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Analysis of Eight Organophosphorus Pesticide Residues in Fresh Vegetables Retailed in Agricultural Product Markets of Nanjing, China

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Abstract A method to effectively remove pigments in fresh vegetables using activated carbon followed cleanup through solid phase extraction (SPE) cartridge to further reduce matrix interference and contamination, was established to determine eight organophosphorous pesticides (OPPs) by gas chromatography (GC) with nitrogen–phosphorus detection (NPD) in this study, and it has been successfully applied for the determination of eight OPPs in various fresh vegetables with the recoveries ranging from 61.8% to 107%. To evaluate eight OPPs residue level, some fresh vegetables retailed at three agricultural product markets (APM) of Nanjing in China were detected, the results showed that phorate in Shanghai green (0.0257 $\mu g\ g^{-1}$) and Chinese cabbage (0.0398 $\mu g\ g^{-1}$), dimethoate in Shanghai green (0.0466–0.0810 $\mu g\ g^{-1}$), Chinese cabbage (0.077 $\mu g\ g^{-1}$), and spinach (0.118–0.124 $\mu g\ g^{-1}$), methyl-parathion in Shanghai green (0.0903 $\mu g\ g^{-1}$), Chinese cabbage (0.157 $\mu g\ g^{-1}$), and spinach (0.0924 $\mu g\ g^{-1}$), malathion in Shanghai green (0.0342–0.0526 $\mu g\ g^{-1}$), chorpyrifos in spinach (0.106–0.204 $\mu g\ g^{-1}$), and Chinese cabbage (0.149 $\mu g\ g^{-1}$), chlorfenvinfos in carrot (0.094–0.131 $\mu g\ g^{-1}$), were found. However, fonofos and fenthion were not detected in all the collected vegetable samples.

Keywords Fresh vegetables · Organophosphorous pesticides · Activated carbon · Gas chromatography · Nitrogen–phosphorus detection

Currently organophosphorus pesticides (OPPs) enjoy wide use in the world as an alternative to pest control with organochlorine pesticides (Lyton et al. 1996; Subhani et al. 2001; Toan et al. 2007), which leads to increased world food production. Nevertheless, this use and additional environmental pollution due to industrial emission during their production have resulted in the occurrence of residues of these chemicals and their metabolites in food commodities, water, and soil (Amoah et al. 2006; Randhawa Atif et al. 2007; Aldana-Madrid et al. 2008). Recent literature reveals that the largest proportion of human acute toxicity is related to OPPs intoxication (Ecobichon 2001; Cabras and Angioni 2000). Therefore, there is an increasing human health concern of OPPs residue in fresh vegetables and fruits (Sun et al. 1977; Torres et al. 1996). Many countries have set maximal residue limits (MRL) of used pesticides and take strong measures to detect their residues in food community (Maybury 1989). GC is one of the most effective methods to determine OPPs residues (Barcelò 1993; Grosser et al. 1993; Tekel and Kovacicova 1993; Sanchez-Brunete et al. 1994). In the analysis of OPPs in fresh vegetables using GC, interference and contamination resulting from pigments and matrix were usually encountered. Although silica gel and Florisil cartridge were commonly used to diminish the effect of matrix (Wang et al. 2004, 2005; Tadeo et al. 2000; Randhawa Atif et al. 2007; Zhang et al. 2007), neither at normal dosage in a cartridge is ideal to eliminate pigments in some fresh vegetables with too much pigments. An effective material is needed to remove pigments in fresh vegetables, activated

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carbon may meet this demand owing to its cheapness and strong adsorption character. The objectives of this study were (1) to establish methods to effectively remove pigments in fresh vegetables using activated carbon and determine eight OPPs residue using GC with NPD, and (2) to investigate the eight OPPs residue level in fresh vegetables retailed in representative agricultural product markets of Nanjing city, Southeastern China. The obtained data are expected to evaluate OPPs residue levels in fresh vegetables in Nanjing city.

Materials and Methods

Eight OPPs (fonofos, methyl-parathion, malathion, chlorpyrifos, fenthion, chlorpyrifos, phorate, and dimethoate) were selected to test. These pesticides are high-performance liquid-chromatography-grade reagents with a purity of 99.5%, provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). *n*-hexane (Shanghai Ludou Chemical Reagents Factory, China), acetone, ethyl acetate, hydrochloric acid, petroleum ether, anhydrous sodium sulfate (Nanjing Chemial Reagent no 1 Factory, China), silical gel (Dalian Institute of Physical Chemistry, Chinese Academy of Sciences, China), and active carbon (Institute of Chemical Engineering, Chinese academy of Forestry Sciences, Beijing, China) were used in this study, and all the preceding chemicals used are of analytical purity grade.

Acidified activated carbon (AAC) was used to eliminate pigments in plant remains during the sample treatment, it was derived from activated carbon which was treated with hydrochloric acid (3 mol L^{-1}) for 24 h, then eluted with distilled water till no chlorin anion was detected, finally dried at 120°C.

The collected fresh vegetable samples were undergone the following treated processes: washed clean, wiped dry, and homogenized. The extraction of OPPs in vegetable material was done by adding 15 g anhydrous sodium sulfate and 20 g ethyl acetate to 10 g of the homogenized sample in a 50 mL centrifuge tube. The mixture was blended for 2 min in a mixer at 3,000 rpm, and was then placed in a water bath at 25°C for a 30 min shaking period. The supernatant liquid was filtered through Whatman No. 1 filter paper. The filtrate (10 mL) was transferred into a 40-mL centrifuge tube, 0.05–0.1 g AAC was added to remove soluble plant pigments, and then shaking and filtering again, as described above. The filtrate was concentrated to 0.5 mL in a rotary evaporator at 40°C, then passed through a solid phase extraction cartridge that had been washed with hexane to remove interfering components. The eluate in hexane from the column was collected in a 25 mL pear flask and concentrated to 1 mL, 1 μL of which was injected into GC–NPD for OPPs analysis. Solid phase extraction cartridges were prepared by the method described by Wang et al. (2004).

A Hewlett-Packard HP6890 GC with a nitrogen–phosphorus detector was used for separation and quantitative analysis, and a Hewlett-Packard HP-5MS 5% phenyl–methyl–siloxane capillary column (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness) was used for gas chromatographic separation. N_2 at a speed of 22.4 mL min^{-1} was used as the carrier gas, hydrogen (4 mL min^{-1}), and air (60 mL min^{-1}) were used for the NPD (nitrogen–phosphorus detector). The column head pressure was set at 85.7 kPa, and the temperatures programmed for OPPs were set as follows: initial temperature was 60°C for 1 min, then up to 140°C at 12°C min^{-1}, and finally up to 300°C at 8°C min^{-1}, whereupon it was held for 5 min. The injector and detector temperatures were set at 250 and 320°C, respectively, and a 1-μL sample was injected for detection. Retention time was used to identify the correct peak on the chromatogram. The limits of detection of the tested eight OPPs are between 0.001 and 0.005 μg g^{-1}. Other equipment included: a rotary evaporator (RE-52, Shanghai Yarong Biochemistry Apparatus Factory, China) used for pre-concentration, a vacuum pump (1065-V2A, Gast Manufacturing Corporation, USA) used to decrease the pressure in the concentrating step, an ultrasonic water bath (Hengao T&D, China) used for extraction, and a centrifuge (Sigma 2-16K, Osterode, Germany) used for separating the supernatant and solvent phases.

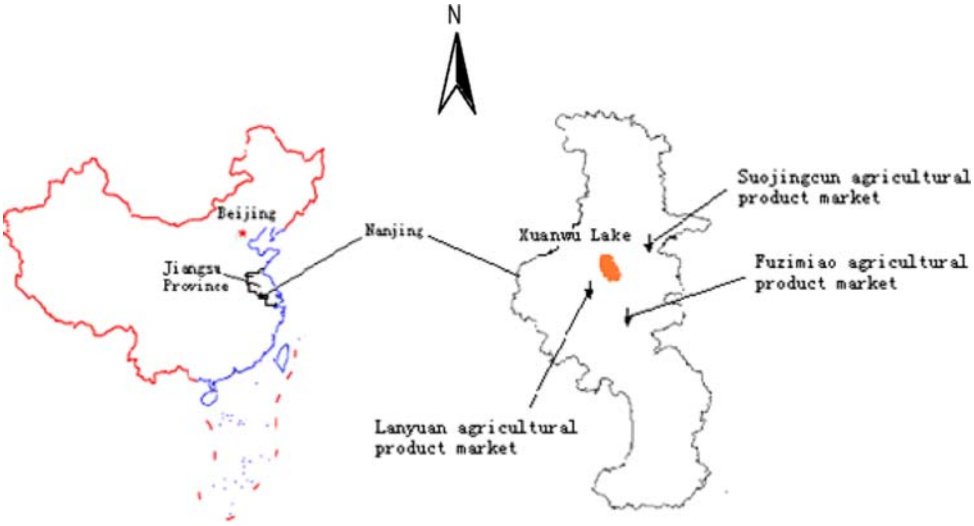
The adsorptive ability of AAC to OPPs was tested. Ten milliliter of ethyl acetate and 1 mL OPPs mixture with a concentration of 1 μg mL^{-1} for each pesticide were sequentially added into 40 mL centrifuge tubes, then 0.05, 0.08, 0.1, 0.2, and 0.5 g AAC were added into the respective tubes, three replicates were conducted for each AAC dosage. After all the tubes were shaken at 25°C for 30 min under water bath, the mixture were filtered through a Whatman No. 1 filter paper, the eluates were collected in 25 mL pear flask and concentrated to 1 mL, 1 μL of which was injected into GC–NPD for OPPs analysis. Recoveries of each pesticide were calculated from the analyzed results and original concentration. The treatment with no added pesticide was served as a control.

Four vegetables (Shanghai green, Chinese cabbage, spinach, and carrot) were sampled from Lanyuan, Suojingcun, and Fuzimiao APM of Nanjing city, China. The schematic sites were presented in Fig. 1. Each fresh vegetable was sampled under normal purchase condition from five randomly selected sellers at each APM.

Results and Discussion

The chromatograms detected by GC–NPD (Fig. 2) showed that eight OPPs (1 μg mL^{-1} each in ethyl acetate) were clearly separated and each had suitable peak height, thereby the selected chromatographic method is practicable.

Fig. 1 Schematic sampling sites of fresh vegetables in Nanjing city, China



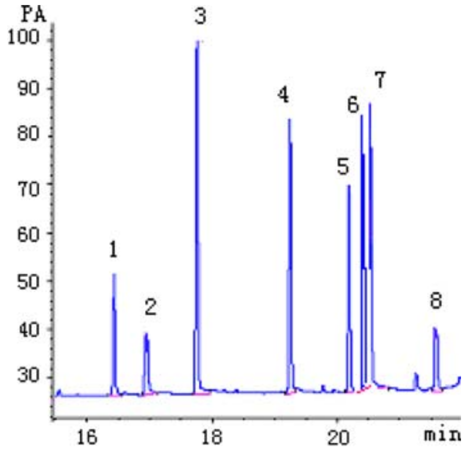


Fig. 2 Chromatograms of Phorate (1), Dimethoate (2) Fonofos (3), Parathion-Methyl (4), Malathion (5), Chlorpyrifos(6), Fenthion (7), and Chlorfenvinfos (8)

AAC was used to eliminate soluble pigments that might lead to contamination and disturbance for chromatographic analysis. However, AAC might adsorb OPPs in vegetable samples so as to cause a decreased recovery which was probably related to the added AAC amount and might become lower with the increased AAC usage amount. Therefore, it is necessary to determine a proper AAC dosage range in which pigments could be substantially eliminated and an acceptable recovery owing to less pesticide adsorption would be ensured. The recoveries of OPPs added in ethyl acetate as solvent were tested followed addition of AAC at 0.05–0.5 g. The obtained results showed (Fig. 3) that the eight OPPs recoveries decreased with the increased dosage of AAC, these indicated a substantial adsorption of AAC to OPPs. However, the recoveries of dimethoate and malathion had a lowest decrease in the AAC usage amount scope (0.05–0.5 g). This should attribute to a weak adsorption of AAC to dimethoate and malathion in ethyl acetate solution. Interestingly, phorate recovery had no obvious variation at this AAC usage range, this might imply a saturated adsorption at 0.1 g dosage. For all tested OPPs in this experiment, their recoveries exceeded 60% at AAC dosage range from 0.05 to 0.2 g. Therefore, 0.05–0.2 g AAC might be an appropriate dosage range.

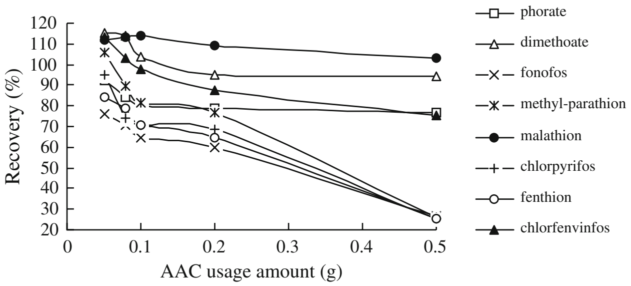


Fig. 3 Recoveries of OPPs in ethyl acetate solution after AAC addition

Fresh Shanghai green vegetable, Chinese cabbage and carrot without OPPs application history during their growth were sampled and homogenized. An aliquot of 10 g homogenized sample was fortified with 1 mL OPPs mixture in which each pesticide had a concentration of 1 μg mL^{-1}, another aliquot without OPPs fortification was served as a control. Through extraction, cleanup, and analysis by the methods described as preceding 2.2, OPPs recoveries were obtained as shown in Table 1. For Shanghai green samples, dimethoate had a little higher recovery (106%), and the other pesticides had ideal recoveries (84.6–102%). For Chinese cabbage samples, all the tested OPPs recoveries are acceptable (78.8–107%). For carrot samples, dimethoate, malathion, and Chlorfenvinfos had proper recoveries (85.1–110%), but the others had a little lower recoveries (51.8–63.6%).

In treated fresh vegetable samples, the added AAC might have a competitive adsorption between pigment and

Table 1 Recoveries of OPPs fortified in fresh vegetables (%)

	PR	DT	FF	MP	MT	CP	FT	CF
Shanghai green	85.9	106	102	99	99.2	93.9	84.6	96.9
Chinese cabbage	107	106	84.6	86.9	105	84.4	78.8	106
Carrot	61.8	105	63.6	63	110	64	68.1	85.1

Note: PR denotes phorate, DT denotes dimethoate, FF denotes fonofos, MP denotes methyl-parathion, MT denotes malathion, CP denotes Chlorpyrifos, FT denotes fenthion, CF denotes Chlorfenvinfos

OPPs. The obtained OPPs recoveries would be related to adsorptive ability of AAC to both OPPs and pigment species in fresh vegetable samples. A stronger adsorption ability of AAC to pigments and a weaker one to OPPs possibly bring higher OPPs recoveries. Otherwise, lower OPPs recoveries would be presented. In this study, all the tested OPPs have suitable recoveries in three fresh vegetable, and this indicates that AAC might have a higher adsorptive ability to pigments than to OPPs.

Shanghai green vegetable without OPPs application history during their growth were sampled and homogenized. An aliquot of 10 g homogenized sample each was fortified at different OPPs levels (0.01–1 μg g^{-1}), another aliquot without OPPs fortification was served as a control. Through extraction, cleanup, and analysis by the methods described as preceding 2.2, OPPs recoveries were obtained as given in Table 2. Chlorfenvinfos and chlorpyrifos had little higher recoveries at fortification levels of 0.5 and 1.0 μg g^{-1}, and 0.2–1 μg g^{-1}, respectively. The other OPPs recoveries at different fortification levels fell in a reasonable extent. The preceding results showed that the established methods were practicable to analyse the selected OPPs.

OPPs residues in fresh vegetables collected from Lanyuan, Suojingcun, and Fuzimiao APM of Nanjing were detected, the results were presented in Table 3.

Table 2 Recoveries of OPPs fortified in Shanghai green samples at different levels

Pesticides	Fortified OPPs level (μg g^{-1})				
	0.01	0.1	0.2	0.5	1.0
Phorate	96.8	101	106	103	104
Dimethoate	107	103	106	97	95.4
Fonofos	70.9	77.4	77	81.6	83
Methyl-parathion	72.8	75.4	79.2	88	92.2
Malathion	101	108	102	106	97.8
Chlorpyrifos	103	105	107	108	106
Fenthion	77.6	70.8	75.6	82.2	91
Chlorfenvinfos	97.9	107	109	124	125

Chlorfenvinfos, a kind of soil insecticide, was found in carrot samples from Lanyuan and Suojingcun APM at concentrations of 0.131 and 0.094 μg g^{-1}, respectively, which exceeded its MRL (0.05 μg g^{-1}) set by Chinese Ministry of Agriculture [Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2001]. This implied that chlorfenvinfos could have been non-professionally used at an excessive dosage in carrot planting area. Malathion residues (0.0342–0.0526 μg g^{-1}) in Shanghai green collected from the three APM exceeded its MRL (non-detectable). Although phorate had relatively lower occurrence, its residue in Shanghai green (0.0257 μg g^{-1}) and Chinese cabbage (0.0398 μg g^{-1}) exceeded MRL (non-detectable). Malathion and phorate were prohibited to be used in vegetable field according to announcements issued by Chinese Ministry of Agriculture [Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2001], owing to their acute toxicity to human. However, this prohibition was not seriously put into practice in vegetable production in China, further investigation would be thereby required.

Dimethoate had the highest occurrence in all the detected OPPs at concentrations of 0.0466–0.124 μg g^{-1} below its MRL (1 μg g^{-1}) [Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2001]. Chlorpyrifos residue was detected in spinach (0.106–0.204 μg g^{-1}) and Chinese cabbage (0.149 μg g^{-1}) below its MRL (1 μg g^{-1}) [Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2001]. Methyl-parathion residue in Shanghai green (0.0903 μg g^{-1}) and Chinese cabbage (0.157 μg g^{-1}) samples from Suojingcun APM did not exceed its MRL (0.2 μg g^{-1}) [Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2001], either. Dimethoate, chlorpyrifos, and methyl-parathion were the most common OPPs applied in vegetable production of China. Nevertheless, their residues in the fresh vegetables in this investigation did not exceed respective MRL. This illustrated that the general OPPs application in field had gradually became more and more professional in China. Fonofos and fenthion were not detected in all vegetable samples, this possibly attributes to the fact that both are seldom or never applied in local vegetable planting area.

It is undoubted that pesticides should be applied correctly according to their recommended dosage and usage frequency. However, in order to benefit from vegetable planting, OPPs were always applied in excessive dosage and frequency during the growing seasons of vegetables and resulted in food contamination (Zhang et al. 2007). The results in this investigation provided important information on the current contamination status of fresh vegetables in Nanjing city of China, and pointed to the need of taking further measures to control the misuse of some OPPs, such as chlorfenvinfos, malathion, and phorate.

Table 3 Eight OPPs residues in four fresh vegetables retailed in agricultural product markets (μg g^{-1} fresh weight)

Site	Vegetable	PR	DT	FF	MP	MT	CP	FT	CF
LY	Shanghai green	0.0257 [2/5]	0.0466 [2/5]	ND	ND	0.0342 [1/5]	ND	ND	ND
	Chinese cabbage	ND	ND	ND	ND	ND	ND	ND	ND
	Carrot	ND	ND	ND	ND	ND	ND	ND	0.131 [1/5]
	Spinach	ND	0.124 [2/5]	ND	ND	ND	0.204	ND	ND
SJC	Shanghai green	ND	0.0804 [2/5]	ND	0.0903 [2/5]	0.0457 [1/5]	ND	ND	ND
	Chinese cabbage	0.0398 [2/5]	ND	ND	0.157 [3/5]	ND	ND	ND	ND
	Carrot	ND	ND	ND	ND	ND	ND	ND	0.094 [2/5]
	Spinach	ND	0.118 [2/5]	ND	ND	ND	0.106 [1/5]	ND	ND
FZM	Shanghai green	ND	0.0810 [2/5]	ND	ND	0.0526 [3/5]	ND	ND	ND
	Chinese cabbage	ND	0.077 [2/5]	ND	ND	ND	0.149 [1/5]	ND	ND
	Spinach	ND	ND	ND	0.0924 [3/5]	ND	0.145 [2/5]	ND	ND
	Carrot	ND	ND	ND	ND	ND	ND	ND	ND

Notes: LY denotes Lanyuan agricultural product market, SJC denotes Suojingcun agricultural product market, FZM denotes Fuzimiao agricultural product market, PR denotes phorate, DT denotes dimethoate, FF denotes fonofos, MP denotes methyl-parathion, MT denotes malathion, CP denotes Chlorpyrifos, FT denotes fenthion, CF denotes Chlorfenvinfos, ND denotes no detected, the figures in square brackets denote numbers of positive samples in the total of five samples

The established method was successfully used to detect eight OPPs residues in fresh vegetables, in which AAC and solid phase extraction cartridge were used to reduce contamination and interference from pigments and matrix. The detected results of fresh vegetables retailed in three APM of Nanjing showed that chlorfenvinfos, malathion, and phorate residues were above their respective MRL set by Chinese government.

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