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# Assessment of Pesticide Residues in Surface Water, Sediment, and Fish from Chhnok Tru, Kampong Chhnang

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**Abstract:** *Pesticide residues in surface water, sediment and fish samples from Chnok Tru Floating Community of Tonle Sap Lake (TSL) was determined. Surface water and sediments from 18 sites were collected while 10 fish species were sampled from different locations of Chhnok Tru Floating Village. Samples were extracted by solid-phase extraction method using PLS3-AC2 cartridge and were subjected to analysis by GC-MS with an automated identification and quantification system. The results showed that 67% of water samples, 44% of sediment samples, and 70% of fish samples were contaminated with at least one pesticide compound. Among the 23 pesticide quantified, o,p'-DDT was the predominant pesticide commonly detected in all sample types. The highest concentration of o,p'-DDT was 2.32 µg/L in water, 18.3 ng/g dry weight in sediment, and 35.8 ng/g dry weight in fish samples. The presence of these pesticides in water, sediments and fish muscle brought about the great concern on not only ecosystem health but also public awareness. Therefore, spatial distribution and seasonal monitoring of these toxic chemicals should be further investigated to ensure the safety of aqua organism as well as human who dependent on this lake.*

**Keywords:** Pesticide analysis; Chhnok Tru; Tonle Sap Lake

## 1. INTRODUCTION

The Tonle Sap Lake (TSL), also known as the Great Lake, is situated in the central plains of Cambodia. The Tonle Sap River, 120 km long, links the lake with the Mekong River. The lake is known for its rich biodiversity and exceptional water regime, with vast seasonal fluctuations in water level and volume (Keskinen, 2006). Fishing and crop cultivation in the TSL basin benefited from ample freshwater, nutrients and rich soils generated by seasonal flood pulse and rainfall; together these ecosystem services have sustained the region's livelihoods for centuries (Lin & Qi, 2017).

Agriculture is the economic backbone of Cambodia in which more than 70% of population use to be involved in agricultural activities (FAO, 2014; Jensen et al., 2011a). However, agriculture sectors face some constrain such as crop losses due to pest infestication. It is reported that global crop losses due to weeds accounted for 33 percent, 26 percent due to plant diseases, 20 percent to insect pests, and 21 percent to pests (Kimkhuy & Chhay, 2014). A number of global monitoring studies have shown the ability of pesticides to contaminate surface and ground water due to runoff, groundwater leaching and spray drift (Jensen et al., 2011b; Kapsi et al., 2019; Papadakis et al., 2015). In Cambodia, pesticides are applied to prevent pests and increase crop yields. However, farmers are not aware of proper pest management particularly in pesticide application (Matsukawa et al., 2015). Cambodia do not produce pesticides; therefore, pesticides are imported from other countries thus labelled in foreign languages which is incomprehensible to local farmers. Heavy uses of highly toxic compounds and improper management of pesticide application cause significant health issues to agricultural workers in low-income countries (Jensen et al., 2011a).

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Each year, over 3 million cases of pesticide poisoning with an estimated 220,000 deaths occur globally especially in developing countries. Most of them were caused by organophosphate groups (Thetkathuek et al., 2014).

Scientific evidences are nessesary to raise awareness of harmful effects of pesticides toward all stakeholders including farmers and government agency. However, there is limited information on pesticide contamination in Cambodia. Therefore, this study aims to evaluate the levels of some banned pesticides in surface water, sediment, and fish from Chhnok Tru, Kampong Chhnang, Cambodia. Chhnok Tru is one of the floating communites of TSL. With its special characteristic, during rainy season, most of the village are under water; whereas, large cultivated lands are available during dry season. This contribute to the abundant of rice and other crop cultivation in this area making it is a nessesary to study the contamination levels of pesticides from agriculture to water and aquasystem.

## 2. METHODOLOGY

### *2.1 Study area*

Chhnok Tru is one of three floating villages located in Chhnok Tru Commune, Kampong Chhnang. Most parts of this area are covered by water, but land is also available for crop cultivation activities in some areas during the dry season. The village has two small islands within its boundaries, namely Chhnok Tru and Mo No, which local people use to grow crops such as rice, potatoes, pumpkins, musk melons, mandrels and water melons in the dry season (Phanith, 2017).

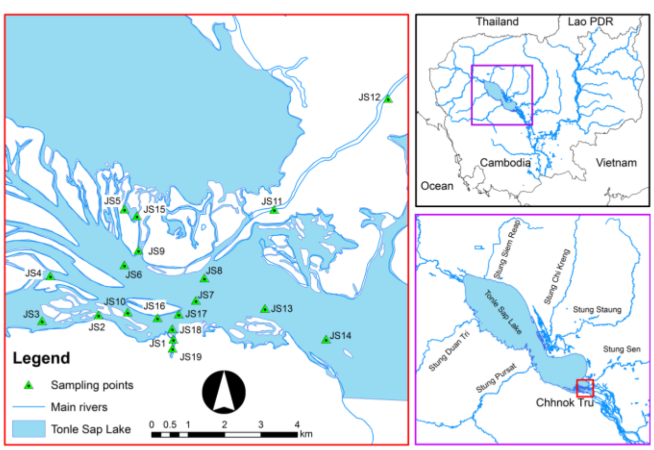


Fig.1. Water and sediment sampling sites at Chhnok Tru

### *2.2 Sample collection*

Surface water (around 20 cm depth) and sediment samples were collected on 07 - 08 march 2018 at different sites around Chhnok Tru community (Fig.1). Cross-section across Chhnok Tru area were sampled for water and sediment samples. Water samples were stored in cleaned 1 L plastic bottles covered with screwed caps and kept in ice-box during transport to the laboratory at Institute of Technology of Cambodia for analysis. The sediment samples were collected from a boat using a sediment grabber, then the core was sliced into 1-cm samples and placed in sealed plastic bags. After collection, sediments were preserved in a cooled icebox immediately in order to avoid pesticide degradation during transport to the laboratory.

Freshwater fish samples (Trey Chhdor (*Channa micropeltes*), Trey Chhkork (*Cyclocheilichthys enoplos*), Trey Chrakaing (*Puntioplites falcifer*), Trey Khlanghaiy (*Belodontichthys truncatus*), Trey Khmann (*Hampala macrolepidota*), Trey Po (*Pangasius larnaudii*), Trey Pra (*Pangasius hypophthalmus*), Trey Proma (*Boesemania microlepis*), Trey Slat (*Notopterus notopterus*), Trey Trasorksor (*Probarbus labeamator*)) were collected from fishermen at the various sites in Chhnok Tru, Kompng Chhnang Province. Sampling was done between November and March, 2018. Fish were also wrapped in sealed plastic bags and kept in cooled iceboxes during transport to the laboratory.

### *2.3 Water sample preparation*

Sample preparation has been adopted from Jinya (2013) in which 1 L of surface water sample has been adjusted with 1 mL of phosphate buffer solution (1 mol/L, pH 7.0) (Jinya, 2013). Water samples were then filtered using 0.45 µm fiber glass filter (Whatman GMF 150) to remove suspended solids. , Suspended solids retained by the filters were eluted with 5 mL of acetone and 5 mL of dichloromethane to dissolve potentially adsorbed compounds . Filtered samples were subjected to extraction process by using solid-phase extraction method (SPE).

### *2.4 Sediment sample preparation*

Sediment was air dried at room condition around 5-7 days. Then, samples were grinded using porcelain mortar pestle and sieved through a stainless steel screen. Extraction method for sediment sample was done following the method of Kafilzadeh with some modification (Kafilzadeh, 2015). First, 10 g of dried sediment sample was placed in beaker Erlenmeyer 250 mL containing 20 g of anhydrous sodium sulfate and 1 ml of solution sodium phosphate buffer pH 7. Then, 100 mL of acetone: n-hexane ratio (1:4) was added and mixed thoroughly. After that, it was stirred overnight with magnetic stirrer. Then, the mixture was filtered with Whatman filter paper with pore size of 2 µm and stored at 4°C in refrigerator before applied to SPE.

*2.5 Fish sample preparation*

At least three fish per species were collected and fileted for muscle. Fish muscles were then grind and freeze-dried. Five grams of freezed dry fish muscle in powder form was homogenized with 50 mL of distilled water and 100 mL of aceton/hexan mixture (50/50, v/v) was added to the suspension and stirred for 15 min. After that, this mixture was filtered with Whatman filter paper (pore size of 2 µm). Then, ten grams of sodium chloride and 1 mL of phosphate buffer solution (1 mol/L, pH 7.0) were added into the filtrated solution and stirred again for 10 min. After that, the decanter was used to separate the layer of organic solvent from fish fat part. Then, anhydrous sodium sulfate was used to remove any remaining water and impurities from the solution. Lastly, the solution was applied to SPE.

*2.6. Solid-phase extraction (SPE)*

SPE was done using a combination of two cartrides according to the method of Jinya (2013). Cartridge were combined from Polymer Sorbent (PLS3) and Activated Carbon (AC2) solid phases. At the beginning, cartridge were conditioned with 5 mL of dichloromethane followed by 5 mL of acetone followed by addition of 10 mL of distilled water during which each solvent was left 1 or 2 min on cartridge before further process. Then, high-pressure solid-phase extraction instrument was set up. One liter of water sample passed through the cartridge at the speed of 15 mL/min. Later, cartridge was dried with nitrogen gas for 40 min. PLS3 was then eluted with 2 mL of acetone followed by 3 mL of dichloromethane, whereas AC2 was eluted with 3 mL of acetone. Each elution process was followed by holding time of 1 or 2 min on cartridge to ensure the well mix of solvent and cartridge. Solutions passed through PLS3 and AC2 were combined and concentrated to a volume of approximately 1 mL by streaming nitrogen gas. Concentrated solution of 1 mL was added with 5 mL of hexane; this combined solution was then transferred to sodium sulfate filter while tube was washed twice with 1 mL of hexane. The hexane was then pipetted into sodium sulfate filter. Solution was left to pass through this sodium sulfate filter followed by washing filer three times with 1 mL of hexane. This combined eluted solution was concentrated again with streaming nitrogen gas to volume of less than 1 mL; then, this solution was combined with washing hexane from tube to make 1 mL volume. Finally, 0.1 mL of internal standard containing 1 ppm of 4-Chlorotoluene-d4, Acenaphthene-d10, 1,4-Dichlorobenzene-d4, Naphthalene-d8, Fluoranthene-d10, Perylene-d12, Chrysene-d12, and Phenanthrene-d10 was added prior to analysis with the use of GC-MS.

*2.6 GC-MS analysis*

The pesticide compounds were analyzed using gas chromatograph and mass spectrometer (GC-MS), model TQ8040 series (Shimadzu, Japan). The column used was DB-5ms with the length of 30 m, thickness 0.25 µm and diameter 0.25 mm. One microliter of sample was injected to GC-MS by auto injector using spitless mode. After that, the column oven temperature was programmed from 40 to 310°C by holding for 2 minutes at 40°C, increased the temperature to 310°C with a rate of 8°C/min, and then held for 5minutes. The carrier gas was ultra-pure helium at the total flow of 50 mL/min while the column flow was 1.23 mL/min. The ion source temperature was 200°C and the temperature of the interface was 300°C. All pesticides were identified by retention time and specific ions, and quantified by the external standard method.

Table 1. Targeted pesticide compounds

| Group | Compounds | Status in Cambodia | Toxicity class (WHO) |
|---|---|---|---|
| Fugicides | Azaconazole | -- | II |
| | Chloroneb | -- | O |
| | Hexachlorobenzen | Banned | Ia |
| | Mefenoxam | -- | II |
| | Metalaxyl | -- | II |
| | Pyroquilon | -- | II |
| | Triadimefon | Permitted | II |
| Herbicides | Anilofos | Permitted | II |
| | Atrazine | Permitted | III |
| | Terbacil | -- | U |
| Insecticides | Aldrin | Banned | O |
| | Chlordane | Banned | II |
| | Dieldrin | Banned | O |
| | Endrin | Banned | O |
| | HCHs | Banned | NL |
| | Heptachlor | Banned | O |
| | Isazofos | Restricted | Ib |
| | Isoxathion | Banned | Ib |
| | Malathion | Permitted | III |
| | Methamidophos | Banned | Ib |
| | Methyl parathion | Banned | Ia |
| | O,p'-DDT | Banned | II |
| | Parathion | Banned | Ia |

--: No available information; Ia: Extremely hazardous; Ib: Highly hazardous; II: Moderately hazardous; III: Slightly hazardous; NL: Not listed; O: Obsolete or discontinued for use as pesticides; U: Unlikely to present acute hazard

## 3. RESULTS AND DISCUSSION

The assessment of pesticides in this study was focused on 23 target compounds which according to various studies are commonly detected in surface water, sediment and fish around the world (Deknock et al., 2019; Houbraken et al., 2017; Kafilzadeh, 2015; Kuranchie-Mensah et al., 2012; Le et al., 2017; Munschy et al., 2016; Papadakis et al., 2015; H. Wang et al., 2018; W. Wang et al., 2017). The concentrations of these compounds were quantified according to external standards solutions (Table 1). The samples analyzed in this studied were surface water from 15 sites, sediment from 18 sites, and 10 fish species from Chhnok Tru, Kampong Chhnang.

### *3.1 Levels of pesticides in surface water*

Table 2. Levels of fungicides in water from Chhnok Tru

| Sites | Fungicides (µg/L) | | | |
|---|---|---|---|---|
| | Chloroneb | Mefenoxam | Metalaxyl | Triadimefon |
| JS1 | - | - | - | - |
| JS3 | - | - | - | - |
| JS7 | - | - | - | - |
| JS8 | 0.10 | 0.14* | 0.06 | - |
| JS9 | - | - | - | - |
| JS10 | - | - | 0.06 | - |
| JS11 | 2.01* | - | 0.06 | - |
| JS12 | 0.17* | 0.15* | 0.06 | - |
| JS13 | - | - | 0.05 | 0.10 |
| JS14 | - | 0.13* | 0.05 | - |
| JS15 | - | 0.14* | 0.06 | - |
| JS16 | 0.05 | - | - | - |
| JS17 | - | 0.11* | 0.05 | - |
| JS18 | - | - | - | - |
| JS19 | - | - | - | - |

- : Not detected; *: Concentration that is above WHO guidline of drinking water (0.1 µg/L for individual pesticide compound)

Among targeted 23 compounds, four active compounds of fungicides, one of herbicides, and two of insecticides were detected in surface water collected from studied sites (Table 2 and 3). Metalaxyl was detected in 8 sites with the average level of 0.056 µg/L. Metalaxyl is a broad-spectrum fungicide that is widly used globlally to protect crops, vegetables and fruits against fungal diseases of damping-off, late blight, stem, downy mildew and fruit rots. Due to its good water solubility, matalaxyl can permeate into soil and result in potential toxicity by the rain-wash and irrigation (F. Wang et al., 2019). The levels of metalaxyl detected in this study was lower if compare to the maximum acceptable values (MAVs) which is set to be 100 µg/L for drinking water in New Zealand (Hamilton et al., 2003). Folowing that, mefenoxam was found in 5 studied sites, chloroneb in 4 sites, and triadimefon in one site. Mefenoxam, also known as metalaxyl-M or R-metalaxyl, is water soluble and broadly used worldwide (Camargo et al., 2019). Mefenoxam in this study was detected with average concentration of 0.134 µg/L which was over WHO guidline of drinking water (0.1 µg/L for individual pesticide compound) (WHO, 1993).

Table 3. Levels of herbicides and insecticides in water from Chhnok Tru

| Sites | Herbicides (µg/L) | Insecticides (µg/L) | |
|---|---|---|---|
| | Atrazine | Malathion | o,p'-DDT |
| JS1 | 0.02 | - | 2.32* |
| JS3 | - | - | 2.26* |
| JS7 | - | - | - |
| JS8 | - | - | 1.35* |
| JS9 | - | - | - |
| JS10 | - | - | - |
| JS11 | - | - | - |
| JS12 | - | - | - |
| JS13 | - | 1.45* | - |
| JS14 | - | - | - |
| JS15 | - | - | - |
| JS16 | - | - | - |
| JS17 | - | - | - |
| JS18 | - | - | - |
| JS19 | - | - | - |

- : Not detected; *: Concentration that is above WHO guidline of drinking water (0.1 µg/L for individual pesticide compound)

O,p'-DDT, a banned insecticide, was found in 3 sites such as JS1, JS3, and JS8 with the concentration of 2.32, 2.26, and 1.35 µg/L, respectively. The presences of this compound might be due to its highly persistence in environment or its illegal application. According to sub degree set by ministry of environment of Cambodia (1999), the standard limit of DDT in surface water is set to be lower than 10 µg/L. Malathion was detected in JS13 with the concentration of 1.45 µg/L. Malathion was also detected in Lucban River, Phillipines with the levels ranged 0.1 µg/L to 3.3 µg/L (Varca, 2012). Malathion is one of the foremost commonly utilized insecticides among vegetable producers since it is cheap and farmers perceive that its smell contributes to its viability to control pests (Varca, 2012). The levels of malathion detected in this study were highly over the WHO single pesticide limit of 0.1 µg/L for drinking water (WHO, 1993) but below the Australian drinking water health value of 50 µg/L and the Canadian drinking water maximum acceptable concentration (190 µg/L) (Varca, 2012).

The presences of pesticides in surface water, is mainly caused by spray drift, runoff water and drainage water. The contributed factors include improper operations such as filling of sprayers, washing of measuring utilities, disposing of packing materials and cleaning of spraying equipment (Mekonen et al., 2016). As the water in these study sites is the main source of drinking water for the population there, the contamination of pesticides bring serious concerns for human health as well as environment especially aqutic system.

*3.2 Levels of pesticides in sediment*

Pesticide residues in sediment were analyzed in this study and the results showed that only three compounds were detected viz chloroneb, mefenoxam, and o,p'-DDT (Table 4). O,p'-DDT were detected with the concentration ranging from 0.2 ng/g in JS4 to 18.3 ng/g in JS12. The average levels of detected o,p'-DDT in sediment was 9.32 ng/g dry weight which was 4.5 times higher than the levels detected in surface water samples. This might be due to its lower water solubulity which make it easily adsorbed on to suspended particulate matter (SPM). SPM can then precipitate in sediments and eventually lower the o,p'-DDT concentrations in water (Liu et al., 2016). In other studies, o,p'-DDT were found in sediment with the mean concentration of 0.51 ng/g dry weight in Yellow River estuary (Da et al., 2014); 72.67 ng/g dry weight in Vasai Creek near Mumbai (Singare, 2015); 4.59 ng/g dry weight in Hanjian river basin (Liu et al., 2016). Mefenoxam was detected only in JS1 with the level of 4.5 ng/g. The dissipation half-life for mefenoxam in soil was 5–8 days (Triantafyllidis et al., 2012). Chloroneb was found in three sites with highest concentration of 768.8 ng/g in JS1. JS1 was sampled at the port of Chhnok Tru community, which was polluted too much by human activities including throwing pesticides bottles into the water. Furthermore, the water in this location was stable that might contribute to the fact that chloroneb probably accumulated in this point. Chloroneb can be readily biodegraded with half-lives of 180 days in soil. If released into water, chloroneb is expected to be adsorbed to suspended solids and sediment (Maloney, 2001).

Table 4. Levels of pesticides detected in sediment from Chhnok Tru

| Sites | Fungicides (ng/g dry weight) | | Insecticides (ng/g dry weight) |
|---|---|---|---|
| | Chloroneb | Mefenoxam | o,p'-DDT |
| JS1 | 768.8 | 4.5 | - |
| JS2 | - | - | - |
| JS3 | - | - | 11.4 |
| JS4 | - | - | 0.2 |
| JS5 | - | - | - |
| JS6 | - | - | 7.9 |
| JS7 | - | - | - |
| JS8 | - | - | - |
| JS9 | 16.7 | - | - |
| JS10 | - | - | - |
| JS11 | - | - | - |
| JS12 | - | - | 18.3 |
| JS14 | - | - | - |
| JS15 | - | - | - |
| JS16 | - | - | - |
| JS17 | 16.8 | - | - |
| JS18 | - | - | - |
| JS19 | - | - | 8.8 |

- : Not detected

*3.3 Levels of pesticides in fish*

Ten species of fish were collected from Chhnok Tru community and analyze for pesticide residues. Two fungicides (chloroneb and metalaxyl) and one insecticide (o,p'-DDT) were detected in fish muscles (Table 5). Chloroneb was found in Trey Chhdor and Trey Trasorksor with the levels of 22 and 60 ng/g dry weight, respectively. Metalaxyl was detected only in Trey Pra with the concentration of 9 ng/g dry weigth. While, o,p'-DDT was found in 60% of fish samples with the maximum detected concentration of 35.8 ng/g dry weight in Trey Slat. Other studies also reported the contamination of DDT in fish (Chouvelon et al., 2017; Munschy et al., 2016; Varol & Sünbül, 2017). O,p'-DDT has become a major environmental problem due to their long persistence, bioaccumulation and adverse effects on animals and humans. In aquatic environments, o,p'-DDT easily enter the food chain due to their hydrophobic and lipophilic properties, finally reaching humans by consumption of edible aquatic organisms (Varol & Sünbül, 2017). O,p'-DDT and other derivatives of DDT are banned in Cambodia due to their possible carcinogenic and mutagenic properties (Ministry of Agriculture, 2019; Varol & Sünbül, 2017).

Table 5. Pesticide levels detected in fish from Chhnok Tru

| Fish sample | Fungicides (ng/g dry weight) | | Insecticides (ng/g dry weight) |
|---|---|---|---|
| | Chloroneb | Metalaxyl | o,p'-DDT |
| Trey Chhdor (*Channa micropeltes*) | 22 | - | 2.6 |
| Trey Chhkork (*Cyclocheilichth*ys *enoplos*) | - | - | - |
| Trey Chrakaing (*Puntioplites falcifer*) | - | - | 13 |
| Trey Khlanghaiy (*Belodontichthys truncates*) | - | - | 1.8 |
| Trey Khmann (*Hampala macrolepidota*) | - | - | 30.7 |
| Trey Po (*Pangasius larnaudii*) | - | - | - |
| Trey Pra (*Pangasius hypophthalmus*) | - | 9 | - |
| Trey Proma (*Boesemania microlepis*) | - | - | - |
| Trey Slat (*Notopterus notopterus*) | - | - | 35.8 |
| Trey Trasorksor (*Probarbus labeamator*) | 60 | - | 2 |

- : Not detected

## 4. CONCLUSIONS

In this study, surface water samples from 15 sites, sediment sample from 18 sites and ten fish species were collected from Chhnok Tru floating community and evaluated the levels pesticide contamination. The results showed that 67% of water samples, 44% of sediment samples, and 70% of fish samples were contaminated with at least one of the 23 pesticide compound measured. Pesticides that were detected frequently in these three sample types were o,p'-DDT, chloroneb, metalaxyl, and mefenoxam. The presences of pesticides in multiple environment of Chhnok Tru community expouse the citizen surrounding that area to various exposure routes which increase the risk of adverse heatlh effects of those pesticide compounds. Regular monitoring studies should be done in order to raise public awareness of pesticide contamination and prevent the worseness of pesticide pollution problem.

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