

Analysis of Pesticide Residues in Sediment from Chhnok Tru, Kampong Chhnang Using Different Extraction MethodsSokheng Kai¹, Sereyvath Yeoun², Chanvorleak Phat^{2*}¹ *Master of Agro-Industry, Graduate School, Institute of Technology of Cambodia, Russian Federation Blvd., P.O. Box 86, Phnom Penh, Cambodia*² *Faculty of Chemical and Food Enigeering, Institute of Technology of Cambodia, Russian Federation Blvd., P.O. Box 86, Phnom Penh, Cambodia*

Received: 29 June 2020; Received: 12 November 2020; Available online: xxxx

Abstract: Tonle Sap lake has been the largest fresh water lake in Southeast Asia, providing a fundamental environment for nurturing more than three million people in the five surrounding provinces, which about 90% lived from the fishery or agriculture. However, the ecosystem and populations living around the lake have experienced environmental pressure, since the lake water, fishes, living organisms are polluted. More than that, sediment is the type of soil which provides beneficial nutrients, and it also has been polluted by discharge of untreated pesticide residues from the agriculture actions. This current study aims to identify the presence of 451 pesticide compounds and quantify the concentration of 23 targeted pesticide residues in sediment at Chhnok Trou community of the Tonle Sap Lake. Furthermore, pesticide residues were extracted by Solid-Phase Extraction (SPE) method and detected by Gas Chromatography-Mass Spectrometry (GC-MS). Different cartridges (C18 and PLS3-AC2) and different ratio of elution solvents were tested to validate the extraction method before the actual sample analysis. The recovery yield of combination of amino and activated carbon cartridges using mixture of methanol (MeOH)/dichloromethane (DCM) 10/90 (v/v) for elution step was higher than those of another two conditions of MeOH/DCM: 5/95 and 50/50 (v/v). For the recovery rate of silica cartridge, using mixture of acetic acid/acetonitrile of 1/99 (v/v) for elution step gave higher recovery rate than those of another two mixing conditions of acetic acid/acetonitrile: 3/97 and 5/95 (v/v). In this study, combination of amino and activated carbon cartridges and 10/90 (v/v) of methanol/dichloromethane as a mixture solvent for elution step were used for pesticide extraction and clean-up for sediment samples. As a result, four pesticide residue compounds were presented in sediment samples; whereas, none of targeted compounds were quantitatively detected. Qualitatively, chlorfenapyr, insecticide and acaricide were found in sample JS2, fluquinconazole and fungicide in JS5, mevinphos and insecticide in JS3, while oxabtrinil and herbicide safener were found in JS4 and JS6.

Keywords: Dichloromethane; Extraction methods; Methanol; Pesticide residues; Sediment**1. INTRODUCTION**

Agriculture has the potential to be an engine for economic growth and food security in Cambodia in which the tropical climate dry and wet is suitable for plants growth (Bansok et al., 2011). Despite of this good weather, it also has a great chance for living and breeding places of pests and crops yields are decreased due to the detrimental effect of pest management is uncontrolled properly (Bansok et al., 2011). Management of pests turn out to be a very considerable aspect for success of cultivation (Aktar et al., 2009). From the time of the agriculture, chemical pesticides have been used in agriculture sector to protect crops against insects, fungi, weeds and other pests due the fact that it could help farmers in a achieving a substantial increase in

agricultural productivity (Aktar et al., 2009). Pesticides were used in many countries in the world especially, it was the most useful in developing country including Cambodia; moreover, Cambodian citizens have been utilizing pesticides since 1996 to extend yields of crop production due to all the applications of pesticides were played a role as reducing potential losses and preventing from any pests (Matsukawa et al., 2016). In Cambodia, the number of pesticides uses have been recorded to increase from year to year (CEDAC, 2010). For instance, in 1994, there were only 30 pesticides in the market; this increased to 241 in 2000 of which 42 were prohibited in Vietnam and another 16 were banned in Thailand; the increase reached 757 in 2009 (CEDAC, 2010). In addition, Cambodia ranks first among 13 countries in the region with the highest pesticide residue on vegetables (Wang et al., 2011). Survey studies of pesticide contamination of vegetables in Cambodian markets found produce pesticide residues of highly toxic chemical such as

* Corresponding author: Chanvorleak Phat
E-mail: phatchanvorleak@itc.edu.kh; Tel: +855-92-916-184

organochlorine, organophosphate and carbamate exceeding the maximum allowance (Wang et al., 2011). Clearly, Cambodia has become a dumping ground for unwanted and dangerous pesticides (EJF, 2002). Thus, the related ministry has classified and banned 163 highly toxic chemical pesticides and have classified 46 others into the attention list due to its negative impacts on human health and environment (MAFF, 2013). In addition, some pesticides compounds are very persistent. In the troposphere, HCB was transported over long distances by virtue of its persistence, but does undergo slow photolytic degradation (the half-life is approximately 80 days) (IPCS, 1997). Chlordane was very resistant to chemical and biological degradation; it was highly immobile and migrates very poorly (Rao and Davidson, 1982). Dissipation of chlordane from soils is mainly due to volatilization. The soil half-life is about 4 years (Rao and Davidson, 1982). Endrin was a foliar insecticide that acted against a wide range of agricultural pests at doses of 0.2–0.5 kg of active material per ha. It had a broad spectrum of control and was particularly effective against Lepidoptera (IPCS, 1992). It was used mainly on cotton but also against pests of rice, sugar-cane, maize and other crops. It was also used as a rodenticide (IPCS, 1992). Dichlorodiphenyltrichloroethane (DDT) was used widely to control pests starting in the mid-1940s. In 1958, the US Department of Agriculture began a program to phase out DDT due to concerns about its persistence in the environment and toxicity to non-target organisms and it was banned 40 years ago (Sitting, 1980). Their persistence was due to low vapor pressure and resistance to further degradation (Sitting, 1980). The Tonle Sap lake has been the largest fresh water lake in Southeast Asia, providing a fundamental environment for nurturing more than three million people in the five surrounding provinces, which about 90% lived from the fishery or agriculture (Uk et al., 2018). The Tonle Sap lake, as component of cultural Cambodia identity, has been essential to the local and national economy by providing water resources, fish production, aquaculture, transportation and tourism (Uk et al., 2018). However, despite its significant value, the ecosystem and populations living around the lake have experienced environmental pressure, since the lake water and its sediment has been polluted by discharge of untreated waste water and pesticides from the agriculture actions (Uk et al., 2018). As mentioned above, assessment of pesticide residues in sediment of Tonle Sap Lake is necessary to give scientific evidences about pesticide pollution. In order to achieve this, pesticide extraction method from sediment has to be chosen carefully to get the highest recovery yields. Therefore, in this study, different extraction methods were compared and the suitable method will be selected to analyze the actual sediment samples. Pesticide analysis in this research will be done for both semi-quantitatively which samples were scanned for the presences of pesticides and

quantitatively which samples were quantified for 23 targeted compounds.

2. METHODOLOGY

2.1 Site description

Chhnok Tru is one of three floating villages located in Chhnok Tru Commune, and shares a border with Phat Sanday Commune in Kampong Svay District, Kampong Thom Province to the north, Kar Chab Commune to the west, Ponley Commune and Boribou District to the south, and Kampong Phrash village in Chhnok Tru Commune to the east. Chhnok Tru is an area which has a lot of agriculture activities and is important part of Tonle Sap lake.

2.2 Sample collection

Sediment samples were collected from 17 sites on 7th to 8th, March 2019 (Table 1) in different areas in Chhnok Tru, by using collection tubes at the bottom of Tonle Sap lake (Fig.1). All the wet raw sediment samples were dried at room temperature (25 °C to 28 °C) and then, it was grinded in to powder by using mortar to make it homogeneous. After that, it was next introduced into cleaned plastic bags and stored in refrigerator at 4 °C until extraction.

Table 1. Latitudes and longitudes of sample sites

Samples	Latitude	Longitude
JS1	12°30'32.94"N	104°27'17.28"E
JS2	12°30'54.59"N	104°26'09.1"E
JS3	12°30'49.25"N	104°25'17.72"E
JS4	12°31'29.54"N	104°25'25.25"E
JS5	12°32'29.61"N	104°26'32.53"E
JS6	12°31'39.53"N	104°26'32.53"E
JS8	12°31'27.79"N	104°27'45.43"E
JS9	12°31'52.63"N	104°26'45.35"E
JS10	12°30'57.22"N	104°26'35.66"E
JS11	12°32'29.49"N	104°28'48.83"E
JS12	12°34'09.16"N	104°30'32.29"E
JS14	12°30'33.27"N	104°29'36.17"E
JS15	12°32'23.63"N	104°26'43.94"E
JS16	12°30'52.19"N	104°27'02.81"E
JS17	12°30'55.51"N	104°27'22.1"E
JS18	12°30'42.36"N	104°27'16.24"E
JS19	12°30'24.76"N	104°27'16.63"E

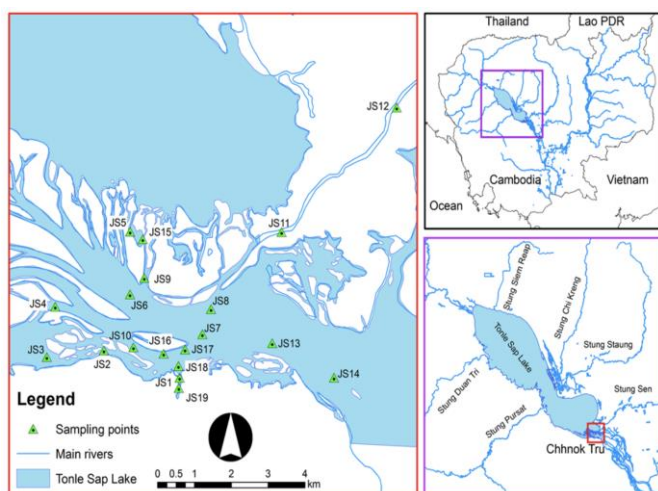


Fig.1. Map of the study area and the sampling points

2.3 Reagents and standards

Reagents used in this study included n-hexane (99%, HPLC grade, Spain), Acetonitrile (MeCN) (99.5%, Spain), Dichloromethane (DCM) (99.99%, for GC, UK) and acetic acid (AA). To quantify concentration of 23 target compounds (Table 2) in sediment sample, stock standard solution was prepared in hexane with a concentration 10 ppm and diluted to 5 different concentrations such as 0.1 ppm, 0.125 ppm, 0.25 ppm, 0.5 ppm and 1 ppm to create standard curve.

Table 2. External standards

No.	Pesticide compounds	Formulations	Natures
1	Isoxathion	C ₁₃ H ₁₆ NO ₄ PS	Insecticide
2	Chloroneb	C ₈ H ₈ Cl ₂ O ₂	Fungicide
3	O, p'- DDT	C ₁₄ H ₉ Cl ₅	Insecticide
4	Aldrin	C ₁₂ H ₈ Cl ₆	Insecticide
5	Dieldrin	C ₁₂ H ₈ C ₁₆ O	Insecticide
6	Lindane	C ₆ H ₆ Cl ₆	Insecticide
7	Methamidophos	C ₂ H ₈ NO ₂ PS	Insecticide
8	Isazophos	C ₉ H ₁₇ ClN ₃ O ₃ PS	Insecticide
9	Terbacil	C ₉ H ₁₃ ClN ₂ O ₂	Herbicide
10	Matalaxyl	C ₁₅ H ₂₁ NO ₄	Fungicide
11	Mefenoxam	C ₁₅ H ₂₁ NO ₄	Fungicide
12	Endrin	C ₁₂ H ₈ Cl ₆ O	Insecticide
13	Heptachlor	C ₁₀ H ₅ Cl ₇	Insecticide
14	Parathion	C ₁₀ H ₁₄ NO ₅ PS	Insecticide
15	Methyl-parathion	C ₈ H ₁₀ NO ₃ PS	Insecticide
16	Malathion	C ₁₀ H ₁₉ O ₆ PS ₂	Insecticide
17	Anilofos	C ₁₃ H ₁₉ ClNO ₃ PS ₂	Herbicide

18	Triadimefon	C ₁₄ H ₁₆ ClN ₃ O ₂	Fungicide
19	Atrazine	C ₈ H ₁₄ ClN ₅	Herbicide
20	Pyroquilon	C ₁₁ H ₁₁ NO	Fungicide
21	Azaconazole	C ₁₂ H ₁₁ Cl ₂ N ₃ O ₂	Fungicide
22	Chlordane	C ₁₀ H ₆ Cl ₈	Fungicide
23	HCB	C ₆ Cl ₆	Fungicide

2.4 Extraction and spiking for recovery test

There were two types of cartridge viz combination of amino (PLS3) and activated carbon (AC2) cartridges and silica cartridges (C18) were used for doing recovery test. PLS3-AC2 cartridges were eluted in mixture of methanol (MeOH) 5%, 10%, and 50% in dichloromethane (v/v). Silica cartridge were eluted with three mixture conditions of acetic acid and acetonitrile; 1%, 3%, and 5% of acetic acid was mixed with acetonitrile (v/v) in elution step.

2.4.1 Combination of amino and activated carbon cartridges (PLS3-AC2)

Extraction of pesticide from sediment sample was done by solid-phase extraction (SPE) (Fig.2). Ten grams of sediment sample was spiked with 1mL of 23 mixture of targeted compounds and then it was rested for 1h. After 1h was done, it was introduced to mix with 20 mL of acetonitrile and then it was introduced to do sonication for 20 min. Supernatant was filtered through 47 mm diameter of glass microfiber filters 2 μm pore size (Whatman, GF/CTM) to remove sediment from filtrate. After that, the filter and flask were washed with 20 mL of acetonitrile to dissolve all compounds which stick in the layer of glass microfiber filters. Next, solution was introduced to evaporate until solution was dried by rotary evaporator (RV 10 C S099), temperature 40 °C, pressure 150 mbar with 200 rpm for 15 min. Then, each 5 mL of three mixture conditions of methanol/dichloromethane (5/95, 10/90, 50/50, v/v) was re-dissolved and it was kept for loading. PLS3-AC2 cartridge were activated by first conditioning with 5 mL of dichloromethane and after with 5 mL of each three mixture conditions of methanol/dichloromethane 5/95, 10/90, 50/50 (v/v). Next, 5 mL of sample was loaded through the cartridges and then 10 mL of each three mixture conditions of methanol/dichloromethane 5/95, 10/90, 50/50 (v/v) was then eluted. After that it was concentrated by nitrogen gas until it dried and then it was re-dissolved 1 mL of acetone. Furthermore, sample were added 100μL of internal standard and it was transferred to vial and subjected to GC-MS analysis.

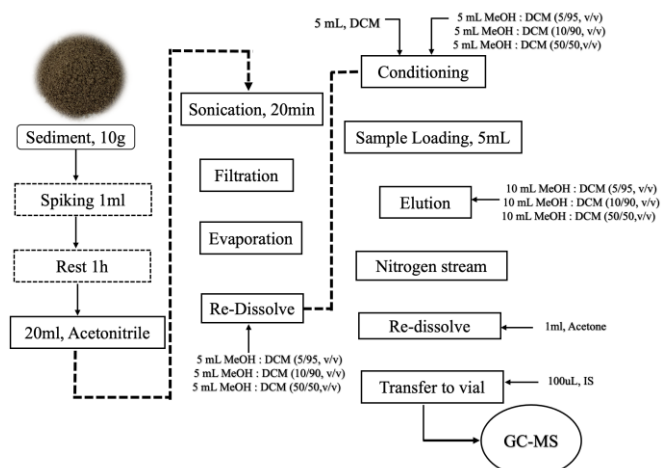


Fig.2. Pesticide extraction using PLS3-AC2 cartridge

2.4.2 Silica cartridge (C18)

Extraction of pesticide from sediment sample was done by SPE (Fig.3). Five grams of sediment sample was spiked with 1 mL of 23 mixture of targeted compounds 1ppm and then it was rested for 1h. After 1h was done, it was introduced to mix with 4g mixture of salt such as magnesium sulfate, sodium chloride, sodium citrate, citric acid disodium salt and 10 mL of acetonitrile was added. Supernatant was done by centrifugation 4 °C, 4000 rpm for 15 min and it was taken 2 mL after finishing centrifugation for loading. The cartridge was activated by conditioning with 5 mL of acetonitrile and then, 2 mL of sample was loading through the cartridge and 5mL of each three mixture conditions of acetic acid/acetonitrile 1/99, 3/97, 5/95 (v/v) was then eluted.

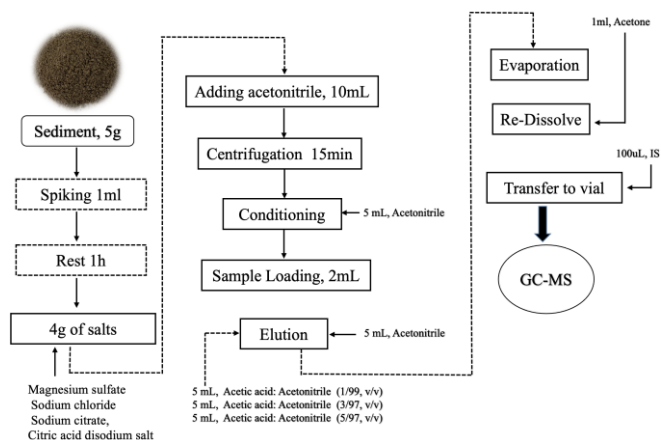


Fig.3. Pesticide extraction using C18 cartridge

Next, solution was introduced to evaporate until solution was dried by rotary evaporator (RV 10 C S099), temperature 40 °C, pressure 150 mbar with 200 rpm for 15

min and then it was re-dissolved 1 mL of acetone. Furthermore, it was injected 100 µl of internal standard and it was transferred to vial and subjected to GC-MS.

2.6 GC-MS

The study will be undertaken by using both qualitative and quantitative method of research of pesticides residues in sediment in Tonle Sap lake and then analyzed with Gas-Chromatography Mass Spectrometry (GC-MS) to identify chemical pesticide composition. Gas Chromatography is a method of measuring and separating volatile chemical constituents of a substance and Mass Spectrometry an analytical tool useful for measuring the mass-to-charge ratio (m/z) of one or more molecules present in a sample. Pesticide residues were analyzed by Shimadzu 2010 GC (GC-MSTQ8040, Shimadzu, Japan). GC-MS was operated by utilizing helium as carrier gas with Agilent DB-5ms column (30 m x 0.25 mm, df – 0.25 µm). Column temperature was set as 40 °C for 1 min, 40 to 310 °C for 4 min at rate of 8 °C/min. Interface temperature was set to 270 °C, and ion source temperature of 200 °C. Injector was set up at temperature of 250 °C with split less mode while electron impact ionization at 70 eV, and sample was analyzed in the scan mode in the range of m/z 45-600 for confirmation in library search.

2.7 Database software for simultaneous analysis

GC-MS (GC-MSTQ8040, Shimadzu, Japan) was equipped with Automated Identification and Quantification System with a Database (AIQS-DB), which was designed by Professor Kiwao Kadokami of the University of Kitakyushu (formerly associated with Kitakyushu City Institute of Environmental Sciences). This database containing the mass spectra, retention times and calibration curves for about 1,000 substances included 451 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), and 8 compounds of CHP (NOS), which permitted simultaneous identification and quantification of about 1,000 plus substances without the use of chemical standards. Maximizing the performance of this database required high-sensitivity instrument together with feature-rich quantitative soft.

3. RESULTS AND DISCUSSION

3.1 4.1. Recovery yield of PLS3-AC2 cartridges

Table 3 showed the recovery yield of combination of amino and activated carbon cartridges using three conditions of mixture of 5%, 10%, and 50% of methanol and dichloromethane. The recovery yield of methanol/

dichloromethane (5:95 v/v) for the 23 mixture compounds ranged from 19.89% to 101.75% while, 3 compounds viz methamidophos, chloroneb, and HCB were not detected and 5 compounds among 23 compounds were higher than 70% and 15 compounds were lower than 70%. The recovery yield of methanol/dichloromethane (10:90 v/v) for 23 compounds ranged 19.21% to 121.99% while 3 compounds viz methamidophos, chloroneb, and anilofos were not detected. Among 23 compounds, 13 compounds were higher than 70% whereas 7 compounds were lower than 70%. The recovery yield of methanol/dichloromethane (50:50 v/v), 23 compounds ranged 14.65% to 128.45%. methamidophos, chloroneb, anilofos and HCB were not detected as and 12 compounds were higher than 70%, and 7 compounds were lower than 70%. Among the three conditions of MeOH/DMC (5/95), MeOH/DMC (10/90) and MeOH/DMC (50/50), MeOH/DMC (10/90) was better than the other mixtures (Table 3). According to Codex Alimentarius Commission (2017), an acceptable recovery should be in range of 70-120%. In this study, PLS3-AC2 cartridges with MeOH/DMC (10/90) as elution solvents was selected for further analysis of sediment samples.

Table 3. The recovery yield of PLS3-AC2 cartridges using three conditions of mixture of MeOH/DMC (5/95), (10/90) and (50/50)

No.	Compound names	Recovery of PLS3-AC2 cartridges (%)		
		5%	10%	50%
1	Isoxathion	19.89	19.21	128.45
2	Chloroneb	-	-	-
3	O, p'- DDT	101.75	121.99	73.17
4	Aldrin	68.01	74.59	59.75
5	Dieldrin	84.01	91.68	101.31
6	Lindane	45.48	57.07	51.94
7	Methamidophos	-	-	-
8	Isazophos	61.63	86.03	66.16
9	Terbacil	61.65	85.80	66.11
10	Metalaxyl	67.96	85.71	79.86
11	Mefenoxam	67.85	85.48	79.69
12	Endrin	83.53	95.00	100.53
13	Heptachlor	67.18	86.44	79.20
14	Parathion	41.43	41.68	14.65
15	Methyl-parathion	21.18	19.89	77.54
16	Malathion	28.33	57.64	104.16
17	Anilofos	46.00	82.88	-
18	Triadimefon	103.60	114.02	82.14
19	Atrazine	70.82	93.64	69.99

20	Pyroquilon	50.18	57.93	35.65
21	Azaconazole	68.28	77.47	71.13
22	Chlordane	53.90	62.05	84.68
23	HCB	-	-	-

3.2 Recovery yield of silica cartridge

Table 4 showed the recovery yield of silica cartridge using three conditions of mixture of 1%, 3%, and 5% of acetic with acetonitrile.

Table 4. The recovery yield of silica cartridge using three conditions of mixture of 1%, 3%, and 5% of acetic with acetonitrile

No.	Compound names	Recovery of C18 cartridge (%)		
		1%	3%	5%
1	Isoxathion	73.25	-	-
2	Chloroneb	-	51.58	51.32
3	O, p'- DDT	51.33	101.41	114.21
4	Aldrin	71.44	66.45	65.84
5	Dieldrin	73.40	71.44	69.69
6	Lindane	79.09	77.88	82.68
7	Methamidophos	-	-	-
8	Isazophos	73.67	69.93	67.96
9	Terbacil	73.53	69.73	68.48
10	Metalaxyl	60.33	64.65	63.45
11	Mefenoxam	60.14	62.77	62.34
12	Endrin	58.54	74.89	76.54
13	Heptachlor	63.07	66.72	64.65
14	Parathion	79.89	76.87	77.16
15	Methyl-parathion	111.14	70.64	84.38
16	Malathion	96.28	86.47	89.39
17	Anilofos	134.93	66.73	80.79
18	Triadimefon	72.29	78.76	74.57
19	Atrazine	76.72	70.21	67.51
20	Pyroquilon	63.98	62.74	62.77
21	Azaconazole	57.15	55.95	54.78
22	Chlordane	71.91	68.43	73.35
23	HCB	64.31	62.57	62.98

The recovery yield of acetic acid/acetonitrile (1/99 v/v) for 23 compounds ranged from 51.33% to 134.93% while, methamidophos and chloroneb were not detected and 13 compounds among 23 compounds were higher than 70% and 8 compounds were lower than 70%. The recovery yield of acetic acid/acetonitrile (3:97 v/v) for 23 mixture compounds ranged from 51.58% to 101.41% while methamidophos and

isoxathion were not detected and 9 compounds were higher than 70% among 23 compounds and 12 compounds were lower than 70%. The recovery yield of acetic acid/acetonitrile (5:95 v/v) for 23 compounds ranged from 51.32% to 114.21%, while mehamidophos and isoxathion were not detected and 12 compounds were lower than 70% while, 9 compounds were higher than 70%. Among the three mixing conditions of acid/acetonitrile: 1/99, 3/97 and 5/95, 1/99 was better than the other mixtures (Table 4). According to Codex Alimentarius Commission (2017), an acceptable recovery should be in range of 70-120%.

4.3. Semi-quantitative identification of pesticide residues in sediment

Table 5 showed semi-quantitative identification of pesticide residues in sediment samples from Chhnok Trou community. Four pesticide compounds among 451 compounds were detected in the samples in which 1 active compound was fungicide, 1 active compound was insecticide, 1 active compound was acaricide and insecticide, and another 1 active compound was herbicide. The results were gotten using amino and activated cartridges with mixture of methanol and dichloromethane 10/90 (v/v) due to this condition was better than another two condition. Fluquinconazole was found in JS5, fluquinconazole was a quinazoline based triazole fungicide, had been evolved to utilize as a seed treatment to manage all in cereals (Dawson and Bateman, 2008) fluquinconazole was proposed to be grouped in the acute subgroup "motor division" based on tremors observed at 1.79 mg/kg bw (EFSA, 2014) and a NOAEL of 0.45 mg/kg bw (Level 1). A detailed evaluation of the database of fluquinconazole showed that tremor in females at a dose of 1.79 mg/kg bw was observed only on day 24 (EFSA, 2014). Mevinphos was found in JS3 which was an organophosphate insecticide, it was to control a range of insects such as aphids, thrips, *Helicoverpa* spp., leafhoppers, webworm etc. in vegetables (Sapbamrer and Hongsibsong, 2014). Mevinphos easily degraded in natural systems, with the first half-life of <1 day in aerobic soil (Hornsby et al., 1995). Mevinphos was highly toxic via the oral route, with reported oral LD50 values of 3 to 12 mg/kg in rats, and 4 to 18 mg/kg in mice (Khan, 2016). The lowest oral dose of mevinphos that produced toxic effects (peripheral nervous system effects) in humans was 690 ug/kg when it was given intermittently over 28 days (Kidd and James, 1991). In humans, symptoms of poisoning have appeared within as little as 15 minutes to 2 hours after exposure to mevinphos, but onset of symptoms has been delayed for as long as 2 days (Kidd and James, 1991). Mevinphos was also very highly toxic to fish, the 96 hours LC50 for technical mevinphos was 0.012 mg/L in rainbow trout, and 0.022 mg/L in bluegill sunfish (Kidd and James, 1991). The 48 hours LC50 for mevinphos is 0.017 mg/L in rainbow trout; the 24 hours LC50 was 0.034 mg/L in

rainbow trout, 0.041 mg/L in bluegills, and 0.8 mg/L in mosquito fish (Pimentel, 1971). Oxabetrinil was a nature of herbicide safener, it was detected in both of JS4 and JS6. The result of these 4 presences of pesticide residues could be justified by the fact that the area surrounding of these sampling locations was becoming conscious about increasing amount of pesticide use varied through period crop growth during dry season.

Table 5. Semi-quantitative identification of pesticide residues in sediment samples from Chhnok Trou community

Name of pesticides	Nature	JS2	JS3	JS4	JS5	JS6
Chlorfenapyr	Insecticide					
	and	+	-	-	-	-
Fluquinconazole	Acaricide					
	Fungicide	-	-	-	+	-
Mevinphos	Insecticide	-	+	-	-	-
Oxabetrinil	Herbicide					
	safener	-	-	+	-	+

4.4. Quantification of 23 target pesticide compounds in sediment

Seventeen sediment samples from different area in Chhnok Tru community were quantified for 23 targeted pesticide compounds viz isoxathion, chloroneb, o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT), aldrin, dieldrin, lindane, methamidophos, isazophos, terbacil, matalaxyl, mefenoxam, endrin, heptachlor, parathion, methyl-parathion, malathion, anilofos, triadimefon, atrazine, pyroquilon, azaconazole, chlordane, and hexachlorobenzene. However, none of these compounds was not detected in this study. Mostly, the 23 targeted compounds were found in water samples in the same area, it could be attribute to the fact that, it may be soluble in water (Chanvorleak et al., 2019). Theirs molecules may bound in water and highly soluble pesticides would travel with water as it would move in the environment (National Pesticide Information).

4. CONCLUSIONS

Analysis of pesticide residues in sediment from Chhnok Tru, Kampong Chhnag was determined. The recovery yield of combination of amino and activated carbon cartridges using mixture of 10% of methanol and dichloromethane in elution step was higher than the other two conditions of 5% and 50% (MeOH/DCM v/v). For the recovery rate of silica cartridge using mixture 1% of acetic acid and acetonitrile (v/v) in elution step was higher than the other two conditions of 3% and 5% of acetic acid and acetonitrile (v/v). In addition to this, silica cartridge was better than combination of amino and activated carbon cartridges. In this study,

pesticide in sediment was extracted by combination of amino and activated carbon cartridge with MeOH/DCM 10/90 (v/v) as a mixture solvent in elution step because it was the best condition among the other two MeOH/DCM mixtures of 5/90 and 50/50 (v/v) and also due to the availability of only these consumable materials and cartridge in the laboratory. Pesticide residues were semi-quantitatively identified and quantified in sediment at Chhnok Trou community. This study showed that 4 pesticide residue compounds were presented in sediment, within which 4 pesticide compounds were semi-quantitatively detected and none of target compounds were quantitatively detected. For semi-quantitative results, 1 compound of Chlorfenapyr (C₁₅H₁₁BrClF₃N₂O) insecticide and acaricide found in JS2, 1 compound of Fluquinconazole (C₁₆H₈Cl₂FN₅O) fungicide found in JS5, 1 compound of mevinphos (C₁₇H₁₃O₆P) insecticide found in JS3 and 1 compound of Oxabtrininil (C₁₂H₁₂N₂O₃) herbicide safener were detected in JS4 and JS6.

ACKNOWLEDGMENTS

This research was financially supported by Research grant provided by the Science and Technology Research Partnership for Sustainable Development (SATREPS - JST/JICA: grant-number JPMJSA1503 - Establishment of Environmental Conservation Platform of Tonle Sap Lake).

REFERENCES

- Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology*, 2(1), 1-12.
- Bansok, R. O. S., Chhun, C., & Phirun, N. (2011). Agricultural development and climate change: the case of Cambodia. CDRI.
- CEDAC (2010). The Cambodia Monitoring Report on the Pesticide Issue in 2009. (Phnom Penh: CEDAC).
- Phat, C., Kouk, F., Mariquit, E.G., Kuriniawan, W., & Hinode, H. (2019). Analysis of Pesticides Residues in Surface Water in Chhnok Tru Floating Community of Tonle Sap Lake during Low Water Season. Proceedings of the 12th Regional Conference on Chemical Engineering (RCCHE2019), Ho Chi Minh City, Vietnam.
- Codex Alimentarius Commission (2017). Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed (Doc. No. CXG 90-2017).
- European Food Safety Authority (2014). Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for amidosulfuron according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal*, 12(3), 3614.
- Hornsby, A. G., Wauchope, R. D., & Herner, A. (1995). Pesticide properties in the environment. Springer Science & Business Media. *Soils. Applied Sciences*, 8(12), 2343.
- IPCS (1992). Endrin. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 130).
- Khan, S.U. (2016). Pesticides in the Soil Environment. Elsevier.
- Jinya, D. (2013). Development of Solid-Phase Extraction Method for Simultaneous Analysis of Semi-Volatile Organic Compounds Using a GC-MS Database System. Shimadzu Corporation. C146-E202, 1-8.
- Kidd, H. & James, D.R. (1991). The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 5-14.
- Ministry of Agriculture, Forestry and Fisheries (2013). Legal standards under the Law on the Management of Pesticides and Fertilizers. Prakas No. 484. Ministry of Agriculture, Forestry and Fisheries.
- Matsukawa, M., Ito, K., Kawakita, K., & Tanaka, T. (2016). Current status of pesticide use among rice farmers in Cambodia. *Applied entomology and zoology*, 51(4), 571-579.
- Pimentel, D. (1971). Ecological Effects of Pesticides on Nontarget Species. Executive Office of the President's Office of Science and Technology. U.S. Government Printing Office, Washington, DC, 1971.5-28.
- Rao, P.S.C, Davidson J.M. (1982). Retention and transformation of selected pesticides and phosphorus in soil systems: a critical review. Athens, GA, US Environmental Protection Agency (EPA-600/53-82-060).
- Sapbamrer, R., Hongsibsong, S.(2014). Organophosphorus pesticide residues in vegetables from farms, markets, and a supermarket around Kwan Phayao Lake of Northern Thailand. *Archives of environmental contamination and toxicology*, 67(1), 60-67.
- Sittig, M. (1980). Priority Toxic Pollutants: Health Effects and Allowable Limits. NOYES DATA CORP., PARK RIDGE, N. J. 1980.
- Wang, H. S., Sthiannopkao, S., Du, J., Chen, Z. J., Kim, K. W., Yasin, M. S. M., & Wong, M. H. (2011). Daily intake and human risk assessment of organochlorine pesticides (OCPs) based on Cambodian market basket data. *Journal of hazardous materials*, 192(3), 1441-1449.
- Uk, S., Yoshimura, C., Siev, S., Try, S., Yang, H., Oeurng, C., & Hul, S. (2018). Tonle Sap Lake: Current status and important research directions for environmental management. *Lakes & Reservoirs: Research & Management*, 23(3), 177-189.