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# Identification of Pesticide Contamination in Water Sources Surrounding Agrochemical-Free Rice Farming in Battambang Province

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**Abstract:** *Pesticide application is a critical factor in the conventional farming system to improve crop protection and yield during growth stage until post-harvest. The agrochemical residue in food product is a serious problem for consumer and also pollutes the local environment like air, soil, and water sources. Water is the main constituent for both conventional and organic paddy farming. Thus agrochemical-free paddy farming can contaminate with pesticide residue from the surrounding environment. In this study, the agrochemical-free paddy field experiment locates at Research and Training Farm, National University of Battambang (NUBB), Battambang, Cambodia. The main purpose of the present investigation was to identify the pesticide residues in common water sources of the agrochemical-free paddy field. Water samples were collected in paddy fields, canal system and nearby upstream of the NUBB's farm in 2021 and 2022. Thirteen collected water samples were analyzed using multiresidue pesticide analysis. Water samples were extracted by solid-phase extraction (SPE) and analyzed by gas chromatography-mass spectrometry (GC-MS). Recovery studies were performed at 1 µg.L$^{-1}$ fortified level. Twenty-one out of 34 reference pesticides presented satisfy range 60-120 % of recovery rate with relative standard deviation, RSD < 30 % at the fortified level. The remaining 13 compounds gave the recovery yield below 70 % with RSD < 20 %. Twenty out of total pesticide standards had a limit of detection (LOD) and limit of quantification (LOQ) less than 0.01 and 0.0333 µg.L$^{-1}$, respectively. After multiresidues pesticide analysis, azoxystrobin, biphenyl, chlorfenapy and methyl parathion were detected in paddy water and water from the canal system. However, 2-phenylphenol, isoprothiolane and terbcarb (mbpmc) were not in the list of 34 reference standards. 2-phenylphenol and isoprothiolane are slight to moderate hazardous while terbcarb (mbpmc) is an obsolete pesticide in the WHO classification. The abovementioned pesticide compounds were detected in water collected from paddy fields and different points in the canal system. This means that the surrounding environment contaminates agrochemical-free paddy fields through agricultural activities and water sources. Further analysis of other samples like paddy grain, soil and sediment should be considered.*

**Keywords:** Agrochemical-free farm; Multiresidue pesticide; Solid phase extraction; Gas chromatography-Mass spectrometry

## 1. INTRODUCTION

Pesticide application is an essential tool as a plant defense agent for raising crop yield and food production during the development of agriculture. These pesticides leave their residues in food and thereby produce problems when concentration pass the maximum residue limit (MRL) [1]. In Cambodia, farmers use pesticides widely in crop, fruit, and vegetable production to increase yield and protect products from any pests, weeds and diseases. Rice is one among the most important crops and dominates most daily dietary intake [2]. In connection to Cambodia, the conventional paddy farming system is performed. The extensive use of pesticide and fertilizer input is one factor of conventional farming for increasing protection efficiency and crop productivity. Since Cambodia has no manufacturing of pesticide products, they are imported from Vietnam and Thailand. Schreinemachers et al. [3] reported the volume of pesticide products imported to Cambodia with an annual growth rate estimated at 61 %. Matsukawa et al. [4] was conducted a survey on the current status of pesticide use among rice farmers

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in Takeo, Prey Veng and Siem Reap in 2014. The survey demonstrated that 9 active ingredients (AIs) of herbicide products like Aryloxyphenoxy propionate herbicide (FOPs), sulfonylurea pyrimidinyl enzoate, amide, chloroace-tamide, quinolinecar-boxylic acid and phenoxycarboxylic acid were commonly used by farmers to control weed. Moreover, 30 AIs in insecticide products frequently used were in carbamate, organic phosphorus, phenylpyrazole, pyrethroid, neoicotinoid, avermectin, pymetrozine, nereis-toxin, benzoyl urea, indoxacarb, buprofezin, and diamide group. For regular usage the fungicide products exist 20 AIs in MBC, QoI, hexopyranosyl antibiotic, glucipyranosyl anti-biotic, tetracycline antibiotic, dithiolane, DMI, MBI-R, MBI-D inorganic, dithiocarbamate, and dicarboximide group.

After applied agrochemical products, they frequently left their residues into surrounding environment and became toxic to humans. A high concentration of these agrochemical residue in food could affect human health. For safety, many alternative farming systems were developed to reduce pesticide consumption in the agricultural sector. Organic farming is the agrochemical-free farming system and one alternative to produce safe agricultural products. Moreover, demanding organic rice in the international markets has been increasing in Cambodia since 2013 [5]. According to Cambodia Rice Federation (CRF), organic rice exported to EU markets reached 9.5 thousand tons in 2020 [6]. However, AIs of pesticides can corporate with soil particles and stay in the soil for years. They further continue to damage flora and fauna in the soil. The pesticides also contaminate water sources through factors like runoff, seepage or draining out [7]. Even agrochemical-free farming is not used for pesticide application through the plant cycle, but pesticide residue can contaminate to the farm from the surrounding area primarily through water sources for irrigation. The advanced analysis of pesticide residues in water is required to respond to the abovementioned hypothesis.

The study aims to identify the pesticide residues in familiar water sources from the agrochemical-free paddy field experiment at Research and Training Farm, National University of Battambang (NUBB), Battambang, Cambodia. The research provides preliminary information of current risk and alters awareness of water contaminated by pesticide residue from agricultural activities.

## 2. METHODOLOGY

### *2.1. Study area*

The agrochemical-free paddy field surrounded by the paddy cultivated lands was investigated at Research and Training Farm, NUBB, Battambang, Cambodia (12°59'45.10"N latitude and 103°19'6.36"E longitude) (see Fig. 1). Farmers commonly use pesticides and chemical fertilizers in the surrounding paddy fields during growth stage. The canal irrigation system in the farm is an earthen dug canal to the river and Srah Keo reservoir that diverts and stores the water for year-round farming activities. Therefore, the main study area is on the canal in/outside NUBB's farm and paddy fields.

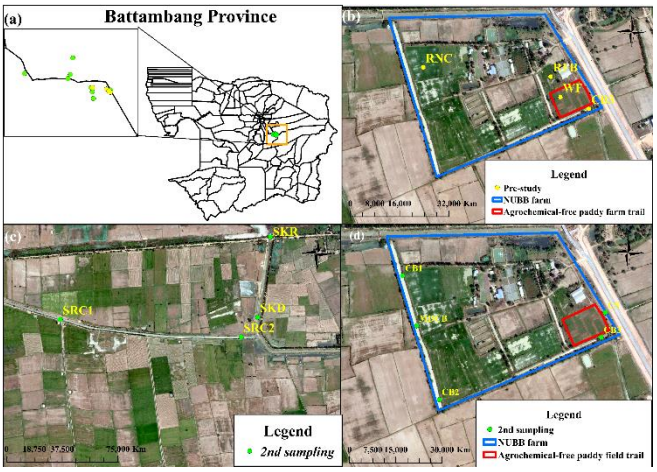


**Fig. 1.** Sampling location of water sample: (a) all sampling points; (b) sampling point during pre-study in 2021, (c) sampling point at nearby upstream in 2022 (d) sampling points inside NUBB's farm in 2022.

### *2.2. Sample collection*

The first pre-study of pesticide residues in the irrigated water of agrochemical-free paddy field trail was conducted in 2021. The water samples were taken from the field trail, two conventional paddy fields and one point in the canal system next to the field trail. According to the result of pesticide analysis in pre-study, water sampling points had extended to outside the NUBB's farm (see Fig. 1). Table 1 presents GPS coordinate of each sampling point during the study. Water samples were collected in duplicate in 1 L plastic bottles and immediately stored in the ice box before transporting to the chemical laboratory at Institute of Technology of Cambodia.

### *2.3. Standards and reagents*

The analysis was investigated using HPLC-grade solvents such as acetone, dichloromethane, n-hexane (RCI Labscane Limited, Thailand); ultrapure water purified by Puric-α ultrapurewater Technology (Organo, Japan); sodium sulfate anhydrous (Sigma-aldrich, Inc.); and InertSep (PLS-3) and InertSep Slim-J (AC2) (GL Sciences, Japan).

Pesticide standards, aldrin, azoxystrobin, buprofezin, carbofuran, chloroneb, chlorothalonil, , dieldrin, heptachlor, hexaconazole, isazofos, isoxathion, lindane, malathion, metalaxyl, methyl parathion, o,p'-DDT, triadimefon, paclobutrazol, propanil, chlorpyrifos, fenobucarb, metolachlor, permethrin, phenthoate, and pretilachlor were purchased from Chem Service Inc. For bifenthrin, difenoconazole, fipronil, propiconazole, biphenyl, chlorfenapyr, dimethamethryn, mevinphos, and pyroquilon were purchased from LGC Ltd and

Fujifilm Wako Pure Chemical Corporation. The purity of pesticide standards are in the range of 96.3% - 99.9%.

**Table 1** GPS coordinate of sampling sites

| Samples | Latitude | Longitude |
|---|---|---|
| WF* | 12°59'41.25"N | 103°19'5.13"E |
| RFB* | 12°59'43.30"N | 103°19'4.72"E |
| RNC* | 12°59'44.77"N | 103°18'51.64"E |
| CB1** | 12°59'45.48"N | 103°18'50.07"E |
| CB2** | 12°59'33.66"N | 103°18'53.43"E |
| CB3** | 12°59'40.46"N | 103°19'8.45"E |
| MDCB** | 12°59'40.80"N | 103°18'51.50"E |
| CS** | 12°59'42.61"N | 103°19'8.70"E |
| SKD** | 12°59'57.40"N | 103°18'31.47"E |
| SKR** | 13° 0'11.02"N | 103°18'34.00"E |
| SRC1** | 12°59'56.04"N | 103°17'52.89"E |
| SRC2** | 12°59'52.37"N | 103°18'28.52"E |

*Paddy water; **Water from canal system

### 2.4. Preparation of standards

Thirty-four reference pesticide compounds are extremely hazardous to humans and commonly found in the environment. Nineteen reference standards are in organochlorine and organophosphate group. Four reference compounds are in carbamate and pyrethroid groups frequently used in agriculture. The other 11 standard compounds are POPs including chlorfennapyr, buprofezin, fipronil, dimethametryn, propanil, biphenyl, metalaxyl, pyroquilon, paclobutrazol, hexaconazole, and triadimefon. The stock standard solution was prepared individually at 1000 $mg.L^{-1}$. In addition, azoxystrobin, buprofezin, carbofuran, chlorothalonil, fipronil, hexaconazole, paclobutrazol, and propanil were also prepared individually in acetone due to their high solubility rate. The remaining compounds were further prepared in hexane. The working standard solution was the mixture of 34 standard compounds at 10 mg. $L^{-1}$ in hexane due to homogeneity mixture of hexane and acetone.

### 2.5. Method validation

The method was validated with respect to accuracy (expressed as recovery), precision (expressed as relative standard deviation, RSD), limit of detection (LOD), and limit of quantification (LOQ). The accuracy and precision were evaluated by performing the recovery study with four replicates. The recovery study was performed using blank water spiked at 1 $\mu g.L^{-1}$ and extract as described in the extraction section. The recovery percentage (%) and RSD values were calculated following Eq. 1 and Eq. 2. Limit of detection (LOD) is defined as the lowest amount of compound in the test sample that the analytical instrument can reliably detect. The limit of quantification (LOQ) is the lowest analyte concentration in a sample that can be quantified with surety. Three replicates were conducted for the test of LOD and LOQ. The LOD and LOQ were calculated using Eqs. 3 and 4. Descriptive statistic was conducted using Microsoft Excel software version 2019.

$$\text{Recovery}(\%) = (A_S - A_B) \times 100 / A_c \quad \text{(Eq. 1)}$$

$$\text{RSD} = (\text{SD} \times 100) / \text{Mean} \quad \text{(Eq. 2)}$$

$$\text{LOD} = 3 \times S/N \quad \text{(Eq. 3)}$$

$$\text{LOQ} = 10 \times S/N \quad \text{(Eq. 4)}$$

where:

$A_S$ = Peak area of spiking sample
$A_B$ = Peak area of blank sample
$A_C$ = Peak area of control sample
SD = Standard deviation of recovery rates
Mean = Average value of recovery rates
S/N = Signal – to – noise ratio

### 2.6. Sample prepation

Water samples were pre-filtration before solid-phase extraction (SPE). Water samples were adjusted with 1 mL of phosphate solution (1 $mol.L^{-1}$, pH 7) in 1 L of water then filtered by 2 µm fiber glass filter (Whatman GMF 2UM) with the vacuum pump to remove suspended solids from the sample. After that, the suspended solids on the surface of filter paper was eluted with 5 mL of acetone and 5 mL of dichloromethane to dissolve pesticides that were adsorbed on the suspended solids layer [8]. Filtrate samples were stored at 4 °C until the extraction step.

### 2.7. Solid-phase extraction (SPE)

One liter of water sample was spiked with the proper amount of working standard solution. The sample was extracted by solid-phase extraction developed by Jinya [8]. This method uses two types of cartridges such as polymer sorbent (PLS3) and activated carbon (AC2). The abovementioned cartridges were conditioned with 5 mL of dichloromethane followed by 5 mL of acetone and 10 mL of ultrapure water. For loading sample, 1 L of water sample passed through the cartridges at maximum speed 15 $mL.min^{-1}$. After that, the cartridges were dried under gentle nitrogen stream for 40 min. After drying, PLS3 cartridge was eluted with 2 mL of acetone followed by 5 mL of dichloromethane whereas AC2 was eluted with 5 mL of acetone. Eluted solution from PLS3 and AC2 cartridge was combined and concentrated to a volume of approximately 1 mL under gentle nitrogen stream. 5 mL of hexane was added to 1 mL of

concentrated solution, and the concentrated tube was washed with 1 mL of hexane 5 times. Then extract solution was dehydrated with sodium sulfate followed by concentrated step for the second time under the gentle nitrogen stream until 1 mL. The extract solution was ready for GC-MS analysis.

### *2.8. GC-MS analysis*

The analysis procedure was adapted from Jinya [8]. The GC-MS analysis was conducted on a Shimadzu GCMS-TQ8040 coupled to a GC-2010 Plus equipped with an AOC-20S autoinjector and -20i autosampler (Shimadzu, Japan). The injected mode was splitless and the injection volume was 1 µL. The capillary column was DB-5ms (30 m x 0.25 mm inner diameter, 0.25 µm film thickness, Agilent, USA). The oven temperature programmed was initialized at 40 °C with holding time for 1 min, and then increased to 310 °C (8 °C.min$^{-1}$) with holding time for 4 min. The injector and interface temperature were 250 °C and 270 °C, respectively. Helium (purity ≥ 99.999 %) was used as carrier gas with flow rate of 50 mL.min$^{-1}$. For MS, EI was 70 eV, ion source and interface temperature were 200 °C and 300 °C, respectively. The sample was analyzed in the full scan mode in the range of m/z 45-600 for confirming in spectral library search of the pesticide compounds. The identification of pesticide was done by the data treatment system and the computer, which calculated the monoisotopic mass, predicted the structural formula of compounds, and compared them using the MS database.

### *2.9. Database software for simultaneous analysis*

The GC-MS works with Automated Identification and Quantification System with a Database (AIQS-DB). This database (AIQS-DB) was designed by professor Kiwao Kadokami. This database contains the mass spectra, retention times, and calibration curves for about 1,000 substances including 450 compounds of pesticide, 194 compounds of CH, 150 compounds of CHO, 113 compounds of CHN(O), 14 compounds of PPCPs, 12 compounds of CHS(NO), 8 compounds of CHP(NOS); permitted simultaneous identification and quantification of about 1,000 plus substances without the use of chemical standards.

## 3. RESULTS AND DISCUSSION

### *3.1. Method validation*

The solid-phase extraction method in this study for simultaneous multiresidues pesticide analysis was validated in terms of accuracy (express as recovery) and precision (express as RSD). The accuracy and precision of proposed method were study with 4 replicates (n = 4) of the recovery experiment at 1 µg.L$^{-1}$ [9, 10]. The proposed method presented satisfactory accuracy (recoveries in the range 70 – 120 %) and precision (RSD < 20 %) for 19 pesticides (55.9 % of the compounds) at fortified concentration (Table 2). Although 13 compounds gave recoveries below 70% but they had good precision (RSD < 20 %). Mevinphos presented 88.2% of recovery rate with high precision (RSD > 20 %) at 1 µg.L$^{-1}$ fortified level. Phenthoate gave recovery yield of 65.7 % with RSD < 20 % at the same fortified level. However, this is also acceptable for concentration below 10 µg.L$^{-1}$ of pesticide residue in water , according to the SANTE guidelines [11].

**Table 2** Recovery yield of 34 reference pesticide compounds

| Pesticides | Accuracy (Recovery, %) | Precision (RSD, %) |
|---|---|---|
| Aldrin | 6.0 | 13.3 |
| Azoxystrobin | 109.4 | 4.3 |
| Bifenthrin | 8.7 | 9.0 |
| Biphenyl | 17.0 | 15.7 |
| Buprofezin | 38.2 | 11.2 |
| Carbofuran | 81.7 | 6.8 |
| Chlorfenapyr | 32.0 | 15.4 |
| Chloroneb | 73.5 | 3.0 |
| Chlorothalonil | 88.1 | 6.2 |
| Chlorpyrifos | 17.0 | 14.8 |
| Dieldrin | 18.3 | 8.5 |
| Difenoconazole | 103.2 | 10.7 |
| Dimethametryn | 96.6 | 2.0 |
| Fenobucarb | 105.5 | 3.0 |
| Fipronil | 111.9 | 2.8 |
| Heptachlor | 6.1 | 8.6 |
| Hexaconazole | 111.7 | 3.7 |
| Isazofos | 101.4 | 3.6 |
| Isoxathion | 41.3 | 16.7 |
| Lindane | 47.6 | 9.7 |
| Malathion | 102.0 | 7.8 |
| Metalaxyl | 106.0 | 5.0 |
| Methyl parathion | 105.0 | 12.3 |
| Metolachlor | 98.8 | 2.1 |
| Meviphos | 88.2 | 24.8 |
| o,p'-DDT | 6.4 | 14.6 |
| Paclobutrazol | 11.8 | 0.8 |
| Permethrin | 8.5 | 1.8 |
| Phenthoate | 65.7 | 10.5 |
| Pretilachlor | 85.7 | 11.1 |
| Propanil | 112.0 | 5.3 |
| Propiconazole | 108.7 | 6.1 |
| Pyroquilon | 27.1 | 13.3 |
| Triadimefon | 106.4 | 5.7 |

**Table 3** Limit of detection (LOD) and limit of quantification (LOQ) of 34 reference pesticide compounds

| Pesticides | LOD (µg.L$^{-1}$) | LOQ (µg.L$^{-1}$) |
|---|---|---|
| Aldrin | 0.0050 | 0.0167 |
| Azoxystrobin | 0.0333 | 0.1110 |
| Bifenthrin | 0.0250 | 0.0833 |
| Biphenyl | 0.0025 | 0.0083 |
| Buprofezin | 0.0192 | 0.0640 |
| Carbofuran | 0.0333 | 0.1110 |
| Chlorfenapyr | 0.0050 | 0.0167 |
| Chloroneb | 0.0050 | 0.0167 |
| Chlorothalonil | 0.0583 | 0.1943 |
| Chlorpyrifos | 0.0050 | 0.0167 |
| Dieldrin | 0.0050 | 0.0167 |
| Difenoconazole | 0.0333 | 0.1110 |
| Dimethametryn | 0.0200 | 0.0667 |
| Fenobucarb | 0.0050 | 0.0167 |
| Fipronil | 0.0025 | 0.0083 |
| Heptachlor | 0.0050 | 0.0167 |
| Hexaconazole | 0.0100 | 0.0333 |
| Isazofos | 0.0050 | 0.0167 |
| Isoxathion | 0.0250 | 0.0833 |
| Lindane | 0.0250 | 0.0833 |
| Malathion | 0.0083 | 0.0277 |
| Metalaxyl | 0.0333 | 0.1110 |
| Methyl parathion | 0.0250 | 0.0833 |
| Metolachlor | 0.0025 | 0.0083 |
| Meviphos | 0.0250 | 0.0833 |
| o,p'-DDT | 0.0025 | 0.0083 |
| Paclobutrazol | 0.0100 | 0.0333 |
| Permethrin | 0.0025 | 0.0083 |
| Phenthoate | 0.0025 | 0.0083 |
| Pretilachlor | 0.0025 | 0.0083 |
| Propanil | 0.0250 | 0.0833 |
| Propiconazole | 0.0050 | 0.0167 |
| Pyroquilon | 0.0050 | 0.0167 |
| Triadimefon | 0.0075 | 0.0250 |

LODs and LOQs in this study were defined by manual injection in the concentration range of 0.0025 – 1 µg.L$^{-1}$. LODs and LOQs were calculated above 3 and 10 signal-to-noise (LOD = 3S/N and LOQ = 10S/N), respectively. LOD study was prepared in triplicate (n = 3) and injected in each equipment. As a result, 20 out of 34 reference pesticide compounds (58.8 % of compounds) had a LOD and LOQ less than 0.01 and 0.0333 µg.L$^{-1}$, respectively. The other 14 pesticide compounds had a LOD and LOQ over 0.01 and 0.0333 µg.L$^{-1}$, respectively (see Table 3). After the validation process, the proposed method for simultaneous multiresidues pesticide analysis proved to be accurate and reliable for analyzing only 21 selected pesticides extracted from canal water. However, the other 13 compounds are also included in this study due to frequently use in agriculture.

### *3.2. Pesticide analysis in water*

The validated method was applied to detect and screen the pesticide residues in paddy and canal water in the agrochemical-free paddy field experiment and the NUBB's farm. The GC-MS spectrum for confirmation of pesticide identification was injecting positive samples, and compared with the instrumental database and the NIST library version 14. Table 4 illustrates presence of biphenyl, chlorfenapyr and methyl parathion in paddy water samples from the field experiment and conventional paddy fields inside the farm during pre-study. At the same time, isoprothiolane and terbcarb (mbpmc) were detected in water samples from rice fields and canal system. Paddy water collected from the field experiment during pre-study in 2021 detected the residue of biphenyl and methyl parathion. Furthermore, biphenyl and chlorfenapyr were found in paddy water collected from one paddy field (sample code RNC) close to the farm's water gate. Only two samples were detected isoprothiolane – one paddy water (sample code RFB) and one irrigated water (sample code CB3) collected. Terbcarb was found in paddy water (sample code RNC) collected from rice field close to water gate of the NUBB's farm.

According to the result of detecting pesticides in the collected samples during pre-study in 2021, sampling points had expended to upstream near the NUBB's farm in 2022. In mid-March 2022, the water collected in the paddy fields were not available because of end harvesting season of paddy cultivation in this area. The results showed that only two samples contained azoxystrobin residue – sample code CB1 and MDCB. Biphenyl was detected in the water sample (sample code CS) collected from the point next to the national road, while a sample from canal connected to Sang Ke river (sample code SRC1) was detected chlorfenapyr. However, terbcarb (mbpmc) were found in all water samples collected from different points in the canal system inside the farm and nearby upstream. Water samples collected from all points in the canal system inside the NUBB's farm noticed presence of isoprothiolane. But 2-phenylphenol was detected in 3 different sampling points (CB1, CB2, and MDCB) in the farm's canal system and in Srah Keo Reservoirs (sample code SKR) (see Table 5). However, 2-phenylphenol and isoprothiolane are fungicide while terbcarb is herbicide. Regarding the WHO classification, isoprothiolane is moderate hazardous, 2-phenylphenol is slight hazardous, and terbcarb is classified as an obsolete pesticide [12].

However, research information from literature was highlighted the presence of pesticide residues in surface water

**Table 4** Pesticide detected in water samples collected for pre-study in 2021

| Pesticide detected | Sample codes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WF | RFB | RNC | CB1 | CB2 | CB3 | MDCB | CS | SKD | SKR | SRC1 | SRC2 |
| Biphenyl | + | - | + | NA | NA | - | NA | NA | NA | NA | NA | NA |
| Chlorfenapyr | - | - | + | NA | NA | - | NA | NA | NA | NA | NA | NA |
| Isoprothiolane | - | + | - | NA | NA | + | NA | NA | NA | NA | NA | NA |
| Methyl parathion | + | - | - | NA | NA | - | NA | NA | NA | NA | NA | NA |
| Terbcarb (MBPMC) | - | - | + | NA | NA | - | NA | NA | NA | NA | NA | NA |

NA: Not available; +: Detected; -: Not detected

**Table 5** Pesticides detected in water samples collected in 2022

| Pesticides detected | Sample codes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WF | RFB | RNC | CB1 | CB2 | CB3 | MDCB | CS | SKD | SKR | SRC1 | SRC2 |
| 2-Phenyl phenol (OPP) | NA | NA | NA | + | + | - | + | - | - | + | - | - |
| Azoxystrobin | NA | NA | NA | + | - | - | + | - | - | - | - | - |
| Biphenyl | NA | NA | NA | - | - | - | - | + | - | - | - | - |
| Chlorfenapyr | NA | NA | NA | - | - | - | - | - | - | - | + | - |
| Isoprothiolane | NA | NA | NA | + | + | + | + | + | - | - | - | - |
| Terbcarb (MBPMC) | NA | NA | NA | + | + | + | + | + | + | + | + | + |

NA: Not available; +: Detected; -: Not detected

for irrigation. For instant, Phat et al. [13] found that at least one pesticide compound were identified in 12 water samples collected from Chhnok Tru, Kampong Chhnang, Cambodia. Recent research, Keo et al. [14] has reported 6 pesticide residues were detected in water samples collected from canal irrigation system of the upper Mekong Delta, Cambodia. In this study, 7 pesticides were detected in water samples collected during pre-study in 2021 and 2022. Only water was analyzed for pesticide residue using gas chromatography – mass spectrometry (GC-MS). Moreover, each pesticide compound has different affinity with various matrices like soil, grain, or biota. This could explain why only 7 pesticides were found in the area. During sample collection for pre-study, water in experimental field was little left over after 2 months harvesting and the other two paddy fields were at tillering stage of new rice cycle. Water in the agrochemical-free rice field experiment was detected biphenyl and methyl parathion residue. However, biphenyl also detected in one paddy water collected (sample code RNC) during pre-study and one canal water sample collected (sample code CS) in 2022. Biphenyl can transport into water via volatilization [15]. Even volatilization is the second dissipation route of methyl parathion. In the United State, methyl parathion was detected in both air and rain samples across the state [16]. Therefore methyl parathion and biphenyl could probably be volatile from pesticide application activities in nearby conventional paddy farms into atmosphere and drop on the ground during rain. This is a possible explanation for its presence in the experimental paddy field. Chlorfenapyr was found in water collected from the paddy field (sample code RNC) collected for pre-study and one sampling point (sample code SRC1) at nearby upstream source collected in 2022. However, azoxystrobin was found in water collected from sampling point CB1 and MDCB locate inside the NUBB's farm in 2022. Chlorfenapyr is an insecticide to control some insects on paddy plants like rice leaf folder, steam borer and so on. At the same time, azoxystrobin is fungicide to treat fungus on leave for rice.

Unlike available pesticide standard compounds, the canal system is possibly a way of pesticide distribution for 2-phenylphenol, isoprothiolane and terbcarb. Besides the agrochemical-free paddy field experiment, farmers were pumping water from the canal system into paddy fields and spraying agrochemical products throughout the rice cycle. OPP residue was detected in Srah Keo reservoir and the other 3 sampling points inside the farm's canal system in 2022. In agriculture, OPP is used in cattle, swine, poultry farms and primises [17]. However, farmers living near Srah Keo reservoir are cultivated not only paddy rice but also have their own small livestock farms. Hence, OPP could contaminate the canal system from those small livestock farms activities. Terbcarb (mbpmc) is a compound with limited information about its environmental fate. Nevertheless, mbpmc was found in one paddy water (sample code RNC) collected during pre-study in 2021 and in all water samples collected from different points in the canal system and nearby upstream in 2022. During sample collection for pre-

study in 2021, the paddy plant in RNC field was at tillering stage. This could expect that farmers applied herbicide during the tillering stage of rice cycle to control weed in the field, which could contaminate and be transported into the water after application. Since terbcarb is an obsolete compound, this could clarify that terbcarb is a hidden ingredient in frequently used herbicide products in this area. However, isoprothiolane was detected in 2 water samples – one paddy water (sample code RFB) and one canal water (sample code CB3) during pre-study. Moreover, this compound also identified in all water samples collected from different sampling points in the farm's canal system in 2022. Despite the presence of isoprothiolane in water samples in this study, isoprothiolane is a rejected pesticide to use in Cambodia because this compound is not registered in pesticide compound list of US Environmental Protection Agency (EPA) [18]. Even so detection of banned pesticides in different environment compartments can be an outcome of illegal uses or their persistence in the environment [19]. For example, Duong et al. (2022) reported that isoprothiolane had contamination rate of 47 % (as medium-risk group) in organic rice production in An Giang province, Vietnam

## 4. CONCLUSION

After applying agrochemical products, they left the toxic residues in the surrounding environment. However, water is the common source of pesticide contamination to organic farming. In this study, water samples were analyzed for multiresidues of pesticide by solid-phase extraction (SPE) and GC-MS. In validation method, 61.8 % of total reference pesticides presented a satifactory result of accuracy and precision at fortified concentration. The remaining 13 compounds gave below minimum acceptable value for recovery yield but their RSD was below 20 %. The limit of detection (LOD) and limit of quantification (LOQ) of 20 out of total reference compounds were less than 0.01 and 0.0333 $\mu g.L^{-1}$, respectively. This proposed method was used to extract pesticides from water sources surrounding the agrochemical-free paddy farm of Research and Training Farm, NUBB, Battambang, Cambodia. The results showed that 7 pesticides were detected in water samples collected from paddy fields and canal system. Three out of 7 pesticides were not in the list of the available reference compounds. The residue of biphenyl and methyl parathion in paddy water from the field experiment and RNC are most likely to contaminate from the atmosphere after pesticides are applied in nearby paddy fields. Additionally, azoxystrobin and chlorfenapyr are fungicide and insecticide to treat fungus and insects on leaves. However, water samples from the paddy fields and canal system presented 2-phenylphenol (OPP), isoprothiolane and terbcarb. Since 2-phenylphenol is used in the livestock farm, the effluent waste from the livestock farm near Srah Keo reservoir is likely the primary source of contamination. Terbcarb could be the hidden compound in the herbicide products that frequently used by farmers in this location. However, presence of isoprothiolane could be an outcome of illegal use or its persistent in the environment. This highlights that agrochemical-free paddy field trail in this study was contaminated from the surrounding environment through water sources and agricultural activities. In this case, further analysis of other factors like paddy grain, soil, and sediment should be considered better to understand pesticide application around the agrochemical-free paddy farm.

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