



Analysis of sediment-associated insecticides using ultrasound assisted microwave extraction and gas chromatography–mass spectrometry

Huizhen Li^{a,b}, Yanli Wei^{a,b}, Jing You^{a,*}, Michael J. Lydy^c

^a State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

^b Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

^c Fisheries and Illinois Aquaculture Center and Department of Zoology, 171 Life Science II, Southern Illinois University, Carbondale, IL 62901, USA

ARTICLE INFO

Article history:

Received 22 June 2010

Received in revised form 31 August 2010

Accepted 3 September 2010

Available online 15 September 2010

Keywords:

Ultrasound assisted microwave extraction (UAME)

Organophosphate insecticides

Pyrethroid insecticides

Sediment

ABSTRACT

An ultrasound assisted microwave extraction (UAME) method was developed to simultaneously extract five organophosphate (OP) and eight pyrethroid insecticides from sediment. The optimized UAME conditions were to use 100 ml of a mixture of hexane and acetone (1:1, v/v) solution as the extraction solvents, and extraction time, microwave and ultrasonic power settings of 6 min, 100 W and 50 W, respectively. Extracts were cleaned using solid phase extraction and analyzed by gas chromatography–mass spectrometry in negative chemical ionization mode and quantification was based on matrix-matched standard solutions along with internal standard calibration. At the spiked concentrations of 1, 5 and 20 ng/g dry weight (dw), recoveries of OPs were 77.6–122%, 65.2–128% and 75.6–141% with relative standard deviations (RSDs) of 10.6–18.1%, 3.1–12.5% and 8.0–35.3%, respectively, while recoveries of pyrethroids were 78.0–101%, 76.4–104% and 71.0–99.5% with RSDs of 10.3–23.5%, 4.7–17.6% and 8.8–18.7%, respectively. Method detection limits ranged from 0.31 to 0.45 ng/g dw for the OP insecticides and from 0.27 to 0.70 ng/g dw for the pyrethroid insecticides. The newly developed UAME method was validated by comparing it to Soxhlet and sonication extraction methods. Better recoveries were achieved for most OPs by the novel UAME method, whereas there was no significant difference in recoveries for most of the pyrethroids. Finally, the UAME method was used to quantify the target insecticides in field-contaminated sediment samples which were collected in Guangzhou, China.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Organophosphate (OP) and pyrethroid insecticides are currently used for pest control in both agricultural and urban areas in China, and their residues are transported to the aquatic environment through runoff events [1]. These insecticides strongly bind to sediment; therefore, their degradation rates are reduced, and as a result, sediment becomes a reservoir for these compounds, and analysis of sediment samples for insecticides is an effective method to quantify insecticide contamination [2].

Due to the strong binding between insecticides and sediment organic carbon (OC), it generally requires an exhaustive extraction method to separate analytes from the complicated matrix components. Different methods have been developed to extract OP and pyrethroid insecticides from sediment [3–11]. Since its introduction in 1879, Soxhlet extraction is still widely used because of its acceptable extraction efficiency and low cost [3]. However, the lengthy extraction time (from 4 to 48 h) which may lead to

potential loss of analytes as well as the requirement of large extraction solvent volumes stimulated studies examining alternative extraction techniques. Sonication extraction [4,5], supercritical fluid extraction (SFE) [6], microwave-assisted extraction (MAE) [7,8] and accelerated solvent extraction (ASE) [9–11] have been employed to extract insecticides from sediment. Although these alternative methods greatly reduced extraction time and solvent usage, most of them, including SFE, pressurized-MAE and ASE, require operation under high pressure which requires sophisticated and expensive instruments.

In contrast, sonication equipment is relatively inexpensive, and has been shown to be an effective technique to extract organic contaminants from solid samples [4,5]. In addition to sonication, MAE operated under atmospheric pressure is another relatively low cost alternative extraction method [12,13]. Since its first introduction in 1986 [14], MAE has been used for extracting organic pollutants from various environmental matrices [12,13,15]. Two types of MAE have been developed, namely focused MAE (FMAE) and pressurized MAE (PMAE), corresponding to an open-style system under atmospheric pressure and a closed-style system under a certain pressure, respectively [16]. The PMAE method has been extensively used since it can simultaneously extract multiple samples, extraction time is exten-

* Corresponding author. Tel.: +86 20 8529 1497; fax: +86 20 8529 0706.

E-mail address: youjing@gig.ac.cn (J. You).

sively reduced by conducting extraction at elevated temperatures, and because PMAE has been adopted as one of the U.S. EPA standard extraction methods [15–18]. On the other hand, the application of FMAE has been less studied [12,13,19]. By performing extractions under atmospheric pressure, FMAE may provide solutions for some of the limitations of PMAE, such as the high instrumentation cost, lengthy cooling time, low sample load, possibility of losing relatively volatile analytes and degradation of labile compounds at high temperature [16]. Additionally, temperature is controlled by the boiling point of the solvent under atmospheric pressure in FMAE, which guarantees more precisely controlled energy delivery [13].

Considering the improved heating delivered by microwave extraction and efficient agitation provided by ultrasound, the combination of the two techniques produced high yields in short reaction time during organic synthesis [20]. However, only a few studies have combined these two techniques as an extraction tool [21–25], and no studies have used sediment as the environmental matrix. Therefore, the objective of the present study was to develop and evaluate an ultrasound assisted microwave extraction (UAME) method to extract five OP and eight pyrethroid insecticides from sediment under atmospheric pressure. The UAME method was optimized using a four-factor-three-level orthogonal fractional factorial experiment and the developed method was validated by applying it to sediment spiked with the target insecticides at different concentrations. The UAME method was also compared to traditional Soxhlet and sonication extraction techniques. Finally, field sediments collected from Guangzhou, China were analyzed with the newly developed UAME method.

2. Experimental

2.1. Chemicals and materials

Five organophosphate (OP) and eight pyrethroid insecticides were analyzed in the present study. The OPs used included chlorpyrifos, diazinon, malathion, terbufos, and tebufos, while the pyrethroid insecticides included bifenthrin, *lambda*-cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, permethrin, and tefluthrin (Table 1). These particular insecticides were selected, because they are currently used in the Pearl River Delta, China. Tefluthrin was purchased from Sigma–Aldrich (St. Louis, MO, USA) and had a purity of 96.8%, and chlorpyrifos with a purity of 97% was obtained from Ultra (Kingstown, RI, USA). The remaining insecticide standards were purchased from ChemService (West Chester, PA, USA) and had purities >97% as certified by the manufacturer.

Decachlorobiphenyl (DCBP) and 4,4'-dibromooctafluorobiphenyl (DBOBF) were used as surrogates and added to the sediment samples prior to extraction to verify the performance of the analytical process and were purchased from Supelco (Bellefonte, PA, USA). Parathion-*d*₁₀ (Cambridge, Andover, MA, USA) and PCB 189 (AccuStandard, New Haven, CT, USA) were used as internal standards (IS), and were added to the solutions before GC/MS analysis to quantify the OPs and pyrethroids, respectively. The stock solution of each insecticide and surrogate was made at 1 mg/ml in hexane.

Hexane (HPLC grade) was purchased from Burdick and Jackson (SK Chemicals, Ulsan, Korea). Analytical grade dichloromethane and acetone were purchased from Tianjin Chemical Reagent Company (Tianjin, China) and re-distilled in a glass system. Copper powder was cleaned by sonication using acetone, and anhydrous sodium sulfate was baked at 450 °C for 4 h prior to use. Dual-layer solid phase extraction (SPE) cartridges, which were packed with 600 mg primary/secondary amine (PSA) and 300 mg graphite carbon black (GCB), were purchased from Supelco.

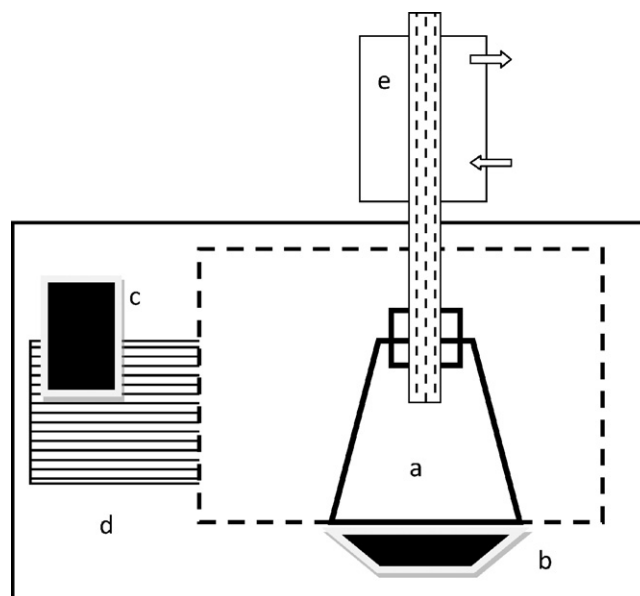


Fig. 1. Schematic diagram of the ultrasound assisted microwave extraction apparatus. (a) Sample flask; (b) ultrasound transducer; (c) microwave magnetron; (d) microwave guide; (e) condenser.

2.2. Sediment spiking and collection

The optimization of the UAME method was conducted using a reference sediment (GIG) prepared from a hydrated soil collected from the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, China, whereas another reference sediment (HB) collected from Hailing Bay, China was used for method validation. No target insecticides were detected in either reference sediment. The GIG soil was air dried at room temperature, ground and sieved through a 2 mm sieve, hydrated to make sediment, and spiked with an appropriate amount of insecticide standard to obtain a concentration of 10 ng/g dry weight (dw). The spiked GIG sediment was mixed thoroughly and stored at 4 °C for 11 days before extraction. The HB sediment samples were spiked with the target insecticides at 1, 5 and 20 ng/g dw to estimate method detection limits (MDL) and assess recoveries and relative standard deviations (RSDs) at the different spiking concentrations. A separate aliquot of HB sediment was spiked at 30 ng/g dw to compare extraction efficiencies among the newly developed UAME and traditional Soxhlet and sonication extraction methods. The spiked HB sediment was thoroughly homogenized and stored at 4 °C overnight before use. All extractions were performed using four replicates; with the exception of the seven replicates used for the MDL study, where the sediment was spiked at 1 ng/g dw.

In addition, five field-contaminated samples collected from the Guangzhou Higher Education Mega Center, Guangzhou, China were used to validate the newly developed UAME method. The upper 1–2 cm of the sediment column was collected with a stainless steel scoop, sieved through a 2 mm sieve, and transported back to the laboratory. After being fully mixed, sediments were stored in the dark at 4 °C until analysis on the next day.

2.3. Sediment Extraction

2.3.1. Ultrasound assisted microwave extraction (UAME)

The UAME was performed on a CW-2000 UAME instrument (Xintuo Company, Shanghai, China) under atmospheric pressure, and the schematic diagram of this extractor is shown in Fig. 1. Ultrasound and microwave energy was provided simultaneously to the sample in the extraction flask to initiate the solvent extraction.

Table 1

Gas chromatography–negative chemical ionization–mass spectrometry method parameters, method detection limits (MDL), and reporting limits (RL) for the target insecticides.

Class	Insecticide	M.W. ^a	t _R (min) ^b	Target ion (m/z)	Qualifier ions (m/z)	MDL (ng/g dw) ^c	RL (ng/g dw) ^c
OP ^d	Terbufos	288.4	10.88	185	187	0.31	0.93
OP	Diazinon	304.3	11.03	169	170	0.38	1.1
PYR ^e	Tefluthrin	418.7	11.18	241	243, 205	0.70	2.1
OP	Tebupirimfos	318.4	11.43	183	167, 275	0.32	1.0
OP	Malathion	330.4	12.64	157	159, 172	0.45	1.4
OP	Chlorpyrifos	350.6	12.88	169	313, 315	0.45	1.4
PYR	Bifenthrin	422.9	18.87	205	241	0.46	1.4
PYR	Lambda-cyhalothrin	449.9	20.68, 21.12	205	241	0.30	0.90
PYR	Permethrin	391.2	22.57, 22.81	207	209	0.58	1.7
PYR	Cyfluthrin	434.3	23.72, 23.82, 23.97, 24.04	207	209, 171	0.42	1.3
PYR	Cypermethrin	416.3	24.21, 24.40, 24.53, 24.60	207	209	0.40	1.2
PYR	Esfenvalerate	419.9	25.88, 26.26	167	211	0.27	0.81
PYR	Deltamethrin	505.2	26.76, 27.16	137	79	0.42	1.3

^a Molecular weight.

^b Retention time.

^c The MDL was calculated from the standard deviations of seven replicates of sediment spiked with insecticides at 1 ng/g dry weight (dw), and the RL was equal to three times the MDL.

^d Organophosphate insecticide.

^e Pyrethroid insecticide.

The extraction was conducted under atmospheric pressure, and a condenser was used to minimize the loss of analytes by solvent evaporation.

A four-factor, three-level orthogonal experiment design was used to optimize the extraction efficiency, and the four factors tested were the type and volume of the extraction solvents, extraction time and microwave power which controlled the time to reach final extraction temperature (boiling point of the extraction solvents), and three levels were investigated for each factor (Table 2). As shown in Table 2, three solvent types were tested and included dichloromethane, a dichloromethane–hexane mixture (1:1, v/v), and an acetone–hexane mixture (1:1, v/v), while the tested solvent volumes were 60, 80 and 100 ml. The optimization levels for extraction time and microwave power were 3, 6 or 9 min and 80, 100 and 150 W, respectively. Because preliminary experiments showed low recoveries without use of the ultrasound feature, ultrasound was included at a fixed power of 50 W and a frequency of 40 kHz for all experiments.

After thorough homogenization and centrifugation to remove excess water, sediment samples were freeze dried at –45 °C with a FD-1C-50 Freeze dryer (Boyikang, Beijing, China). The freeze dried sediment (5 g) and 2 g activated copper powder were placed into a 250 ml extraction flask and 50 ng of each surrogate was added before extraction. Nine treatments were conducted in duplicate to optimize the UAME parameters (Table 2) with the spiked GIG sediment samples, and the optimized conditions were used for

Table 2

Experimental design of the four-factor-three-level orthogonal fractional optimization of the ultrasound assisted microwave extraction method.

Solvent type	Factor ^a		
	Solvent volume (ml)	Extraction time (min)	Microwave power (W)
Dichloromethane	60	3	100
Dichloromethane	80	6	150
Dichloromethane	100	9	80
Dichloromethane–hexane	60	6	80
Dichloromethane–hexane	80	9	100
Dichloromethane–hexane	100	3	150
Acetone–hexane	60	9	150
Acetone–hexane	80	3	80
Acetone–hexane	100	6	100

^a Experiments were conducted in duplicate.

method validation with both spiked and field-collected sediment samples.

After extraction, the extract was decanted and filtered and an additional 50 ml of solvent was added to the extraction flask, and the extraction repeated. The filtered extracts were combined, concentrated and solvent exchanged to 1 ml of hexane using a rotary evaporator (IKA Company, Staufen, Germany) for further cleanup.

2.3.2. Sonication extraction

Sonication extraction was performed using a JY92-IIN high intensity ultrasonic processor (Scientz Biotechnology, Ningbo, China) following a previously validated method [5]. In brief, 5 g of freeze dried sediment, 2 g activated copper powder, 50 ml of a hexane and acetone (1:1, v/v) solution and 50 ng of the surrogates were placed into a 100 ml flask. The extraction was carried out for 5 min in a 3 s work with 3 s pause mode and the ultrasonic power was fixed at 400 W. The extract was filtered, and 50 ml of the solvent mixture was added. The sonication procedure was repeated two additional times, and the extracts were filtered, combined, concentrated, and solvent exchanged to 1 ml of hexane.

2.3.3. Soxhlet extraction

Freeze dried sediment (5 g dw) and 2 g of copper powder were wrapped in filter paper and placed into the Soxhlet extraction apparatus, and 220 ml of a mixture of hexane and acetone (1:1, v/v) was added as extraction solvents. The extraction was conducted for 48 h at 57 °C. After extraction, the extract was transferred to the rotary evaporator, concentrated and solvent exchanged to 1 ml of hexane.

2.4. Extract cleanup

A dual-layer PSA/GCB SPE cartridge, which was capped with 1 cm of anhydrous Na₂SO₄ and prewashed with 3 ml of hexane, was used for the sediment extract cleanup [9]. After loading the concentrated extract into the cartridge, 7 ml of a dichloromethane:hexane (3:7, v/v) solution was used as elution solvents to recover the target compounds. The eluents were concentrated and solvent exchange to 1 ml of hexane under a gentle flow of nitrogen using a K17-0002 Reactivap (Bafang Company, Shanghai, China). After evaporation, 50 ng of internal standards were added to the solution before gas chromatograph/mass spectrometer (GC/MS) analysis.

Table 3
Results of the four-factor-three-level orthogonal fractional factorial optimization of the ultrasound assisted microwave extraction method for the organophosphate insecticide, malathion (Mal) and a pyrethroid insecticide, esfenvalerate (Esf).

Factor	Solvent type	Peak area ^a		Extraction time (min)	Peak area	
		Mal	Esf		Mal	Esf
Mean ^b	Dichloromethane	6866	88,096	3	10,847	82,766
	Dichloromethane–hexane	5631	77,072	6	10,529	91,400
	Acetone–hexane	13,510	80,004	9	4631	71,005
Range ^c		7879	11,023		6215	20,395

Factor	Solvent volume (ml)	Peak area		Microwave power (W)	Peak area	
		Mal	Esf		Mal	Esf
Mean ^b	60	12,687	81,337	80	10,220	84,878
	80	5989	77,439	100	10,043	82,364
	100	7332	86,396	150	5744	77,930
Range ^c		6698	8957		4477	6948

^a Experiments were conducted in duplicate.

^b Mean peak areas of malathion and esfenvalerate of three treatments at each level for each factor.

^c Range peak areas of malathion and esfenvalerate among the three levels for each factor.

2.5. Instrumental analysis

Analysis of OP and pyrethroid insecticides was performed using a QP 2010 plus series GC/MS (Shimadzu, Japan) in negative chemical ionization (NCI) mode. A Rtx-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Restek, Bellefonte, PA) was used for separating the analytes. The oven was held at the initial temperature of 60 °C for 2 min, heated to 180 °C at 25 °C/min, held at 180 °C for 1 min, heated to 240 °C at 10 °C/min, held at 240 °C for 5 min, then heated to 290 °C at 7 °C/min, and held at 290 °C for 10 min. The cleaned extract (1 μl) was injected with a programmable temperature vaporizing (PTV) injector, and the initial PTV temperature was 50 °C, then heated to 300 °C at 230 °C/min after 0.1 min holding time at 50 °C, and held at 300 °C for 5 min. Helium was used as the carrier gas at a flow rate of 1 ml/min, while methane was used as the NCI reaction gas. The temperatures of the ion source and the interface were set at 250 and 260 °C, respectively. The emission current for the NCI was set at 60 μA, and perfluorotributylamine was used as the tuning compound.

The MS was operated in selected ion monitoring (SIM) mode, and the characteristic ions for the target compounds were listed in Table 1. Quantification was performed using IS calibration with matrix-matched standards at concentrations of 5, 10, 50, 100, 250, 500 and 1000 ng/ml of each insecticide and surrogate. The IS, parathion-d₁₀ and PCB 189, were added at 50 ng/ml into each of the seven calibration standard solutions and samples.

2.6. Statistical analysis

Statistical comparison among three extraction methods (UAME, Soxhlet extraction and sonication extraction) was conducted using a Tukey's HSD test with SAS version 8.02 software (SAS Institute Inc., Cary, NC, USA), and $\alpha = 0.05$.

3. Results and discussion

3.1. UAME method development

As a hybrid of MAE and ultrasonic extraction, UAME provides energy by simultaneous irradiation with microwaves and ultrasound. Thus, extraction conditions needed to be optimized for a better usage of both types of energy. Four parameters (factors) were optimized in the current study, including the types and volume of the extraction solvents, extraction time and microwave power, using a four-factor-three-level orthogonal fractional factorial design (Table 2). Three levels were used to optimize each factor.

The three extraction solvents studied included dichloromethane, dichloromethane–hexane, and acetone–hexane mixtures, while the solvent volumes tested were 60, 80, and 100 ml. Three extraction times were studied including 3, 6, and 9 min, and three microwave power settings were used including 80, 100, and 150 W. Ultrasonic power was fixed at 50 W for all nine treatments.

The mean peak areas and the range of the means for malathion and esfenvalerate at the three levels for each of the four extraction factors were shown in Table 3. Malathion and esfenvalerate were selected as the representative insecticides for the OPs and pyrethroids, respectively, and the other insecticides showed similar trends. As shown in Table 3, the type of extraction solvents was the most important factor affecting the extraction efficiencies of OPs. Acetonitrile, dichloromethane, acetone and a hexane–acetone mixture were the recommended extraction solvents for MAE from the literature [26]. To avoid the extensive time to evaporate the high boiling point solvents (e.g. acetonitrile), the more volatile solvents including dichloromethane, hexane, acetone and their mixtures were investigated in the current study. A mixture of acetone and hexane (1:1, v/v) provided the highest recoveries for malathion and the other OP insecticides (Table 3). This result may be partially explained by the effect of solvent polarity on MAE. The matrix absorbs microwave energy through a dielectric mechanism which involves dipolar polarization and favors polar solvents [27]. Therefore, the dipolar aprotic acetone had a better ability to absorb microwave energy and produce bulk heating. On the other hand, solvent type affected pyrethroids less than the OPs. Compared to pyrethroids, OPs were more polar, and favored extraction solvents with a higher polarity.

With the greatest range among the three levels, extraction time was the most important factor influencing pyrethroid extraction (Table 3). The peak area of esfenvalerate increased as extraction time was increased from 3 to 6 min, but decreased when the extraction time was increased to 9 min. Reduction in peak area after 9 min of extraction time was also observed for malathion (Table 3). The decrease in extraction efficiency may be attributed to an increase loss of compound through evaporation of solvents, and degradation of some labile OP insecticides due to the prolonged extraction time. In the current study, a condenser was positioned above the sampling flask in the UAME system (Fig. 1), which should have reduced the loss of the more volatile pesticides [16].

The other two factors, namely solvent volume and microwave power, showed less effect on extraction efficiencies for the two classes of insecticides (Table 3). To avoid loss of chemicals due to the incomplete transfer of extraction solvents, an additional 50 ml of solvent was used to wash the sample after decanting the

Table 4

Mean recoveries (%) and relative standard deviation (RSD, %) of the target insecticides in sediment samples spiked at various concentrations. Insecticides were analyzed by gas chromatography–negative chemical ionization–mass spectrometry after ultrasound assisted microwave extraction and solid phase extraction cleanup. Matrix-matched standards with internal calibration were used for quantification. $n =$ replicates.

Insecticide	1 ng/g dry weight ($n = 7$)		5 ng/g dry weight ($n = 4$)		20 ng/g dry weight ($n = 4$)	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Terbufos	77.6	12.6	65.2	3.1	75.6	11.1
Diazinon	93.0	12.8	78.6	4.7	79.4	8.9
Tefluthrin	122.8	18.1	101.6	3.8	95.0	8.5
Tebupirimfos	95.8	10.6	128.1	6.5	141.0	12.8
Malathion	81.4	17.7	115.0	12.5	99.6	35.2
Chlorpyrifos	97.5	14.8	106.4	4.0	99.5	8.0
Bifenthrin	96.6	15.1	95.8	4.7	99.5	12.6
<i>Lambda</i> -cyhalothrin	67.8	14.1	76.4	4.8	71.0	18.7
Permethrin	78.0	23.5	93.2	10.3	99.3	14.5
Cyfluthrin	101.2	13.1	84.7	6.5	81.7	12.2
Cypermethrin	82.1	15.4	103.6	15.3	80.7	10.9
Esfenvalerate	84.0	10.3	89.0	17.6	76.6	8.8
Deltamethrin	89.4	15.0	82.1	10.1	80.3	11.9

extracts. Microwave power corresponded to extraction temperature. Compared to traditional conductive heating, bulk heating produced by microwave radiation simultaneously increased the temperature of the entire sample, which resulted in shortened extraction time. Although temperature has been reported to be one of the most important factors affecting extraction efficiency in PMAE [19], it has less effect on FMAE, where temperature was controlled by the boiling point of the extraction solvents. Therefore, no significant temperature effects were observed on insecticides' recoveries when the microwave power was increased from 80 to 100 W. Conversely, with the increase of the microwave power from 100 to 150 W, losses of analytes might occur (Table 3). The higher microwave powers shorten the time to reach the maximum extraction temperature (e.g. the boiling point of the extraction solvents), and prolonged the exposure time of the analytes at the elevated temperatures. As the result, some labile OPs may have experienced thermal-degradation.

In summary, the optimized methods included the use of a 100 ml mixture of hexane and acetone (1:1, v/v) as extraction solvents, and extraction was conducted with simultaneous radiation of 100 W of microwave and 50 W of ultrasound for 6 min. After extraction, the sample was decanted and washed with an additional 50 ml of extraction solvents.

During the UAME optimization process, extremely high recoveries were noted for the pyrethroid insecticides (172–279%) when the calibration standards were made in pure hexane solution. Similar phenomenon has been previously reported, and has been termed matrix-induced chromatographic response enhancement [28]. Compared to the calibration standards made in pure hexane, analytes provided greater responses in sediment extracts due to the coverage of active sites on the GC inlet where adsorption and isomerization of pyrethroids can occur [29]. In addition, the complicated ionization process that occurs during GC/MS analysis may magnify the matrix effect, therefore using matrix-matched standard calibration solutions along with IS calibration was suggested to help improve quantification accuracy [11]. However, the use of ^{13}C -pyrethroid as an IS for the GC–NCI–MS analysis was practically impossible because the labeled carbon is located in the benzene ring in the commercialized ^{13}C -pyrethroid and it could not be detected in NCI mode. Thus, in the current study, calibration standards were made in matrix-matched extracts, and parathion- d_{10} was used as the IS for quantification for the OPs, while PCB-189 was used as the IS for the pyrethroids and surrogates (DBOFB and DCBP).

Conversely, low recoveries (<60% in general and <10% for terbufos) were found for the OPs. After spiking, GIG sediment was stored in the dark at 4 °C for 11 days before extraction, and degradation

of the OPs occurred during the aging period which resulted in the low recoveries. It has been previously reported that some OPs have short half lives in sediment [30]; therefore, the spiked sediment was only aged for 12 h prior to extraction for the method validation experiment.

3.2. Method validation

3.2.1. Accuracy, precision and sensitivity of the developed method

Sediments spiked at 1, 5 and 20 ng/g dw were used to evaluate the accuracy and precision of the newly developed UAME method and recoveries and relative standard deviations (RSDs) of the target insecticides at various concentrations are listed in Table 4. Method accuracy was expressed as recoveries of the target insecticides, which ranged from 67.8% to 123%, from 65.2% to 128%, and from 71.0% to 141% for at the spiking levels of 1, 5 and 20 ng/g dw, respectively. Moreover RSDs were used to evaluate the precision of the analytical method. The RSDs were less than 20% with the exception of permethrin at 1 ng/g dw (23.5%) and malathion at 20 ng/g dw (35.2%). Results showed that the matrix-induced chromatographic response enhancement encountered for the pyrethroids during the method development experiment was sufficiently compensated for by the combination of matrix-matched calibration standards and internal standard calibration during GC/MS quantification.

The sensitivity of the method was assessed by calculating a MDL, which was defined as the minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration was greater than zero [31]. The MDL was derived from the standard deviations of the target insecticides at 1 ng/g dw using Eq. (1) as follows:

$$\text{MDL} = st_{(0.99, n-1)} \quad (1)$$

where s was the standard deviation of the replicates, and $t_{(0.99, n-1)} = 3.14$ was a t -distribution value taken at a confidence level of 0.99 and degrees of freedom of 6. As shown in Table 1, the MDLs varied from 0.31 to 0.45 ng/g dw and from 0.27 to 0.70 ng/g dw for the OPs and pyrethroids, respectively.

3.2.2. Comparison of ultrasound assisted microwave extraction, Soxhlet extraction and sonication extraction

The newly developed UAME method was also validated by comparing it to the traditional Soxhlet extraction and previously developed sonication extraction [5] to analyze HB sediment spiked with the target insecticides at 30 ng/g dw. Four replicates were used for each extraction technique and recoveries (\pm SD) of pyrethroids ranged from $86 \pm 8\%$ to $117 \pm 11\%$, from $73 \pm 7\%$ to $94 \pm 8\%$, and from $76 \pm 4\%$ to $92 \pm 11\%$ for UAME, Soxhlet and sonication extrac-

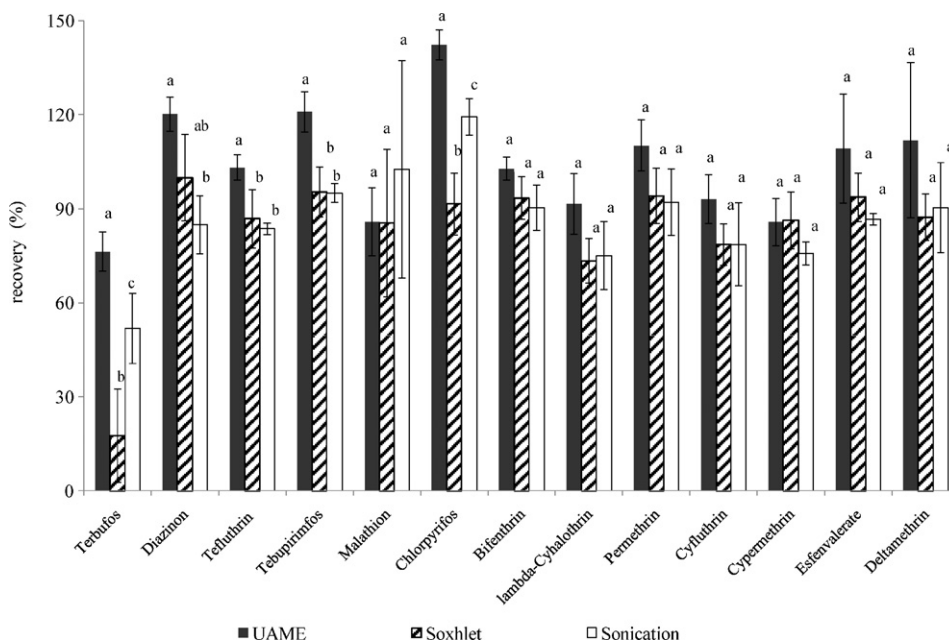


Fig. 2. Comparison of ultrasound assisted microwave extraction (UAME), Soxhlet extraction and sonication extraction methods for analyzing sediment spiked with the target insecticides at 30 ng/g dry weight. Different letters ((a), (b), (c)) indicated significant difference among extraction methods for the same insecticide, while the same letter showed no significant difference between methods. Statistical analysis was conducted using a Tukey's HSD test with SAS version 8.02 (SAS Institute Inc., Cary, NC, USA), $\alpha=0.05$.

tion methods, respectively. With the exception of terbufos, OPs' recoveries (\pm SD) ranged from $86 \pm 11\%$ to $142 \pm 5\%$, from $84 \pm 24\%$ to $100 \pm 14\%$ and from $85 \pm 9\%$ to $119 \pm 6\%$ for UAME, Soxhlet and sonication extraction methods, respectively. Recoveries (\pm SD) of terbufos were lower at $76 \pm 6\%$, $18 \pm 15\%$, and $52 \pm 11\%$ for UAME, Soxhlet extraction and sonication extraction, respectively. Extraction efficiencies of the target insecticides using the three methods were compared using a Tukey's HSD test, and the results are shown in Fig. 2. No significant difference ($p > 0.05$) was observed between the extraction efficiencies of Soxhlet and sonication extraction methods for most of the insecticides with the exception that sonication recovered more terbufos and chlorpyrifos. In contrast, the UAME method provided greater recoveries for four out of five OPs in comparison to the other two extraction methods ($p < 0.05$). The three extraction methods showed no significant difference in recoveries of the pyrethroids with the exception of tefluthrin (Fig. 2).

Some OPs are labile and prone to degrade in the environment [30,32], and our preliminary data showed low recoveries for the OPs (<60% in general and <10% for terbufos) from the spiked sediment aged for 11 days. In contrast, acceptable recoveries were achieved using the UAME and sonication methods when the spiked sediment was only aged for 12 h, although recovery of terbufos was low using Soxhlet extraction. Therefore, the loss of compound during the aging period was most likely not the reason for the low terbufos recovery during Soxhlet extraction. With a boiling point

of 69°C , terbufos was the most volatile compound of the target insecticides, thus it may experience loss by volatilization and/or thermal-degradation during the 48 h Soxhlet extraction. Therefore, UAME was a better choice for extracting the thermally labile and volatile compounds (OPs in the current study) by extensively reducing the exposure time for these compounds to heat.

The overall better extraction efficiency of the UAME method compared to the traditional extraction methods may be attributed to its synergistic effect by hybridizing two energetic radiations [21]. Through the dielectric mechanism, the matrix absorbs microwave radiation and forms bulk heating, which provides energy for the extraction process [19]. Different from microwave radiation, the ultrasound effect was a result of cavitation, which was a phenomenon of the formation, growth, and implosive collapse of bubbles in the liquid. The collapse of bubbles and associated shock wave produced high velocity inter-particle collisions and intense local heating and high pressures conditions [33]. By coupling microwave and ultrasound radiations in the UAME technique, the extraction process was accelerated by improved dielectric heating, effective agitation, and enhanced mass transfer. As a result, extraction efficiency was improved, extraction time was reduced, and loss of compounds was minimized.

3.2.3. Analysis of field-contaminated sediments

Five field-contaminated sediment samples were collected from creeks in Guangzhou, China and extracted using the newly devel-

Table 5
Concentrations (ng/g dry weight) of organophosphate and pyrethroid insecticides in field-contaminated sediments collected from Guangzhou, China. Extracts were analyzed by gas chromatography–negative chemical ionization–mass spectrometry after ultrasound assisted microwave extraction and solid phase extraction cleanup.

Sample ID	GZ (1)	GZ (2)	GZ (3)	GZ (4)	GZ (5)
Terbufos	1.8	<RL	<RL	<RL	<RL
Chlorpyrifos	1.3	1.3	ND	27.4	<RL
Lambda-cyhalothrin	1.7	<RL	4.5	<RL	<RL
Permethrin	12.2	<RL	<RL	<RL	<RL
Cypermethrin	8.1	1.4	27.9	15.5	6.7
Esfenvalerate	1.4	<RL	<RL	2.27	<RL

<RL: less than reporting limits which equalled three times the method detection limits and were reported in Table 1.

oped UAME method, cleaned by SPE, and the target insecticides analyzed by GC/MS. Analytical results of the five sediment samples are shown in Table 5. Chlorpyrifos was the most frequently detected OP, and was found in three of the five samples with the highest concentration of 27.4 ng/g dw. Another OP terbufos was detected in one sample at 1.8 ng/g dw. Four pyrethroids were detected in the sediments and included *lambda*-cyhalothrin, permethrin, cypermethrin, and esfenvalerate. Cypermethrin was detected in every sample at concentrations ranging from 1.4 to 27.9 ng/g dw. No detectable amounts of diazinon, malathion, tebuirimfos, tefluthrin, bifenthrin, cyfluthrin, and deltamethrin were found in any of the five samples, therefore they were not included in Table 5.

After banning of the persistent organochlorine insecticides in 1984, OPs dominated the insecticide market in China; however, the use of pyrethroids has increased steadily. In 2007, some OPs were banned in China due to their high mammalian toxicity, and their replacements, such as chlorpyrifos and pyrethroids, noted significant increases in their usage. The high frequency of detection of chlorpyrifos and pyrethroids in the field-collected sediment samples reflected this trend. It has been reported that bifenthrin was the most frequently detected pyrethroid insecticide in sediments in the USA [2,34,35], however, cypermethrin was the greatest concern in our sediment samples from China (Table 5). To our knowledge, there is no reported data on pyrethroid sediment contamination in Southeast China. Cypermethrin and esfenvalerate were reported as the highest used pyrethroids in Guangdong Province, China where Guangzhou is located, and a previous study reported cypermethrin was the most frequently detected pesticide residues in vegetables collected in Guangdong Province with cypermethrin detected in 21.7–31.8% in various types of vegetable samples [36]. These results are consistent with our findings.

4. Conclusions

In the current study, a UAME method was developed to extract current-use insecticides from sediment, and the target insecticides were quantified by GC/MS in NCI mode after SPE cleanup. By combining microwave and ultrasound technologies, the UAME method provided satisfactory recoveries for the target compounds. Due to a significant reduction in extraction time, the UAME method greatly improved extraction efficiency of the thermally labile and volatile insecticides.

Acknowledgments

This work was partially supported by the State Key Laboratory of Organic Geochemistry (Grant No. SKLOG2009A04), the Hundred

Talents Program of the Chinese Academy of Sciences (Grant No. kzcx2-yw-BR-05) and the National Natural Science Foundation of China (Grant No. 40971263) GIGCAS contribution 1244.

References

- [1] Z.L. Zhang, H.S. Hong, J.L. Zhou, G. Yu, J. Environ. Monit. 4 (2002) 498.
- [2] D.P. Weston, J. You, M.J. Lydy, Environ. Sci. Technol. 38 (2004) 2752.
- [3] M.B. Woudneh, D.R. Oros, J. Chromatogr. A 1135 (2006) 71.
- [4] E.R. Bennett, M.T. Moore, C.M. Cooper, S. Smith Jr., Bull. Environ. Contam. Toxicol. 64 (2000) 825.
- [5] J. You, D.P. Weston, M.J. Lydy, Arch. Environ. Contam. Toxicol. 47 (2004) 141.
- [6] W.A. House, J.L.A. Long, J.E. Rae, A. Parker, D.R. Orr, Pest Manag. Sci. 56 (2000) 597.
- [7] F.A. Esteve-Turrillas, C.S. Aman, A. Pastor, M. de la Gurdia, Anal. Chim. Acta 522 (2004) 73.
- [8] K.L. Smalling, K.M. Kuivila, J. Chromatogr. A 1210 (2008) 8.
- [9] J. You, D.P. Weston, M.J. Lydy, ACS Symp. Ser. 991 (2008) 87.
- [10] J. You, D. Wang, M.J. Lydy, Talanta 81 (2010) 136.
- [11] D. Wang, J. You, M.J. Lydy, Arch. Environ. Contam. Toxicol. 59 (2010) 382.
- [12] J. Szpunar, V.O. Schmitt, O.F.X. Donard, R. Lobifiski, Trends Anal. Chem. 15 (1996) 181.
- [13] Y.Y. Shu, T.L. Lai, H.-s. Lin, T.C. Yang, C.-P. Chang, Chemosphere 52 (2003) 1667.
- [14] K. Ganzler, A. Salgo, K. Valko, J. Chromatogr. 371 (1986) 299.
- [15] I. Silgoner, R. Kraska, E. Lombas, O. Gans, E. Rosenberg, M. Grasserbauer, Fresenius J. Anal. Chem. 362 (1998) 120.
- [16] V. Camel, Trends Anal. Chem. 19 (2000) 229.
- [17] M.C. Hernández-Soriano, A. Peña, M.D. Mingorance, Anal. Bioanal. Chem. 389 (2007) 619.
- [18] U.S.EPA, EPA 3546, US Environmental Protection Agency, Washington, DC, 2007.
- [19] J.R. Dean, Sci. Total Environ. 15 (2000) 2914.
- [20] G. Cravotto, P. Cintas, Chem. Eur. J. 13 (2007) 1902.
- [21] G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro, P. Cintas, Ultrason. Sonochem. 15 (2008) 898.
- [22] C. Domini, L. Vidal, G. Cravotto, A. Canals, Ultrason. Sonochem. 16 (2009) 564.
- [23] S. Chemat, A. Lagha, H.A. Amar, F. Chemat, Ultrason. Sonochem. 11 (2004) 5.
- [24] Z. Lou, H. Wang, S. Zhu, M. Zhang, Y. Gao, C. Ma, Z. Wang, J. Chromatogr. A 1217 (2010) 2441.
- [25] L. Zhang, Z. Liu., Ultrason. Sonochem. 15 (2008) 731.
- [26] J. Ji, C. Deng, H. Zhang, Y. Wu, X. Zhang, Talanta 71 (2007) 1068.
- [27] M. Ericsson, Doctoral Dissertation, Stockholm University, Sweden, 2003.
- [28] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. 638 (1993) 57.
- [29] J. You, M.J. Lydy, J. Chromatogr. A 1166 (2007) 181.
- [30] J.A. Noblet, L.A. Smith, I.H. Suffet, J. Agric. Food Chem. 44 (1996) 3685.
- [31] J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, Environ. Sci. Technol. 15 (1981) 1426.
- [32] K.V. Ragnarsdottir, J. Geol. Soc. 157 (2000) 859.
- [33] S.J. Doktycz, K.S. Suslick, Science 247 (1990) 1067.
- [34] D.P. Weston, R.W. Holmes, J. You, M.J. Lydy, Environ. Sci. Technol. 39 (2005) 9778.
- [35] E.L. Amweg, D.P. Weston, J. You, M.J. Lydy, Environ. Sci. Technol. 40 (2006) 1700.
- [36] F. Deng, C. Liang, W. Huang, H. Li, X. Xu, S. Hu, M. Chen, S. Fan, C. Long, F. Lv, X. Liang, H. Liang, Z. Zhong, Y. Gao, L. Lu, Chin. J. Food Hyg. 19 (2007) 1.