ANTIBIOTICS AND PESTICIDES IN WATER AND SEDIMENTS FROM INTENSIVE SHRIMP FARMS IN SOUTHERN VIETNAM

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ABSTRACT

In recent years, shrimp farming has developed rapidly and become one of the major export sectors of Vietnam. In particular, Mekong Delta Vietnam (MDVN) accounts for over 90 % shrimp farming area in Vietnam. Shrimp industry development leads to use a large amount of antibiotics (ABs) and pesticides for disease prevention, treatment and sanitation pond. These compounds are usually discharged directly into the environment through water and sludge after harvesting. The presence of antibiotics and pesticides in the environment cause negative impacts on ecosystems and human health. In this study, the passive sampling method with POCIS sampler (Polar Organic Chemical Integrative Samplers) was used to monitor the presence of 12 ABs and 2 pesticides in water of intensive shrimp ponds and the surrounding canals at Tan Tru District (Long An province) and Can Gio District (Ho Chi Minh city) following a production cycle. The results showed that ABs and pesticides were detected in water and sediment in the pond and canals in both research sites. Two pesticides (atrazine and diuron) were detected in the pond and leading canals in both study areas with low concentrations from several ng/L to several tens of ng/L. In Tan Tru, three antibiotics (Trimethoprim, Sulfamethoxazole and Enrofloxacin) were detected in water at concentrations ranging from several tens of ng/L to 300 ng/L; in sediments of shrimp pond and canal the levels of pesticides and ABs ranged from 10 to 200 µg/kg. In Can Gio - HCMC, 4 ABs (Trimethoprim, Sulfamethoxazole, Ciprofloxacin and Enrofloxacin) were detected ranging from a few ng/L to several µg/L in water, and a few mg/kg in sediments of the pond. The results also showed that in the pond, ABs type and concentration detection varied by region and shrimp growth cycles.

Keywords: shrimp farming; antibiotics; pesticides; water; passive sampling.

1. INTRODUCTION

Vietnam is one of top ten countries in shrimp production [1]. Shrimp export contributes an important part of the national economy. However, at present, the intensive shrimp farms in Vietnam are facing the risk of decreased yields due to the disease [2, 3]. Therefore a large amount of ABs is used continuously to prevent and treat diseases in shrimp. Besides that, the pesticides are also used for sanitation and eliminating contaminants in the pond. Moreover a large number of ABs and pesticides are discharged directly into the environment through water and sludge ponds after shrimp harvest without any treatment. The presence of ABs and pesticides in the environment can cause negative impacts on human health and ecosystems.

Many studies show that ABs is detected in water and sediment, sulfamethoxazole and trimethoprim are the most frequently detected in surface water at concentrations ranging from several tens ng/L to over 1900 ng/L for sulfamethoxazole [4 – 6]. Concentration of ABs in the sediment is often more important than in water [7], oxytetracyclin and flumequine detected at concentrations up to 246 mg/kg and 578 mg/kg in sediment of the fish farms [8]. In Vietnam, only a few studies on the presence of ABs in wastewater and urban hospitals, very few researches on the ABs in the shrimp farms [9]. Moreover, these studies only used the methods that the concentration of the ABs could be detected at a certain time. There was no research about tracking the changes of ABs concentration and the behavior of ABs during shrimp culture. Can Gio District (Ho Chi Minh City) and Tan Tru District (Long An Province) are two intensive shrimp farming in the Mekong Delta with over 3000 hectares shrimp farming. The shrimp farmers use ABs continuously feeds during shrimp culture. The ABs types and doses are usually according to the experience of the farmers, not through any guidance of management agencies. Moreover, ABs in the ban list are still used for shrimp farming. Therefore, in this study, we used the passive sampling method with POCIS sampler (Polar Organic Chemical Integrative Samplers) to evaluate continuously the presence and behavior of 12 ABs and 2 pesticides in water and the surrounding canals of intensive shrimp farms in two regions Tan Tru, and Can Gio. In addition, the sediment in shrimp farms also was evaluated.

2. MATERIALS AND METHODS

2.1. Materials Chemicals and reagents

Erythromycin (ERM), Sulfamethoxazole (SMX), Trimethoprim (TMP), Atrazin (ATZ), Diuron (DRN), Enrofloxacin (EFX), of Dr Ehrenstorfer were purchased from Toronto Research Chemicals. Ciprofloxacin (CFX), Norfloxacin (NOR), Ofloxacin (OFL), Sulfamethazin (SMT), Tetracycline (TC), Chlotetracyclin (CTC), Tylosin (TLS) and Vancomycin (VAN) were obtained from Sigma-Aldrich (Vietnam). All standards have a purity level higher than 99 %. The internal standards, Ciprofloxacin-d8, CIP-d8, Sulfamethoxazole-d4, Trimethoprim-d3 (TMR-d3), Erofloxacin-d5 (EFX-d5), Tetracycline-d6 (TC-d6), Diuron-d6 (DIU- d6) and Atrazin-d5 (ATR-d5) were purchased from Sigma-Aldrich with a purity level greater than 95 %. Deuterium Compounds Performance Reference Substances (PRC) labeled atrazine-desisopropyl (DIA-d5) were purchased from Sigma-Aldrich with 99 % purity. The solvent: Methanol (MeOH) (VWR), acetonitrile (ACN) (Merck) qualified used for LC-MS/MS. Formic acid (99 %), ortho-phosphoris acid (85 %), NaOH 50 % were purchased from Sigma-Aldrich. Ultrapure water (Up-water) taken from ultrapure water system (Elga Labwater). OASIS HLB extraction column (200 mL, 6 mL) was bought from Waters. Polyethersulfone membrane (PES) (0.1 µm 90 mm) were purchased from Pall Corporation (Ann Arbor, USA). Liquid chromatography combined with

dual probe Mass spectrometry (LC-MS/MS) (Agilent 1200 RRLC).The individual standard solutions are diluted at 1 mg/mL in MeOH, for CIP, NOR, OFL were added NaOH 1M to increase the solubility at 1 ‰, the standard solution was stored at -18 ^{o}C. Then a mixture of standards called the source solution included all of the standard mixture at a concentration of 1 µg/mL diluted in a mixture of MeOH/Up-water (5/95, v/v), then this source solution was diluted in the different concentrations at 200-100-50-10 and 5 ng/mL to run baseline on LC-MS/MS. This diluted solutions were stored at 4 °C and renewed after 2 weeks. POCIS samplers used for passive sampling. Each consists of two membranes POCIS polyethersulfone (PES), 0.1 µm thick and 90 mm diameter, its middle is 200 mg film-absorbing material OASIS – HLB. All materials are fixed by 2 stainless steel rings. DIA-d5 used as Performance Reference Compound (PRC), the desorption rate's PCR is corresponding to the percentage of Abs (or pesticides) that we want to detect adsorbed into the adsorbent of POCIS.

2.2. Methods

2.2.1. Sampling area

This study was conducted at 2 intensive shrimp farms in Tan Tru District – Long An province and Can Gio – HCMC. These are two areas of intensive shrimp farming in the Mekong Delta that have completely different characteristics. Tan Tru District – Long An, the shrimp pond area are about 5000 m^2 belong to different owners, groundwater with low salinity (about 7 ‰) are being used in these shrimp ponds, while at Gio HCMC shrimp farms have larger areas (tens of hectares) and are invested professionally, water is used as the Saigon river surface water with high salinity (about 23 ‰) and processed through many different stages before leading to the shrimp pond. Water and sediment were taken in the shrimp pond and the canals in Tan Tru, while in Can Gio samples were taken at canals, treatment pond and shrimp pond.

2.2.2. Sampling collection

For water samples, samples were taken by Passive Sampling with POCIS samplers. POCIS samplers were stored at 4 ^{o}C after preparation. POCIS were fixed in a stainless steel protection cage, each cage will be fixed 2 POCIS, then it would be fixed on a plastic rod and placed in the environment that needed to be analyzed. On each sampling, there were 2 POCIS (keeping in one cage) placed at every point during 14 days and there were 2 POCIS samplers used as controls. At Tan Tru, Long An, due to well-developed shrimp, shrimp ponds continuously surveyed two consecutive breeding cycle from March to December in 2015 and in Can Gio, the survey conducted only from March to June in 2015 because of the shrimp death after two months. The chemical and physical parameters of environmental sampling at locations including: temperature, salinity, conductivity, pH, DO, flow rate was measured in place.

For sediment samples, at each sampling point about 100 g surface sediment was taken by the plastic spoon or stainless steel mud sampler, sediment samples were kept in 148eighb and stored at 4 °C before transferring to the laboratory. In the laboratory, water in sediment was removed by the lyophilized, then sieved through a 1 mm sieve to remove impurities and then extracted with ultrasonic waves. If the sediments cannot extract immediately, they will be stored at -18 ^{o}C and extract later.

2.2.3. Sample preparation and analysis

After transferring to the laboratory, the POCIS were detached each parts and then Oasis HLB absorbent in membranes was collected stuffed back into the column, then eluted with 5 mL MeOH and concentrated with a stream of nitrogen at 40 ^{o}C. Then it was reconstituted with 0.5 mL of MeOH (10 %) with 0.1 % formic acid, and filtered through a 0.2 µm syringe before analyzing by liquid chromatography combined mass spectrometry (LC-MS/MS) [10].

For sediments samples, 2 g dried sediment were placed in a 30 mL glass tube and extracted twice (the first time under the acid condition and the second time under base condition) with 10 mL (MeOH/1M H_3PO_4 80/20 v/v, + 0.1% EDTA 1 M), then 10 mL (MeOH / 5 % NaOH 20/80 v/v, + 0.1 % EDTA 1 M). Two extraction solutions were mixed together and were blown with N_2 at 40 ^{0}C until remaining a few mL volume. This concentrated solution were added 200 mL Milli-Q water and adjusted to pH = 7 with orthophosphoric acid (50 %). Then proceed the solid phase extraction prior to analysis by LC-MS/MS described by Dinh et al. [10].

3. RESULTS AND DISCUSSION

3.1. Physical and chemical parameters in environment

In general, in both sampling areas had temperature (24 – 32 ^{o}C) and salinity (6 -7 ‰ at Tan Tru and 20 – 25 ‰ at Can Gio) that increased along with sampling time. pH ranged from 6.8 to 8.0 and the dissolve oxygen (DO) in the pond changed from 8 to 10 mg/L, while the canal DO had large variations from 1 to 10 mg/L. The rate of flow had a major difference between canals (0 – 15 cm/s). These phenomena can be explained by warming and the intensification of solar radiation at the dry season and the tidal phenomenon had changed the rate of flow. The dissolve oxygen also varied significantly in the canals at Tan Tru, Long An. This could be due to the disposal of the large pig manure amounts at upstream that caused eutrophic phenomenon. However, the dissolve oxygen concentration is not a significant factor to affect the rate of absorption of polar compounds in POCIS [11].

3.2. Antibiotics and pesticides in the shrimp pond and the surrounding canals

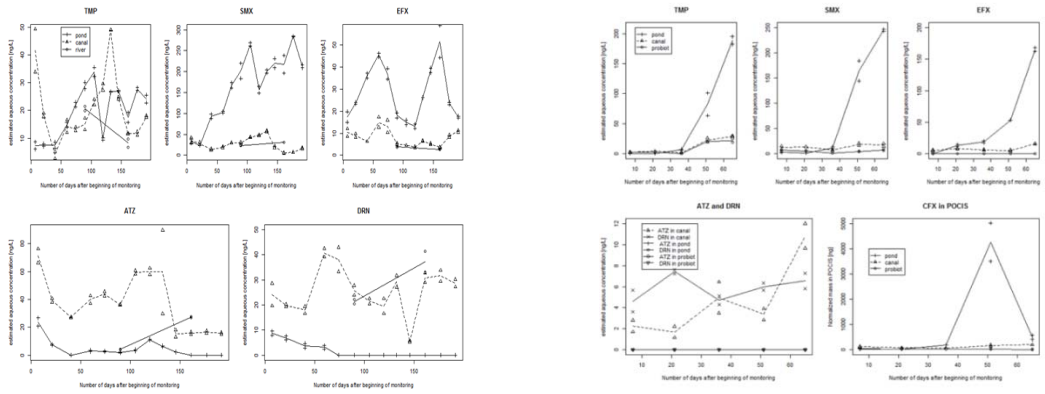
3.2.1. Tan Tru – Long An

The changes in concentrations of Abs and pesticides in the shrimp ponds had been tracked during 199 days, from March to May 2015 (Figure 1). At the beginning, shrimp pond was filled by half of groundwater and half of surface water for the first 30 days. Then, shrimps (*Penaeus vannamei*) were cultured and harvested after 80 days. After that the pond was kept to stabilize the physical and chemical parameters in 20 days. Then shrimps were cultured for the second harvest and harvested in the day of 190th. After two harvests, water and sediment of the pond were discharged directly into the canal and released to Vam Co Dong river. The Figure 1 shows that the concentration of TMP, SMX and EFX in the shrimp pond were detected ranged from several dozen ng/L to 300 ng/L. This proves that these Abs had been used during feeding. ATZ and DRN pesticides were also found in the pond with low level is from several to 40 ng/L, these pesticides were present in the pond that could come from groundwater and surface water supply. The other Abs had concentrations lower than the limits of detections (LODs) (LODs were in the range 0.5 – 13.0 ng/L of Abs and 0.3 – 0.7 ng/L of pesticides and the recoveries were higher than 80 % of both). The concentrations of EFX, SMX were high in both two harvest and significantly higher than TMP, especially for SMX. This can be explained by the ratio of SMX and TMP, the farmers usually use these Abs with the ratio TMP : SMX = 1 : 5. Moreover, the

farmers tend to use more quantity of SMX when shrimps were diseased. In addition, the baby shrimps were fed with a large of EFX, while the old shrimp were not fed with EFX. These led to EFX concentration were high in the first stage of the shrimp production cycle. Besides that, shrimps in this study could be diseased in the end of the production cycle, leading to concentration of SMX increased significantly in this period. In the surrounding canals and river SMX, TMP and EFX were also detected with low concentration,10 – 40 ng/L. DRN and ATZ pesticides in the canals had concentrations higher than Abs, this shows that these pesticides may be from agriculture (rice) around the shrimp ponds.

In general, except SMX, almost Abs concentration in the shrimp pond and the canals were not too high. TMP concentration lightly increased in the pond and decreased in the canals. The SMX and TMP concentration's tendency were similar; however, SMX concentration promptly increased on the second sampling. The other Abs were not found, it could say that the farm owner did not use these Abs during shrimp farming. The presence of CFX, TMP and EFX in the canals may be explained by the pig feed from some households where were closed to the canals or the other polluted sources in Vam Co Dong river.

ATZ's trend in the shrimp pond and the canals were similar. ATZ presence was due to the farm owner used it to control weed around the shrimp pond or it was available in water supply. However, ATZ concentration in the canals in the third sampling periods maybe lightly increased, this may be due to ATZ used to remove grass from the other farms or 150eighbor rice fields.



Tân Trụ - Long An

Can Gio – Hochiminh

Figure 1. Antibiotic and pesticide concentrations (ng/L) in the shrimp pond and the canals at Tan Tru-Long An and Can Gio-Hociminh City.

Abs and pesticides concentrations in the sediment of shrimp pond and the canals are shown in Figure 2. In which, ATZ and DRN in the shrimp pond sediments were lower than the canal sediments (0 – 20.1 µg/kg) with 23.8 µg/kg and 63.8 µg/kg respectively, these results were suitable to ATZ and DRN surveyed results in the shrimp pond and the canals. Similarly, ERM, EFX and TMP in the shrimp pond sediments were corresponding with 156.6; 4152 and 146.8 µg/kg that were higher than the canal sediments (5.6; 12.4 and 24.2 µg/kg). EFX concentration in sediment significantly higher than other Abs can be explained by the absorption coefficient (K_d) of these Abs into sediment. K_d of EFX is from 3037 – 5612 L/kg, while K_d of ERM and TMP were 8 – 290 and 76 L/kg, respectively [12]. Therefore, a high concentration of EFX had existed in the sediment of shrimp pond for a long time.

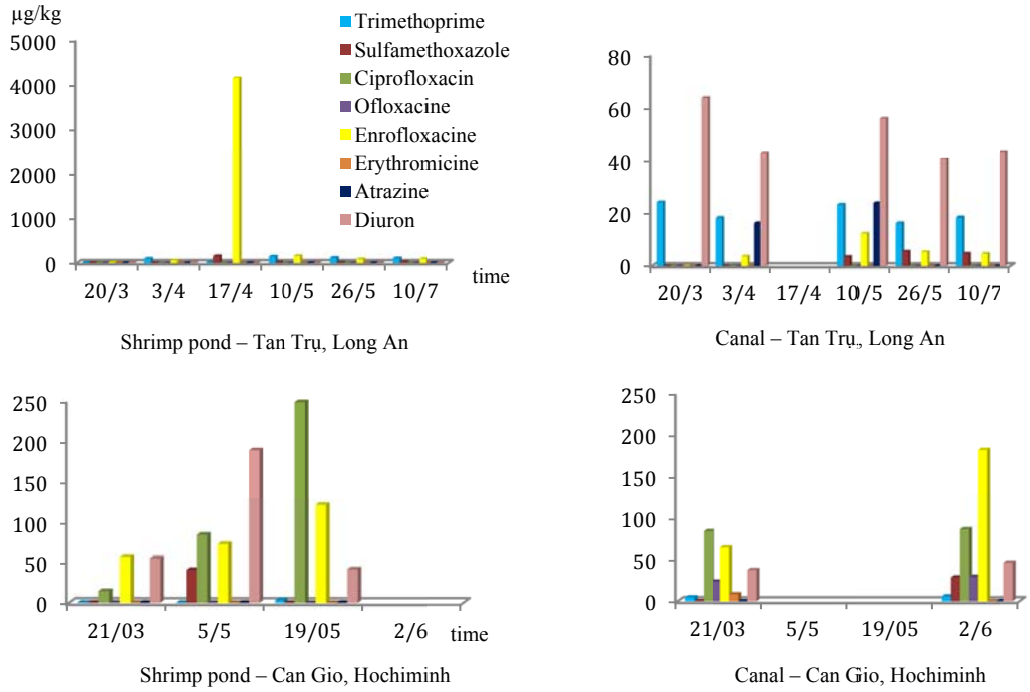


Figure 2. Antibiotics and pesticides concentrations (µg/Kg) in sediments in the shrimp pond and the canals at Tan Tru – Long An and Can Gio – Hochiminh City.

3.2.2. Can Gio – Ho Chi Minh City

Abs and pesticides concentrations in water were monitored in 10 weeks along with sampling 5 times. Sampling process was stopped at the fifth times because all of the shrimp were died. Abs and pesticides concentrations were found in the treatment pond, shrimp pond and canals water that are shown in Figure 1. This indicated that TMP, SMX, CFX and EFX were available in shrimp feed, while CFX was used in high doses and also mixed into food feeding during the diseased shrimp treatment. In this study, shrimps got the disease after roughly 30 days, and the farmers raised dramatically dose antibiotic usage. Consequently, the concentrations of TMP, SMX and EFX detected in shrimp pond were high in this period, reached 160 – 200 ng/L. In the canals, the Abs and pesticides above were also identified with low levels of a few dozen ng/L. While in the pond pesticides were almost undetectable.

The concentration of Abs and pesticides in sediment pond and canals are shown in Figure 2. In particular, OFL and ATZ had not found the sediment pond, but appeared in the canals, the results were consistent with the results of the analysis in the pond and canal water. DRN was not detected in the sediment, but in the water, indicating DRN had been used in previous harvest or DRN was present in the inlet water. SMX, TMP, CFX and EFX were detected in sediment ponds and canals, but the levels were not high.

The different concentrations of Abs and pesticides detected in the surface water and sediment in Long An and Can Gio can be explained by the use of different Abs and the effects of urban areas (Ho Chi Minh city) on the Can Gio while Long An was affected by rural areas.

4. CONCLUSIONS

Applying passive sampling with POCIS sampler in monitoring the antibiotic and pesticide concentrations in the intensive shrimp farm water give accurate and continuous results. Survey results at two shrimp farming show ABs and pesticides which were chosen in this study were commonly existed in the water and sediments. Antibiotic concentrations in the shrimp pond was high, however each shrimp farm used the different ABs types and ABs doses. Using antibiotic to prevent and cure shrimp diseases, and stimulate the shrimp growth can threat to the ecological environment of the surrounding areas. There should be a legal mechanism and training courses to the shrimp farm owners. On the other hand, it is necessary to apply simple and inexpensive techniques to treat ABs and pesticides after harvesting such as constructed wetland technology.

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