticides as follows:

$$MDL = S \times t_{(N-1,0.99)} \quad (3)$$

$$MQL = 3 \times MDL \quad (4)$$

where S is the standard deviation (SD) of the three blank matrixes at the three spiked concentrations, $n = 1$ degree of freedom; 0.99 is the confidence interval of 99%. MDLs, MQLs, and recovery rates are presented in Table S3.

Matrix blanks (blank soil, chili and chili leaves) were determined and the level of residual target pesticides were all below the IDLs. The recovery rates at gradient concentrations of 0.1, 1.0, and 10 mg/kg for each of the six target pesticides were conducted to ensure accuracy of the method (Table S3). Determined concentrations higher than 10 mg/kg were diluted to less than 10 mg/kg. During the analysis of every 24 samples, two whole procedure blanks (reagent blanks) and two matrix blanks were analysed and the QA/QC of sample recovery and SD of parallel samples are reported. Instrument analysis of three injections of standard solutions consisting of the six target pesticides and the internal standard at 1000 $\mu\text{g/L}$ was also performed daily to ensure QA/QC.

2.7 Risk assessment

The concentrations of the six target pesticides determined in the edible fruit of vegetable samples collected, i.e. chili, cucumber, eggplant, and tomato, were compared with the maximum residue limits (MRLs) of the target compounds (listed in Table 1) from different countries or international organisations.

Health risks have been evaluated by numerous methods but risk to human health is most commonly computed in terms of target hazard quotients (HQs) (Schleier et al. 2008; Islam et al. 2015). Dietary exposure evaluation is a relevant criterion for risk assessment. Exposure is not the same because of different levels of contamination and dietary habits in various parts of the world (Ramírez-Bustos et al. 2019; USEPA 2003). Based on the acceptable daily intake reference dose (ADI) and acute reference dose (ARfD) values derived by the World Health Organization (WHO) in the context of the Food and Agriculture Organization of the United Nations (FAO)/WHO Joint

Meeting on Pesticide Residues (JMPR) (2018) and the European Food Safety Authority (EFSA) (2019), health risk assessment was performed by comparing the determined residue concentrations of the target pesticides and the established values. Residue levels greater than the MQL were considered as MQL values in the calculation.

2.7.1 Short-term (acute) health risks

The short-term/acute consumer health risk was calculated based on the Pesticide Residue Intake Model- EFSA PRIMo revision 3.1 calculation model (2019), National estimated short-term intake (NESTI), and ARfD. Relevant formulas for acute intake risk assessment are as follows (FAO 2016). Pesticide residual concentrations were below their MQLs for chili and cucumber, and thus only eggplant and tomato were taken into account of this assessment. For food products with a U_{RAC} (Unit weight of raw agricultural commodity) greater than 25 g, where the meal sized portion is $< U_{ep}$, the acute intake risk of pesticide residues could be calculated as follows:

$$\%ARfD = \frac{U_{ep} \times HR \times v}{BW \times ARfD} \times 100\% = \frac{NESTI}{ARfD} \times 100\% \quad (5)$$

where U_{ep} is Unit weight of edible portion, which is 0.268 kg for eggplants and 0.1206 kg for tomatoes (GEMS/food 2015); HR is the highest residue according to residue definition for enforcement in composite sample (mg/kg), which is 0.276 mg/kg for eggplants and 0.919 mg/kg for tomatoes; v is the variability factor of individual vegetables, which is usually 3 (Lin et al. 2019); LP is large portion reported (kg/day) (97.5th percentile of eaters), which is 0.3379 for young children (0–6 years old) and 0.4489 for older children and adults (7–70 years old) for eggplants, 0.2793 for young children and 0.4576 for older children and adults for tomatoes (GEMS/food 2015); BW means body weight for the subgroup of the population related to LP or mean consumption (kg). BW is 24.5 kg for the 0–6 age group and 61.75 kg for the 7–70 age group in China (Ma et al. 2019). $\%ARfD$ is the acute intake risk of pesticide residues and $ARfD$ is the acute reference dose (mg/kg BW). When $NESTI$ is less than $ARfD$, the acute risk is considered acceptable, and when the $NESTI$ exceeds $ARfD$, the acute risk is considered unacceptable.

2.7.2 Long-term (chronic) health risks

The chronic/long-term consumer health risk was calculated based on the International Estimated Daily Intake (IEDI) and ADI. The relevant formulas for chronic intake risk assessment are as follows.

Table 1 Parameters of target pesticides in target vegetables for health risk assessment

Pesticide	Vegetable	Maximum residue limits (MRLs) (mg/kg)				Acceptable daily intake (ADI) (mg/kg BW) ^e				Acute reference dose (ARfD) (mg/kg BW) ^e		Corresponding reference dose (RfD) (mg/kg/d) China ^f	
		China ^a		EU ^b		Canada ^c		FAO/WHO ^d		WHO			
		GB 2763-2016	Value	Document	Value	Document	Value	Document	Value	Year	Value		Document
Bifenthrin	Chili	0.5	0.5	Reg. (EU) 2018/687	–	PMRL2015-40	0.5	2011	0–0.01	JMPR_2009_report	0.01	JMPR_2009_report	0.013
	Cucumber	–	0.01		0.5		–	–					
	Eggplant	0.3	0.3		0.5		0.3	2011					
	Tomato	0.5	0.3		0.5		0.3	2011					
Chlorfenapyr	Chili	–	0.01	Reg. (EU) No 899/2012	–	–	0.3	2019	0–0.03	JMPR_2018_Report	0.03	JMPR_2018_Report	0.05
	Cucumber	0.5	0.01		0.5	PMRL2019-41	–	–					
	Eggplant	–	0.01		2	PMRL2013-02	–	–					
	Tomato	–	0.01		2		0.4	2019					
Lambda-cyhalothrin	Chili	0.5	0.1	Reg. (EU) 2019/1015	–	EMRL2011-68	0.3	2009	0–0.02	JMPR_2018_Report	0.02	JMPR_2018_Report	0.001
	Cucumber	0.2	0.05		0.05		–	–					
	Eggplant	0.5	0.3		0.2		–	–					
	Tomato	0.5	0.07		0.2		–	–					
Pyridaben	Chili	2	0.01	Reg. (EU) 2019/90	1	Canada Gazette II Prior to 16 June 2008	–	–					0.022
	Cucumber	0.1	0.15		0.1		–	–					
	Eggplant	–	0.15		–		–	–					
	Tomato	–	0.15		0.15		–	–					
Pyrimethanil	Chili	–	2.0	Reg. (EU) 2018/852	–	–	–	–	0–0.2	JMPR_2007_report	Unnecessary	JMPR_2007_report	0.17
	Cucumber	2	0.8		1.5	PMRL2016-38	0.7	2016					
	Eggplant	–	1.0		–		–	–					
	Tomato	1	1.0		0.5	EMRL2008-02	0.7	2008					
Pyriproxyfen	Chili	–	1	Reg. (EU) 2016/1902	–	–	0.6	2019	0–0.2	JMPR_2006_report	Unnecessary	JMPR_2006_report	0.35
	Cucumber	–	0.1		0.1	PMRL2014-77	0.04						
	Eggplant	–	1		0.8	PMRL2013-21	0.6						
	Tomato	1	1		0.25	EMRL2008-03	0.4						

–, not available (has not been assessed); BW, body weight

^aChinese MRLs and RfD values are from the National Food Safety Standard (GB 2763-2016)

^bEU MRLs are from <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database>

^cHealth Canada, Maximum Residue Limits for Pesticide, <https://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php>

^d<https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/en/>

^eADI and ARfD values were from WHO JMPR (<https://apps.who.int/pesticide-residues-jmpr-database>)

^fChinese Ministry of Ecology and Environment (2019). National environmental protection Standards of the People’s Republic of China. Technical guidelines for risk assessment of soil contamination of land for construction (HJ-25.3-2019). 2019-12-5

$$IEDI = \sum_{x=i}^n \frac{STMR_i \times MC_i}{BW} \quad (6)$$

$$\%ADI = \frac{IEDI}{ADI} \times 100\% \quad (7)$$

where $STMR_i$ is the Supervised Trials Median Residue for RAC concerned (mg/kg); MC_i is the mean consumption for a given RAC calculated for the whole survey/subgroup of the survey, including processed products (recalculated to the unprocessed RAC) (kg/day), which is 0.0343 kg/day for young children (0–6 years old) and 0.0382 kg/day for older children and adults (7–70 years old) for eggplants, and 0.1628 kg/day for young children and 0.2843 kg/day for older children and adults for tomatoes (GEMS/food 2015).

2.7.3 Carcinogenic risks

Carcinogenicity is a chronic effect from long-term exposure. Acute exposure leads to adverse effects from a single high dose (e.g. neurotox). In principle, genotoxic modes of actions may also cause cancer after a single dose, but it is still assessed in terms of life-time risk. Therefore, the chronic toxicity effect should be compared to the chronic exposure. In this study, cancer risk was assessed using the same exposure estimates as discussed in Unit IV.B.1.ii., i.e. chronic exposure.

2.7.4 Non-carcinogenic risks

Non-carcinogenic risks of the six target pesticides were evaluated according to the risk assessment guidelines recommended by EPA following the description of Ma et al. (2019) The hazard index (HI) value was used to quantify the non-carcinogenic risk posed by the six pesticides. An $HI < 1$ indicates no significant risk of non-carcinogenic effects and an $HI > 1$ indicates that non-carcinogenic effects may occur (IARC 2018). Vegetable intake, soil ingestion, dermal absorption, and inhalation were calculated as follows. Dermal absorption, inhalation, and probably soil ingestion are relevant to workers in the greenhouse, but not consumers, who buy foods.

$$HQ_{intake} = \frac{C_{vegetable} \times IRF \times EF \times ED \times CF}{BW \times AT \times RfD} \quad (8)$$

$$HQ_{ingestion} = \frac{C_{soil} \times IRS \times EF \times ED \times CF}{BW \times AT \times RfD} \quad (9)$$

$$HQ_{dermal} = \frac{C_{soil} \times SA \times AF \times ABS \times EF \times ED \times CF}{BW \times AT \times RfD} \quad (10)$$

$$HQ_{inhale} = \frac{C_{soil} \times EF \times ED \times 1000}{PEF \times AT \times RfD} \quad (11)$$

$$HI = \sum_{i=6} (HQ_{intake} + HQ_{ingestion} + HQ_{dermal} + HQ_{inhale}) \\ = \sum_{i=6} (HQ_{intake} + HQ_{soil}) \quad (12)$$

where $C_{vegetable}$ is the average pesticide concentration in vegetable fruits ($\mu\text{g}/\text{kg}$); IRF is the (non-leafy) daily vegetable intake rate, which is 0.125 for young children and 0.054 for older children and adults [g dry weight (kg BW/day)]; C_{soil} is the pesticide concentration in soil samples (mg/kg DW); IRS is the soil ingestion rate (mg/d), which is 200 mg/d for young children and 100 mg/d for older children and adults; EF is the exposure frequency of each year, which is working days in one year (334/3 d for young children and 334 d for older children and adults) (d/a); ED is the exposure duration, which is the number of years of greenhouse exposure (a); CF is a conversion factor (10^{-6} kg/mg); AT is the average time of life, which is $365 \times ED$ for HQ calculation (d); SA is the soil surface area, which was 5700 (cm^2/d); AF is the soil adherence factor, which was 0.07 (mg/cm); ABS is the fraction of contaminant absorbed dermally from the soil, which was 0.1; PEF is the particle emission factor, which was 1.36×10^9 (m^3/kg) (Ma et al. 2019). The values of HQ were calculated individually, and the sums of the HQ were calculated for the HI to estimate total risk of multiple pesticides (USEPA 2000). When the HI value is less than 1, there are no non-carcinogenic effects to the target population, but when the HI is higher than 1, non-carcinogenic effects are considered to exist (USEPA 2001).

2.8 Statistical analysis

All data were processed with Microsoft Excel 2013 and the Origin 2017 software package. Pesticide concentrations are listed as the average values (quintuplicate in each greenhouse) \pm the standard deviations (SD) of quintuplicates for that particular greenhouse.

3 Results and discussion

3.1 Optimisation of pretreatment methods

QuEChERS has been well established for the simultaneous analysis of multiple pesticides up to several hundred in number. However, the recovery rate of each analyte is usually not that satisfactory. Recovery rates approaching 100% could promise a better accuracy. To obtain the most accurate results, recovery rates of the modified QuEChERS

method were compared in this study. The compared recovery rates of the four pretreatment methods in blank soil and chili fruits at 1000 µg/kg are shown in Fig. 3. For soil samples (Fig. 3a), the recovery rates of six target compounds ranged from about 93.1% ± 2.0% to 98.9% ± 1.7% for bifenthrin; 90.8% ± 1.3% to 95.5% ± 2.1% for chlorfenapyr; 96.7% ± 2.0% to 99.8% ± 3.3% for lambda-cyhalothrin; 83.6% ± 2.8% to 95.8% ± 1.4% for pyridaben; 90.5% ± 2.8% to 98.2% ± 1.6% for pyrimethanil; and 92.6% ± 3.2% to 99.6% ± 4.3% for pyriproxyfen. For plant samples (Fig. 3b), the recovery rates of the six target compounds

ranged from about 93.2% ± 2.0% to 97.7% ± 2.1% for bifenthrin; 91.8% ± 2.1% to 95.6% ± 2.0% for chlorfenapyr; 95.5% ± 2.6% to 98.2% ± 2.0% for lambda-cyhalothrin; 81.9% ± 3.3% to 92.1% ± 3.1% for pyridaben; 88.3% ± 3.2% to 93.6% ± 2.1% for pyrimethanil; and 90.5% ± 3.0% to 94.8% ± 2.2% for pyriproxyfen. In terms of the different methods, the recovery rates of the six target compounds in soils ranged from about 83.6% ± 2.8% to 99.6% ± 4.3% for Method 1; 88.1% ± 2.7% to 96.7% ± 2.0% for Method 2; 94.1% ± 1.3% to 98.9% ± 1.7% for Method 3; and 86.2% ± 3.2% to 99.8% ± 3.3% for Method 4 (Fig. 3a).

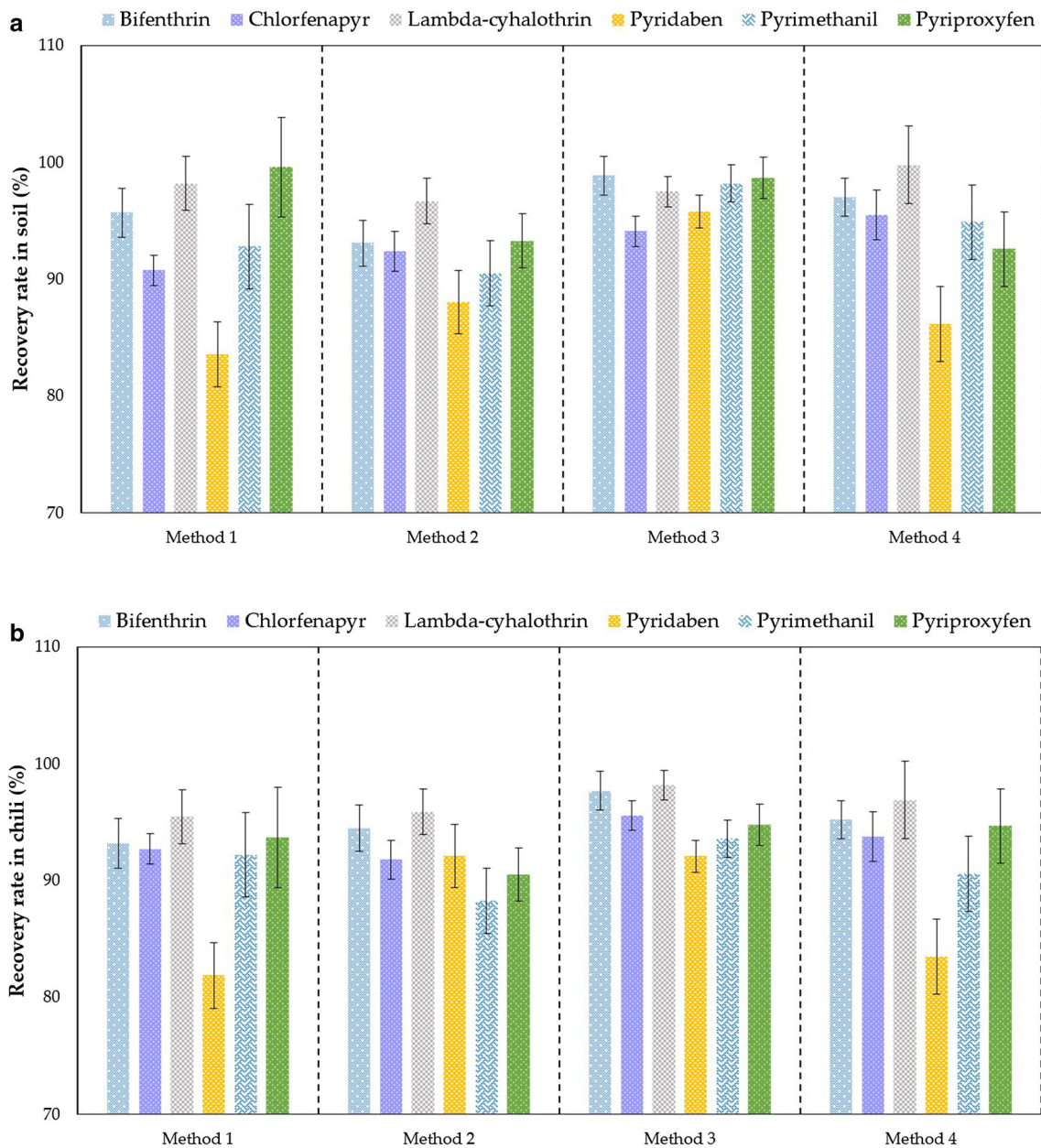


Fig. 3 Comparison of recovery rates using four pretreatment methods

The recovery rates of the six target compounds in plants ranged from about $81.9\% \pm 3.3\%$ to $95.5\% \pm 2.6\%$ for Method 1; $88.3\% \pm 3.2\%$ to $95.9\% \pm 2.3\%$ for Method 2; $92.1\% \pm 2.0\%$ to $98.2\% \pm 2.0\%$ for Method 3; and $83.5\% \pm 3.8\%$ to $96.9\% \pm 4.0\%$ for Method 4 (Fig. 3b).

In general, the average recovery rates of the six target pesticides were more stable and closer to 100% in Method 3. The recovery rates ranged from about 94–99% with SDs < 2% (Fig. 3a). Method 2 was also acceptable with recovery rates of 88–97% and SDs < 3%. Methods 1 and 4 were less satisfactory. Figure 3b shows that Method 3 also gave better recovery rates near 100% and smaller SDs, < 2.5% compared with the other three methods. Thus, Method 3 was selected as the optimised pretreatment method and used in the following QA/QC procedures.

Methods 1 and 2 differed in the processes of column pre-washing, sample loading, and elution. The results of recovery rates indicated that Method 2 with more complicated washing steps achieved better results. Methods 3 and 4 differed in the extraction process, with the original QuEChERS used in Method 4 and modified QuEChERS in Method 3. After the modification of the extraction step, the total recovery rates became more satisfactory, which proved the success of the modification for target compound detection in both soil and plant samples. As a result, Method 3 was selected as the optimised method for sample pretreatment.

3.2 QA/QC comparison of the optimised method

Calibration equations of the six individual target pesticides based on the internal standard chlorpyrifos D10 at standard mixture solutions of the seven concentrations (20, 50, 100, 200, 500, 1000, and 2000 $\mu\text{g/L}$) are presented in Table S2. The correlation coefficients (R^2) were all > 0.996. Overall, the calculated MDLs of the individual pesticides in the three blank matrixes ranged from 0.1 to 0.8 $\mu\text{g/kg}$ (Table S3)—0.1 to 0.2 $\mu\text{g/kg}$ for blank soil and 0.2 to 0.8 $\mu\text{g/kg}$ for chili leaves and chili fruits. All the blank soils and blank vegetables were spiked at three concentrations of the target pesticide mixture at 100, 1000, and 10,000 $\mu\text{g/kg}$. The average recovery rates of the six target compounds were between 80.6 and 118.3% with SD values between 2.4 and 8.4%. Soil matrix and vegetable matrix samples were both carefully collected or planted to minimise any background contamination of the six target pesticides. With Method 3 (and all the other three methods), none of the six pesticides was detected in the matrix soils or vegetables (Table S3), verifying the reliability of spike matrixes.

Previous studies often applied QuEChERS to validate multiresidue analysis of pesticides. For example, residues of 20 pesticides, such as bifenthrin, lambda-cyhalothrin,

and pyriproxyfen, in commercialised maize flour samples were investigated (Milhome et al. 2019). The LOQs of the three common target pesticides were 0.0016, 0.0165 and 0.0016, respectively, whereas the MQLs in our study were 0.0005–0.0017, 0.0002–0.0024, and 0.0003–0.0017 for soil and vegetable matrixes, respectively. Compared with the recovery rates at 0.1 mg/kg, which were 97.2%, 77.3%, and 119.1% for bifenthrin, lambda-cyhalothrin, and pyriproxyfen, respectively, the recovery rates were 80.6–108.3%, 82.2–117.4%, and 82.7–116.7%, respectively, in our study. The optimised method presented better recovery rates and lower MQLs. Badawy et al. (2020) extracted and cleaned abamectin, chlorfenapyr, and pyridaben in pods of green beans (*Phaseolus vulgaris* L.) using QuEChERS and analysed them via HPLC. The LOD and LOQ of chlorfenapyr and pyridaben determined via HPLC were 0.0022 and 0.0072, and 0.0089 and 0.0296 mg/L, respectively, whereas the values determined via GC–MS in our study were 0.0002 and 0.0004, and 0.0005 and 0.0008, respectively. Secondly, the recovery rates at 1 mg/kg (85.54 ± 2.47 for chlorfenapyr and 84.66 ± 1.78 for pyridaben) and 10 mg/kg (91.39 ± 1.64 for chlorfenapyr and 88.83 ± 0.63 for pyridaben) were not as good as those of our study (95.0 ± 7.3 for chlorfenapyr and 94.4 ± 5.2 for pyridaben at 1 mg/kg; 93.0 ± 4.9 for chlorfenapyr and 93.8 ± 5.7 for pyridaben at 10 mg/kg). Although SDs were higher for Method 3, the mean values of the recovery rates were much closer to 100%. In another study of pesticide residues in pepper, chili peppers, and sauce products, bifenthrin, lambda-cyhalothrin, pyrimethanil, and pyridaben were analysed via GC–MS/MS and LC–MS/MS (Song et al. 2018). The LOQs of the four common target pesticides were 0.01 mg/kg and the recovery rates at 0.1 mg/kg were 100–111% for bifenthrin, 98–114% for lambda-cyhalothrin, 38–84% for pyrimethanil, and 93–116% for pyridaben, whereas their values were 80.6–108.3%, 82.2–117.4%, 81.3–109.9%, and 80.9–118.3%, respectively, in our study. The recovery rates of our optimised method were more stable and closer to 100%. In China, the modified QuEChERS has been applied more in the determination of toxins, pesticides, and their metabolites from open field soils, vegetables, fruits, and other food items, but rarely applied to pesticides in greenhouses (Feng et al. 2020a, b; Xing et al. 2019). Guo et al. (2020) used QuEChERS and HPLC monitoring to quantify pesticide residues on plastic mulching films in typical farmlands of Shandong, Tianjin, and Hebei, north China. Bifenthrin, chlorfenapyr, and pyridaben were among the 35 detected pesticides and the general LOD of the 35 pesticides ranged from 0.01 to 2.0 $\mu\text{g/kg}$ and the LOQ ranged from 0.05 to 5.0 $\mu\text{g/kg}$ (Guo et al. 2020). In our present study, MDL and MQL results of bifenthrin,

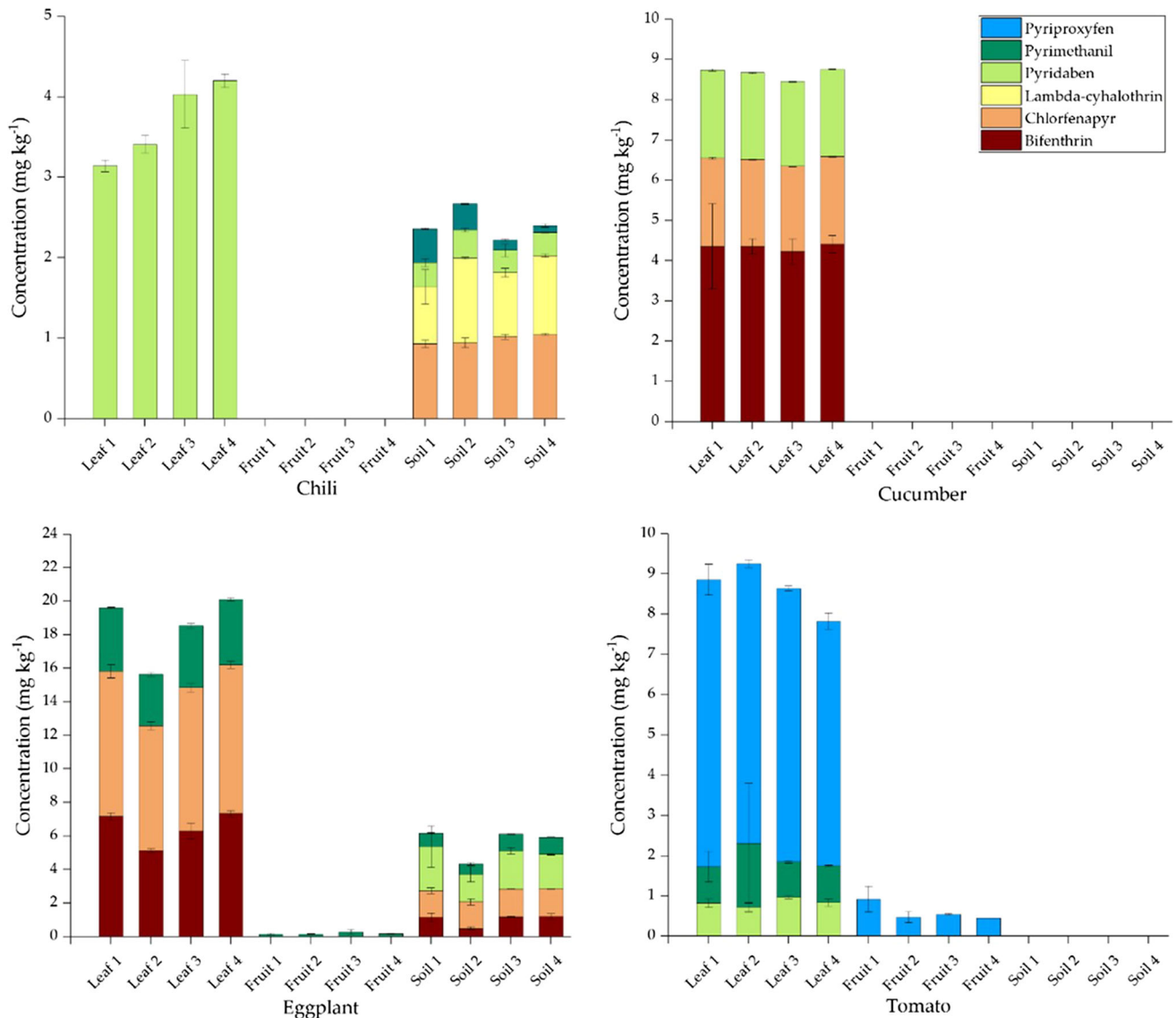


Fig. 4 Residual concentrations of six target pesticides in soil and plant samples from Pengdun

chlorfenapyr, and pyridaben in soil ranged from 0.1 to 0.2 $\mu\text{g}/\text{kg}$ and 0.3 to 0.5 $\mu\text{g}/\text{kg}$, showing better results.

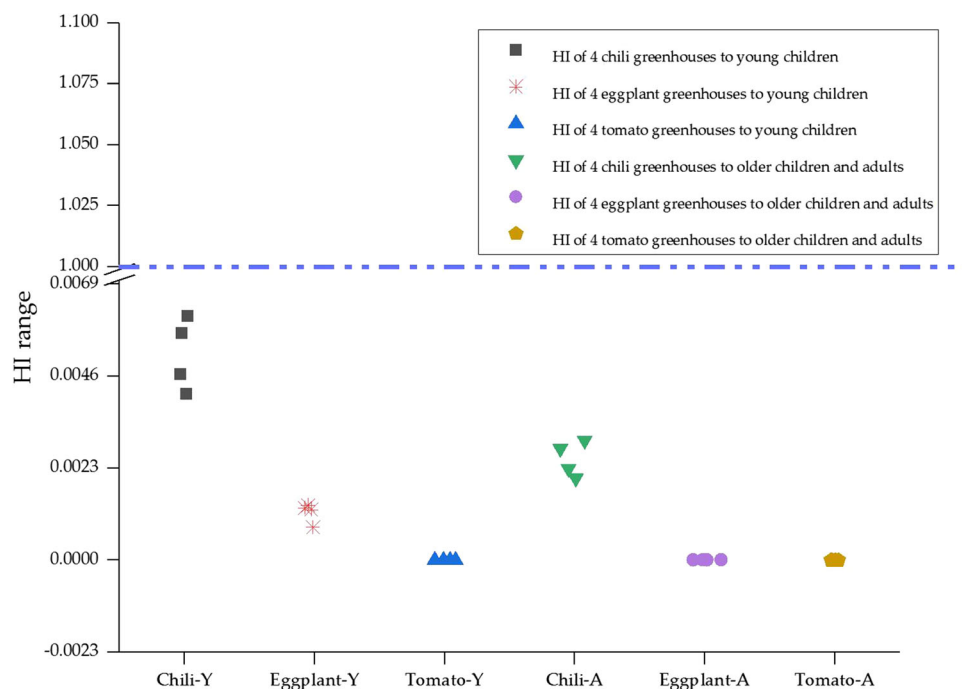
A single target pesticide or two of these six target pesticides have been considered in the determination process in other investigations. In the case of a single bifenthrin determined in wheat residue (You et al. 2013) the MQL ranged from 0.4 to 30 $\mu\text{g}/\text{kg}$, a value much lower than that of our present study (0.2 to 2.5 $\mu\text{g}/\text{kg}$ for the six pesticides). In combined determinations of bifenthrin and lambda-cyhalothrin in green beans the MDLs calculated were 6 and 10 $\mu\text{g}/\text{kg}$, respectively (Bouri et al. 2012), whereas our MDL values ranged from 0.2 to 0.8 $\mu\text{g}/\text{kg}$ for the six pesticides. Although SPE was developed to replace liquid–liquid extraction for the convenience of saving time and reagents, it may also be combined for higher efficiency (Sobhanzadeh et al. 2013). Simultaneous analysis of

multiple pesticide residues might become routine and deserve further investigation in view of the deteriorating environmental and food pollution problems (Yang et al. 2018).

3.3 Target pesticide residues in soil and vegetable samples

The results of the contaminated soils and vegetables are shown in Fig. 4 and Table S4. The six target pesticides were detected mostly in the leaves in all plant samples. Despite the production of different vegetables in different greenhouses and the application of different pesticides, the leaves consistently showed the highest contamination followed by the soils and fruits. The highest average total concentration of the six target pesticides was almost

Fig. 5 HI values of samples collected in Pengdun



20,000 $\mu\text{g}/\text{kg}$ in eggplant leaves, < 10,000 $\mu\text{g}/\text{kg}$ in both cucumber and tomato leaves, and < 5000 $\mu\text{g}/\text{kg}$ in chili leaves. All the pesticides were sprayed on leaves but some landed on the soil or on the fruits. Pesticides remaining in the soil may be degraded more by soil microorganisms and less by photolysis due to the shade of leaves, but those on the leaves may be more subject to photolysis, resulting in differences in pesticide residues in soil and leaves.

In all the greenhouses sampled, pyridaben showed the highest values above MQL, with the highest residue levels in the leaves of chili, followed by chlorfenapyr with even higher residue concentrations in the leaves of eggplant ($8370 \pm 279.8 \mu\text{g}/\text{kg}$) and cucumber ($2156 \pm 14.4 \mu\text{g}/\text{kg}$). Aphids and thrips are the most commonly found piercing-sucking insect pests in almost all greenhouse vegetables. Therefore, pyridaben, targeting mitochondrial respiration, is the most commonly used pesticide in greenhouses (Inak et al. 2019). Chlorfenapyr is a recommended pesticide in Chinese greenhouses because it is effective against thrips, caterpillars, mites, leaf miners, and aphids and thus it is also commonly detected (Ahmad et al. 2018). Pyrimethanil and pyriproxyfen were only detected in eggplant and tomato, respectively, ranging from 446 to 919 $\mu\text{g}/\text{kg}$ with an SD of 3.8–315.3 and from 138–276 $\mu\text{g}/\text{kg}$ with an SD of 30.7–139.4, respectively. Although the total pesticide residues in cucumber leaves were high, no pesticide residues were detected in the soil or cucumber fruits. City dwellers are offered the opportunity to pick their own fruits and vegetables in many villages such as Pengdun and the mini-cucumbers grown in Pengdun are

very popular in this regard. The cucumbers are often eaten fresh after rinsing with tap water and our results might help the consumers gain more confidence in food safety. The yellow small round tomatoes grown in this area are also a popular specialty. The health risk assessment of vegetables produced in Pengdun needs to reconsider the pick-your-own marketing scheme because conventionally home-cooked vegetables may have lower health risks because of the cooking process (Hamilton et al. 2017).

3.4 Risk assessment

3.4.1 Risk for sale

Pyrimethanil residues in eggplant were between 138 and 276 $\mu\text{g}/\text{kg}$ with an SD of 30.7–139.4 $\mu\text{g}/\text{kg}$, while those in tomato were between 446 and 919 $\mu\text{g}/\text{kg}$ with an SD of 3.8–315.3 $\mu\text{g}/\text{kg}$. Although residues of pyrimethanil in eggplant and pyriproxyfen in tomato fruits were detected, the concentration of pyrimethanil in eggplants was below the corresponding MRL of pyrimethanil, which is 1000 $\mu\text{g}/\text{kg}$ according to the EU. For tomato, the MRL of pyriproxyfen is 1000 $\mu\text{g}/\text{kg}$ according to both China and the EU, but it is 250 $\mu\text{g}/\text{kg}$ and 400 $\mu\text{g}/\text{kg}$ according to Canada and FAO/WHO, respectively (Table 1). The results indicate that sales of these tomatoes are acceptable in the Chinese market but would not comply with legal limits established in Canada or with Codex MRLs.

3.4.2 Short-term (acute) health risks

Based on the Pesticide Residue Intake Model-EFSA PRIMo revision 3.1 calculation model (2019), the short-term/acute consumer health risk of consuming the produced eggplants and tomatoes could be calculated. However, ARfD values for pyrimethanil and pyriproxyfen were considered as “Unnecessary” due to their low acute toxicity. Therefore, no acute consumer health risk could be observed in this study.

3.4.3 Long-term (chronic) health risks

The calculated %ADI values for young children (0–6 years old) and older children and adults (7–70 years old) for eggplant consumption are 0.1932% and 0.8537%, respectively, while those for tomato consumption are 3.053% and 2.116%, respectively. Based on the calculated results, the chronic toxicity risk is not major for the consumption of vegetables produced in the study area.

3.4.4 Carcinogenic and non-carcinogenic risks

Pyrimethanil and pyriproxyfen are the only two above-MQL target pesticides in the edible vegetable portion. Nevertheless, their slope factor for oral exposure (SFO) are not available. Therefore, only non-carcinogenic risks were assessed here based on vegetable consumption, ingestion of soil, dermal contact with soil, and soil particle inhalation.

The HQ_{intake} values for eggplant and tomato consumption were 7.73×10^{-6} (< 1) for young children and 3.32×10^{-6} (< 1) for older children and adults, indicating that the non-carcinogenic risks of vegetable consumption are acceptable. Residual concentrations of the six target pesticides in soils were used to obtain the HQ associated with the ingestion of soil, dermal contact with soil, and soil particle inhalation and HI. In greenhouse soils with planted chili, eggplant, and tomato, target pesticides were found to be over their MQLs, and thus HI values could be observed in these greenhouses. The HI values of all 16 greenhouses are shown in Fig. 5 and Table S5. None of the HQ_{intake} values or HQ_{soil} values reached the value of 1, indicating that there was no non-carcinogenic risk to greenhouse vegetable consumers or greenhouse workers in the study area. Greenhouse workers are more likely than other indoor workers to encounter pollutants such as pesticides, fertilisers, phthalates, and antibiotics during their work (Li et al. 2015; Ma et al. 2015). Operators are exposed to chemical products during pesticide application and dermal exposure has been reported to be the principal means of exposure for growers (Rincón et al. 2018). On the other hand, in most studies on the dietary intake of environmental contaminants, analyses are generally carried out

only for raw food products. However, the majority of food products are consumed after cooking (Trabalón et al. 2018). Detailed detection of pesticide concentrations should be conducted in the future for the calculation of more accurate non-carcinogenic risk rather than only HI values from soils and the vegetables. The consequences of simultaneous use of multiple pesticides should also be considered.

4 Conclusions

This study determined an optimised method for the analysis of six commonly used pesticides (bifenthrin, chlorfenapyr, lambda-cyhalothrin, pyridaben, pyrimethanil and pyriproxyfen) in greenhouses. Soil and vegetable samples from intensively managed greenhouses were used for the simultaneous monitoring of pesticide residues. The optimal analysis method was found to be the pretreatment procedure comprising shaking extraction with acetonitrile, florisil SPE column clean-up, and hexane:acetone (3:1 v:v) elution combined with GC–MS determination. The IDLs, IQLs, MDLs, MQLs, and spiked recovery rates in three gradient concentrations all verified the accuracy and reliability of this method. Compared with the local MRLs, all the vegetables produced in greenhouses at Pengdun were safe to eat or sell. Pyriproxyfen in tomatoes exceeded the Canadian and Codex MRLs and thus, they may be restricted for export and sale abroad. According to the chronic risk, acute risk, and non-carcinogenic risk assessment results, the vegetables produced in these greenhouses posed no risk to either farmers working in the greenhouse or consumers of different ages. The results indicate that chlorfenapyr, bifenthrin, and pyridaben represent the most popular pesticide combination in eggplant and cucumber greenhouses. Pyridaben is the most often quantified pesticide. Simultaneous detection of multiple contaminants and health risk assessment in greenhouses merit further investigation. The modified QuEChERS followed by GC–MS showed advantages in terms of accuracy and applicability to places not equipped with MS–MS instruments.

Acknowledgements This study was funded by the National Natural Science Foundation of China (41877127), the Local S&T Special Project of China (ECJF 968 No.109-2016) and the Training Fund Program for Scientific Research of Hubei University of Arts and Science (2019 KYPY001).

Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Ahmad M, Farid A, Saeed M (2018) Resistance to new insecticides and their synergism in *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan. *Crop Protect* 107:79–86. <https://doi.org/10.1016/j.cropro.2017.12.028>
- Araújo CVM, Shinn C, Vasconcelos AM, Ribeiro R, Espíndola ELG (2014) Preference and avoidance responses by tadpoles: the fungicide pyrimethanil as a habitat disturber. *Ecotoxicology* 23(5):851–860. <https://doi.org/10.1007/s10646-014-1225-7>
- Badawy MEI, Mahmoud MS, Khattab MM (2020) Residues and dissipation kinetic of abamectin, chlorfenapyr and pyridaben acaricides in green beans (*Phaseolus vulgaris* L.) under field conditions using QuEChERS method and HPLC. *J Environ Sci Health Part B*. <https://doi.org/10.1080/03601234.2020.1726701>
- Bouri M, Salghi R, Bazzi L, Zarrouk A, Rios A, Zougagh M (2012) Pesticide residue levels in green beans cultivated in Souss Masa Valley (Morocco) after multiple applications of bifenthrin and λ -cyhalothrin. *Bull Environ Contam Toxicol* 89(3):638–643. <https://doi.org/10.1007/s00128-012-0722-8>
- Camara S, Ahoua Alou LP, Koffi AA, Clegban YCM, Kabran J-P, Koffi FM, Koffi K, Pennetier C (2018) Efficacy of Interceptor® G2, a new long-lasting insecticidal net against wild pyrethroid-resistant *Anopheles gambiae* s.s. from Côte d'Ivoire: a semi-field trial. *Parasite* 25:42. <https://doi.org/10.1051/parasite/2018042>
- Cayir A, Coskun M, Coskun M, Cobanoglu H (2019) Comet assay for assessment of DNA damage in greenhouse workers exposed to pesticides. *Biomarkers* 24:592–599. <https://doi.org/10.1080/1354750X.2019.1610498>
- Chen J, Zhang W, Shu Y, Ma X, Cao X (2017) Detection of organophosphorus pesticide residues in leaf lettuce and cucumber through molecularly imprinted solid-phase extraction coupled to gas chromatography. *Food Anal Methods* 10(10):3452–3461. <https://doi.org/10.1007/s12161-017-0875-5>
- Chen L, Chen J, Ma M, Chen L, Yang H, Zhang G (2010) Determination of Lambda-cyhalothrin residue in tea and soil using gas chromatography. *Chin J Chromatogr* 28:817–820. <https://doi.org/10.3724/SP.J.1123.2010.00817>
- Du P, Wu X, Xu J, Dong F, Shi Y, Li Y, Liu X, Zheng Y (2018) Different residue behaviors of four pesticides in mushroom using two different application methods. *Environ Sci Pollut Res* 25(9):8377–8387. <https://doi.org/10.1007/s11356-017-1142-4>
- European Food Safety Authority (EFSA) (2019) Technical report on the Pesticide Residue Intake Model- EFSA PRIMo revision 3.1 (update of EFSA PRIMo revision 3). EFSA supporting publication 2019: EN-1605, 15. <https://doi.org/10.2903/sp.efsa.2019.EN-1605>
- Fang Y, Nie Z, Die Q, Tian Y, Liu F, He J, Huang Q (2017) Organochlorine pesticides in soil and air at and around a compound contaminated site: vertical distribution, soil–air exchange and risk evaluation. *Stoch Environ Res Risk Assess* 32(4):1179–1188. <https://doi.org/10.1007/s00477-017-1412-1>
- Feng C, Xu Q, Qiu X, Jin Y, Ji J, Lin Y, Le S, Wang G, Lu D (2020a) Comprehensive strategy for analysis of pesticide multi-residues in food by GC-MS/MS and UPLC-Q-Orbitrap. *Food Chem*. <https://doi.org/10.1016/j.foodchem.2020.126576>
- Feng YZ, Qi XX, Wang XY, Liang L, Zuo BJ (2020b) Residue dissipation and dietary risk assessment of trifloxystrobin, trifloxystrobin acid, and tebuconazole in wheat under field conditions. *Int J Environ Anal Chem*. <https://doi.org/10.1080/03067319.2020.1739667>
- Food and Agriculture Organization of the United Nations (FAO) (2016) Submission and evaluation of pesticide residues data for the estimation of Maximum Residue Levels in food and feed. Pesticide Residues. 3rd Ed. FAO Plant Production and Protection Paper 225
- GEMS/food (2015) Highest reported 97.5th percentile consumption figures (eaters only) for various commodities by the general population and children ages 6 and under [DB/OL]. [2015-11-07]. https://www.who.int/foodsafety/chem/en/acute_hazard_db1.pdf
- Goncalves C, Alpendurada M (2005) Assessment of pesticide contamination in soil samples from an intensive horticulture area, using ultrasonic extraction and gas chromatography–mass spectrometry. *Talanta* 65(5):1179–1189. <https://doi.org/10.1016/j.talanta.2004.08.057>
- Guo LJ, Wang Y (2018) Study on overdose of pesticides in rice production in China: based on the perspective of maximizing social and private interests. *J Ecol Rural Environ* 34:401–407. <https://doi.org/10.11934/j.issn.1673-4831.2018.05.003>
- Guo B, Meng J, Wang X, Yin C, Hao W, Ma B, Tao Z (2020) Quantification of pesticide residues on plastic mulching films in typical farmlands of the North China. *Front Environ Sci Eng* 14(1):2–11. <https://doi.org/10.1007/s11783-019-1181-9>
- Gupta S, Sharma RK, Gupta RK, Sinha SR, Singh R, Gajbhiye VT (2008) Persistence of new insecticides and their efficacy against insect pests of okra. *Bull Environ Contam Toxicol* 82(2):243–247. <https://doi.org/10.1007/s00128-008-9581-8>
- Hamilton D, Yoshida M, Wolterink G (2017) Pesticide Specifications and their methods for analysis and testing. In: Ambrus A, Hamilton D (eds) Food safety assessment of pesticide residues. World Scientific, New Jersey, pp 283–326. https://doi.org/10.1142/9781786341693_0004
- İnak E, AlpKent YN, Çobanoğlu S, Dermauw W, Van Leeuwen T (2019) Resistance incidence and presence of resistance mutations in populations of *Tetranychus urticae* from vegetable crops in Turkey. *Exp Appl Acarol* 78:343–360. <https://doi.org/10.1007/s10493-019-00398-w>
- International Agency for Research on Cancer (IARC) (2018) IARC Mode of action classification scheme. Version 8.4. <https://www.iarc-online.org>
- Islam MS, Ahmed MK, Habibullah-Al-Mamun M (2015) Apportionment of heavy metals in soil and vegetables and associated health risks assessment. *Stoch Environ Res Risk Assess* 30(1):365–377. <https://doi.org/10.1007/s00477-015-1126-1>
- Kurz MHS, Batista JLS, de Oliveira LG, Hoff R, Martins ML, Gonçalves FF (2018) Clean-up procedure development and method validation for pesticide residues analysis in carrots. *Food Anal Methods* 12:282–292. <https://doi.org/10.1007/s12161-018-1359-y>
- Li C, Chen J, Wang J, Ma Z, Han P, Luan Y, Lu A (2015) Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. *Sci Total Environ* 521–522:101–107. <https://doi.org/10.1016/j.scitotenv.2015.03.070>
- Li J, Dong L, Tian D, Zhao Y, Yang H, Zhi X, Zhu L (2018) Association between pesticide exposure intensity and self-rated health among greenhouse vegetable farmers in Ningxia, China. *PLoS ONE* 13(12):e0209566. <https://doi.org/10.1371/journal.pone.0209566>
- Lin H, Zhao S, Fan X, Ma Y, Wu X, Su Y, Hu J (2019) Residue behavior and dietary risk assessment of chlorothalonil and its metabolite SDS-3701 in water spinach to propose maximum residue limit (MRL). *Regul Toxicol Pharmacol* 107:104416–104423. <https://doi.org/10.1016/j.yrtph.2019.104416>
- Lozowicka B, Abzeitova E, Sagitov A, Kaczynski P, Toleubayev K, Li A (2015) Studies of pesticide residues in tomatoes and cucumbers from Kazakhstan and the associated health risks.

- Environ Monit Assess 187:609. <https://doi.org/10.1007/s10661-015-4818-6>
- Lu RK (1999) Soil Science Society of China. Analysis methods of soil agricultural chemicals. Science and Technology of China Press China Press, Beijing
- Ma TT, Wu LH, Chen L, Zhang HB, Teng Y, Luo YM (2015) Phthalate esters contamination in soils and vegetables of plastic film greenhouses of suburb Nanjing, China and the potential human health risk. Environ Sci Pollut Res 22(16):12018–12028. <https://doi.org/10.1007/s11356-015-4401-2>
- Ma T, Teng Y, Christie P, Luo Y, Chen Y, Ye M, Huang Y (2013) A new procedure combining GC-MS with accelerated solvent extraction for the analysis of phthalic acid esters in contaminated soils. Front Environ Sci Eng 7(1):31–42. <https://doi.org/10.1007/s11783-012-0463-2>
- Ma T, Zhou W, Chen L, Christie P, Luo Y, Wu P (2019) Phthalate ester contamination in intensively managed greenhouse facilities and the assessment of carcinogenic and non-carcinogenic risk: a regional study. Int J Environ Res Public Health 16(16):2818. <https://doi.org/10.3390/ijerph16162818>
- Mathirajan VG, Natarajan K, Kuttalam S, Chandrasekaran S, Regupathy A (2000) Efficacy of lambda-cyhalothrin (Karate 5 EC) against brinjal shoot and fruits borer (*Leucinodes orbonalis* Guen.). Pestic Res J 12:117–119
- Milhome MAL, Vieira SKV, Reges BM, Fernandes DR, Uchoa MLP, Pinheiro AI, Castro RC, Silva VPA, Nobre CA, Menezes MGG, Silva RO, do Nascimento RF (2019) Multiresidue analysis and evaluation of the matrix effect on 20 pesticides in Brazilian maize (*Zea mays* L.) flour. J Environ Sci Health Part B. <https://doi.org/10.1080/03601234.2019.1640586>
- Ministry of Agriculture and Rural Affairs of the People's Republic of China (MOA China) (2007-09-19) https://www.moa.gov.cn/ztlz/ncpxzz/fbg/200709/t20070919_893058.htm
- Mukherjee I, Gopal M, Mathur DS (2007) Behaviour of β -Cyfluthrin after foliar application on chickpea (*Cicer arietinum* L.) and pigeon pea (*Cajanus cajan* L.). Bull Environ Contam Toxicol 78(1):85–89. <https://doi.org/10.1007/s00128-007-9018-9>
- National food safety standard (GB 2763-2016)—maximum residue limits for pesticides in food, the State Standard of the People's Republic of China. Issued on 2016-12-18. Implemented on 2017-06-18. Chinese National Health and Family Planning Commission, The Ministry of Agriculture of the People's Republic of China, The State Food and Drug Administration of the People's Republic of China
- Ramírez-Bustos II, Saldarriaga-Noreña H, Fernández-Herrera E, Juárez-López P, Alia-Tejacal I, Guillén-Sánchez D, Rivera-León I, López-Martínez V (2019) Dissipation behavior of three pesticides in prickly pear (*Opuntia ficus-indica* (L.) Mill.) pads in Morelos, Mexico. Int J Environ Res Public Health 16:2922. <https://doi.org/10.3390/ijerph16162922>
- Razzaghi N, Ziarati P, Rastegar H, Shoeibi S, Amirahmadi M, Conti GO, Ferrante M, Fakhri Y, Khanghah AM (2018) The concentration and probabilistic health risk assessment of pesticide residues in commercially available olive oils in Iran. Food Chem Toxicol 120:32–40. <https://doi.org/10.1016/j.fct.2018.07.002>
- Ren Z, Xiao R, Zhang Z, Lv X, Fei X (2019) Risk assessment and source identification of heavy metals in agricultural soil: a case study in the coastal city of Zhejiang Province, China. Stoch Environ Res Risk Assess 33:2109–2118. <https://doi.org/10.1007/s00477-019-01741-8>
- Rincón VJ, Páez FC, Sánchez-Hermosilla J (2018) Potential dermal exposure to operators applying pesticide on greenhouse crops using low-cost equipment. Sci Total Environ 630:1181–1187. <https://doi.org/10.1016/j.scitotenv.2018.02.235>
- Saidi I, Mouhouche F, Abri H (2017) Determination of pesticide residues on tomatoes from greenhouses in Boudouaou and Douaouda, Algeria. Qual Assur Saf Crops Foods 9(2):207–212. <https://doi.org/10.3920/qas2015.0716>
- Schleier JJ, Macedo PA, Davis RS, Shama LM, Peterson RKD (2008) A two-dimensional probabilistic acute human-health risk assessment of insecticide exposure after adult mosquito management. Stoch Environ Res Risk Assess 23(5):555–563. <https://doi.org/10.1007/s00477-008-0227-5>
- Shahrbabki PE, Hajimohammadi B, Shoeibi S, Elmi M, Yousefzadeh A, Conti GO, Ferrante M, Amirahmadi M, Fakhri Y, Khanghah AM (2018) Probabilistic non-carcinogenic and carcinogenic risk assessments (Monte Carlo simulation method) of the measured acrylamide content in Tah-dig using QuEChERS extraction and UHPLC-MS/MS. Food Chem Toxicol 118:361–370. <https://doi.org/10.1016/j.fct.2018.05.038>
- Sihuincha M, Zamora-perea E, Orellana-rios W, Stancil JD, López-sifuentes V, Vidal-oré C, Devine GJ (2005) Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Perú. J Med Entomol 42(4):620–630. [https://doi.org/10.1603/0022-2585\(2005\)042\[0620](https://doi.org/10.1603/0022-2585(2005)042[0620)
- Sobhanzadeh E, Nemati K (2013) Liquid–liquid extraction/low-temperature purification (LLE/LTP) followed by dispersive solid-phase extraction (d-SPE) cleanup for multiresidue analysis in palm oil by LC-QTOF-MS. J Chem 2013:1–9. <https://doi.org/10.1155/2013/915048>
- Song L, Han Y, Yang J, Qin Y, Zeng W, Xu S, Pan C (2018) Rapid single-step cleanup method for analyzing 47 pesticide residues in pepper, chili peppers and its sauce product by high performance liquid and gas chromatography–tandem mass spectrometry. Food Chem. <https://doi.org/10.1016/j.foodchem.2018.12.017>
- Sun P, Gao Y, Wang J, Liu X (2016) Dispersive liquid-liquid microextraction based on the solidification of a floating organic droplet followed by gas chromatography for the determination of five pyrethroid pesticides residues in liquid milk samples. Chin J Pestic Sci 18:497–502. <https://doi.org/10.16801/j.issn.1008-7303.2016.0069>
- Tang Z, Huang Q, Nie Z, Yang Y, Yang J, Qu D, Cheng J (2015) Levels and distribution of organochlorine pesticides and hexachlorobutadiene in soils and terrestrial organisms from a former pesticide-producing area in Southwest China. Stoch Environ Res Risk Assess 30(4):1249–1262. <https://doi.org/10.1007/s00477-015-1144-z>
- Trabalón L, Alves RN, Castro Ó, Nadal M, Borrull F, Pocurull E, Marques A (2018) Preliminary assessment of galaxolide bioaccessibility in raw and cooked fish. Food Chem Toxicol 122:33–37. <https://doi.org/10.1016/j.fct.2018.09.075>
- USEPA (US final rule, Sept 26, 2003) has regulated the chlorfenapyr by fixing MRLs 1 mg kg⁻¹ in or on raw agricultural food commodities
- USEPA (2000) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum Technical Panel. United States Environmental Protection Agency Office of EPA/630/R-00/002
- USEPA (United States Environmental Protection Agency) (2001) Supplemental Guidance for Developing Soil Screening Level for Superfund Sites; OSWER 9355.4-24; Office of Solid Waste and Emergency Response; USEPA: Washington, DC, USA.
- World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO) (2018) https://apps.who.int/pesticide-residues-jmpr-database.JMPR_2018Report
- Xing L, Zou L, Luo R, Wang Y (2019) Determination of five Alternaria toxins in wolfberry using modified QuEChERS and ultra-high performance liquid chromatography–tandem mass spectrometry. Food Chem. <https://doi.org/10.1016/j.foodchem.2019.125975>
- Yang X, Luo J, Duan Y, Li S, Liu C (2018) Simultaneous analysis of multiple pesticide residues in minor fruits by ultrahigh-

- performance liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry. *Food Chem* 241:188–198. <https://doi.org/10.1016/j.foodchem.2017.08.102>
- Zainudin BH, Salleh S (2017) Method development, optimization and validation of matrix hydration effect on pesticide residues in Cocoa Beans using modified QuEChERS method and gas chromatography tandem mass spectrometry. *Food Anal Methods* 10(12):3874–3885. <https://doi.org/10.1007/s12161-017-0954-7>
- Zhao X, Cui H, Wang Y, Sun C, Cui B, Zeng Z (2017) Development strategies and prospects of nano-based smart pesticide formulation. *J Agric and Food Chem* 66:6504–6512. <https://doi.org/10.1021/acs.jafc.7b02004>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Affiliations

Tingting Ma^{1,2}  · Shengli Li³  · Yuqi Li⁴ · Xiuhua Li² · Yongming Luo²

¹ College of Resource Environment and Tourism, Hubei University of Arts and Science, Xiangyang, China

² Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

³ Institute of Physical Education, Hubei University of Arts and Science, Xiangyang, China

⁴ School of Food Science and Technology and School of Chemical Engineering, Hubei University of Arts and Science, Xiangyang, China