

# OCCURRENCE OF HUNDREDS OF WATER SOLUBLE CHEMICALS IN VIETNAMESE RIVER WATER

Hong CTC<sup>1</sup>, Shirasaka H<sup>1</sup>, Nguyen QT<sup>2</sup>, Kadokami K<sup>1\*</sup>

<sup>1</sup>The University of Kitakyushu, 1-1 Hibikino, Wakamatsu, Kitakyushu, 808-0135 Japan;

<sup>2</sup>Institute of Environmental Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam

## Introduction

In Vietnam, pollution of waterways (rivers, lakes, canals) has become worse because of the lack of connection to wastewater treatment plants. Hanoi and Ho Chi Minh City are the most densely populated cities and have many warnings about environmental pollution. Cities focused on economic development, such as Danang and Hue, are at risk of developing similar pollution problems to Hanoi and Ho Chi Minh City. In Vietnam, there have been quite a large number of studies on priority organic contaminants (e.g. PAHs, POPs.), but the number of studies on the environmental status of emerging contaminants, such as water soluble pharmaceutical and personal care products (PPCPs) is rather limited.

In this study, we have applied a multi-residue method using liquid chromatography-time of flight-mass spectrometry linked to an accurate mass database of 270 compounds to determine the number and levels of water soluble chemicals in river water in Hanoi, Ho Chi Minh City, Danang and Hue in Vietnam. This method is a straightforward approach for the quantitation and identification of these compounds with good sensitivity. The target compounds were agrichemicals and PPCPs which have become of increasing interest recently. The purposes of this research were to obtain a picture of the pollution status of water soluble chemicals in Vietnamese rivers, and to find characteristic substances in Vietnamese cities.

## Materials and methods

### *Sampling and sample preparation*

In February 2013, twenty eight river samples were taken from urban and suburban zones of 4 cities (Hanoi, Hue, Danang, Ho Chi Minh City (HCMC)) located in the north, centre and south of Vietnam (Fig.1).

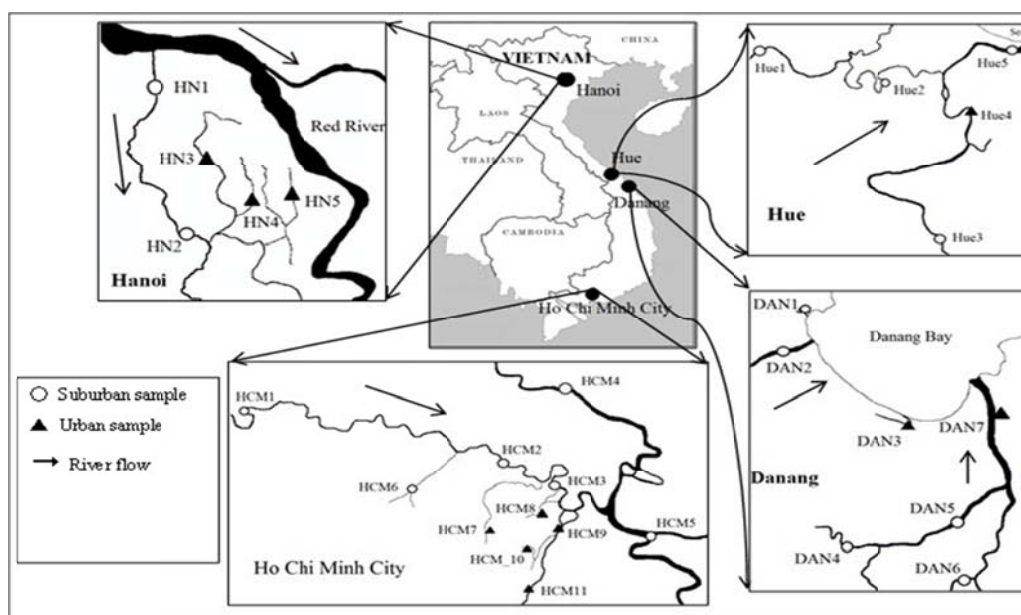


Fig.1 Location of sampling sites

To each water sample (500 mL) was added 1 mL of phosphate buffer (1 M, pH 7.0), and the mixture then filtered through a 1.2 µm glass fiber filter (Whatman, GF/C). A solid-phase extraction (SPE) was used for pre-concentration of the water sample. SPE was performed with combination of an InertSep PLS-3 cartridge (GL Science) and a Sep-Pak Plus AC2 cartridge (Waters) using a Chratec Sep-Pak Concentrator (SPC 10-C; Chratec, Kyoto, Japan). The PLS-3 cartridge was preconditioned with dichloromethane, methanol and MilliQ purified water; the AC2 cartridge was preconditioned with methanol and MilliQ purified water. The filtered sample was passed through the cartridges at a flow rate of 10 mL/min and then rinsed with 10 mL of MilliQ purified water. After that, the cartridges were dried by nitrogen to remove water and finally the analytes were eluted with 5 mL of methanol and dichloromethane. The sample extract was spiked with 40 µL of 5 µg/mL internal standard (mixture of methomyl-D3, pirimicarb-D6, imazalil-D5) and evaporated to a final volume of 50 µL. The concentrate was diluted with 1 mL of MilliQ purified water. Then, the solution was filtered through 0.2 µm syringe filters (Millex-LG) into an analysis vial and subsequently analyzed by LC/TOF/MS.

#### Liquid chromatography time of flight mass spectrometry

The analytes were separated using an HPLC system consisting of a vacuum degasser, an auto-sampler and a binary pump (Agilent Series 1200, Agilent Technologies) equipped with a reversed-phase ODS-4 HP column (2.1 mm x 150 mm and 3 µm particle size; GL Sciences). Column temperature was maintained at 40°C. The sample volume was 2 µL. Mobile phase A and B were water and methanol with 5 mmol ammonium acetate, respectively. The optimized chromatographic method held the initial mobile phase composition (95% A) constant for 5 min, followed by a linear gradient to 5% A up to 30 min, and kept for 20 min at 5% A. The flow rate used was 0.3 mL/min. A 15-min post-run time was used after each analysis. This HPLC system was connected to a time-of-flight mass spectrometer (Agilent 6220 MSD) equipped with a dual electrospray interface operating in positive ion mode, and the following operation parameters: capillary voltage, 3500V; nebulizer pressure, 50 psig; drying gas, 10 L/min; gas temperature, 325°C; fragmentor voltage, 100V; skimmer voltage, 65V. The mass spectra were recorded across the range 100 – 1000 m/z, and the internal reference masses were m/z 121.0509 and m/z 922.0098.

### Results and discussion

Thirty three compounds out of the total 270 target compounds were detected one or more times, including 20 PPCPs, 12 agricultural chemicals and 1 industrial chemical (Table 1); maximum total concentrations of PPCPs and pesticides were 15.54 µg/L and 0.64 µg/L, respectively (Fig. 2).

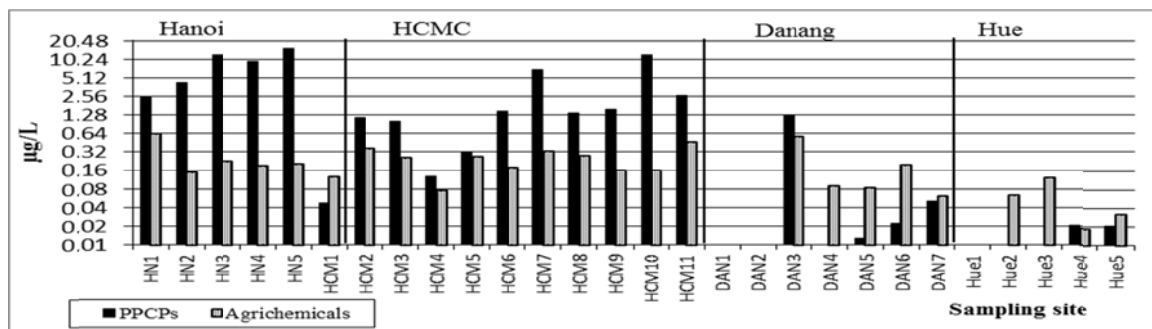
**Table 1** Distribution of types of detected compounds

Group	Detected compounds	Number of detected compounds			
		Hanoi	HCMC	Danang	Hue
PPCPs	20	15	16	6	1
Agricultural chemicals	12	7	7	5	4
Industrial chemicals	1	1	1	1	0
<b>Total</b>	<b>33</b>	<b>23</b>	<b>24</b>	<b>12</b>	<b>5</b>

#### Difference in concentrations among sampling sites

As shown in Table 1, the number of compounds detected in samples collected from Hanoi and HCMC was nearly the same (23 and 24 compounds), and was much larger than the number detected in Danang and Hue (12 and 5, respectively; Table 1). Of 20 detected PPCPs, 15 were detected in HCMC samples, 16 were detected in Hanoi, higher than the number of PPCPs detected in Danang and Hue (6 and 1 compound, respectively). In general, the total concentration of PPCPs in all the samples from Hanoi and HCMC was also higher than in samples from Danang and Hue. However, on the case of agricultural chemicals, there is less difference in the detected number (Table 1) and the concentrations between four cities (Fig. 2). Sampling sites in Hanoi urban area (HN3, HN4 and HN5) had high concentrations of PPCPs compared to those in suburban areas (HN1 and HN2). This pattern was also observed amongst samples collected in urban area and suburban area of HCMC, Danang and Hue. It is clear that differences in population density and economic activities are one of the main reasons leading to the disparity in the number and concentration of detected compounds. Sources of the detected PPCPs were not

only household but also hospitals and health care clinics without wastewater treatment system or ineffective treatment systems. Most of the samples collected of Danang and Hue had numbers of detected compounds each at low concentration, with some sites producing no detected compounds (DAN1, DAN2 and Hue1) (Fig.2). Nevertheless, like Hanoi and HCMC, environmental risks will remain high if wastewater treatment systems are not commonly applied.



**Fig.2** Total concentrations of PPCPs and agrichemicals of each sampling site

### Detected compounds

Since many sampling sites were in densely populated areas, PPCPs were detected in a large numbers and often at high concentrations. The most common type of PPCPs detected was antibiotic (11 compounds). Lincomycin and sulfamethoxazole were determined in high percentage of detection (54%). Maximum concentrations of lincomycin and sulfamethoxazole were found in urban river samples (2.7µg/L, 2.1 µg/L, respectively), their concentrations were higher than the concentrations in surface waters in Spain (0.047µg/L and 0.03 µg/L)<sup>1</sup>, and Germany (0.48µg/L of sulfamethoxazole)<sup>2</sup>. Five derivatives of 2-quinolinecarboxylic acid (2-QCA), cotinine, fenobucarb, tricyclazole and triphenylphosphate were detected in more than 60% of samples. Cotinine was the most frequently detected pharmaceutical analyzed in this study (75% of all samples) at an average concentration of 0.57 µg/L (maximum concentration of 2.9µg/L in Hanoi samples) and higher than the average concentration (0.04 µg/L) of cotinine detected in 22% of US surface waters<sup>3</sup>. One possible source of this cotinine is untreated wastewater from cigarette factories contained high level of cotinine, being discharged directly into rivers. Acetaminophen was detected in urban river water samples from Hanoi and HCMC at maximum concentrations of 5.6 µg/L and 5.2µg/L, respectively; these concentrations are higher than the maximum value (1.97µg/L) reported in surface water samples in Spain<sup>1</sup>. 2-QCA, pharmaceutical for animals, was detected in 64% of all samples (100% of Hanoi and HCMC samples), with maximum concentration of Hanoi and HCMC samples (0.61 and 0.57µg/L, respectively). The median value of urban and suburban samples collected in Hanoi and HCMC were 0.41 and 0.30 µg/L, respectively. 2-QCA is a metabolite of carbadox used as veterinary medicine for improving feed efficiency, promoting growth, increasing the rate of weight gain and therapeutic control for swine dysentery and bacterial enteritis in swine. However, use of carbadox in animal feeds has been warned or banned in many countries like the European Union (EU)<sup>4</sup> and Vietnam<sup>5</sup> because of its carcinogenicity. In Vietnam, registered pig farms are concentrated in the regions surrounding or near Hanoi and Ho Chi Minh City<sup>6</sup>. Therefore, it is hypothesized that the illegal use of carbadox in raising pigs leads to frequent detection and high concentration of 2-QCA in the river water of Hanoi and HCMC. It is apparent that the lack of widespread connection to effectively operating wastewater treatment systems in Vietnam has resulted in PPCP concentrations higher than river samples collected from developed countries. However, this may not be the only issue.

About detected agrichemicals, some compounds were detected frequently in samples of Hanoi and HCMC but infrequently in Danang and Hue samples. These included duron (herbicide), carbendazim (fungicide). Carbendazim was detected in all of Hanoi and HCMC samples with almost the same maximum and mean value of these two cities samples (0.20 µg/L and 0.14µg/L in Hanoi samples; 0.21µg/L and 0.10 µg/L in HCMC samples). Duron was detected in 100% of Hanoi samples with 0.092µg/L of maximum concentration, and was detected in more than 70% of HCMC samples with 0.14µg/L of maximum concentration. Some compounds were detected frequently in HCMC samples but infrequently in Hanoi samples such as fenobucarb (insecticide)

and tricyclazole (fungicide). Fenobucarb was detected in 90% of HCMC samples and 60% of Hue samples with maximum concentration of 0.043µg/L (HCMC) and 0.033µg/L (Hue). Tricyclazole was detected in 100% of HCMC samples with maximum concentration of 0.098 µg/L and median value of 0.056µg/L. However, it was detected in only one Hanoi sample with 0.009 µg/L of concentration. Tricyclazole was also detected in 57% of Danang samples with 0.036µg/L of maximum concentration and 0.026µg/L of median value. These compounds are used commonly in agricultural of Vietnam. But in each area, the amount of consumption of these compounds is different. Therefore, the kind of detected agricultural chemicals and detected concentration were different in river samples of the studied cities.

**Table 2.** Concentration of substances detected in river samples in the 4 cities (µg/L)

Compound	Type	LOQ	% Detection	Hanoi		Ho Chi Minh City		Danang		Hue	
				Max	Median	Max	Median	Max	Median	Max	Median
2-Quinoxalinecarboxylic acid	PPCP	0.008	64	0.61	0.41	0.57	0.30	0.23	n.d.	n.d.	n.d.
Acetaminophen	PPCP	0.02	25	5.6	0.71	5.2	n.d.	n.d.	n.d.	n.d.	n.d.
Ampicillin	PPCP	0.08	11	0.64	0.37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antipyrine	PPCP	0.008	7	n.d.	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	n.d.
Cimetidine	PPCP	0.008	7	0.14	n.d.	0.18	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	PPCP	0.008	32	0.17	0.06	0.07	n.d.	0.10	n.d.	n.d.	n.d.
Cotinine	PPCP	0.008	75	2.9	1.8	1.6	0.05	0.04	n.d.	0.02	n.d.
Erythromycin	PPCP	0.02	4	n.d.	n.d.	0.09	n.d.	n.d.	n.d.	n.d.	n.d.
Griseofulvin	PPCP	0.02	11	n.d.	n.d.	0.05	n.d.	n.d.	n.d.	n.d.	n.d.
Lincomycin	PPCP	0.04	54	1.4	1.2	2.7	0.47	0.75	n.d.	n.d.	n.d.
Losartan	PPCP	0.008	21	0.16	0.09	0.30	n.d.	n.d.	n.d.	n.d.	n.d.
Oleandomycin	PPCP	0.008	21	0.57	0.23	0.03	n.d.	n.d.	n.d.	n.d.	n.d.
Phenacetin	PPCP	0.008	4	0.071	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Roxithromycin	PPCP	0.008	14	0.05	0.02	0.02	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	PPCP	0.02	54	2.1	1.7	1.0	0.22	0.11	n.d.	n.d.	n.d.
Sulfanilamide	PPCP	0.04	18	0.30	n.d.	0.59	n.d.	0.09	n.d.	n.d.	n.d.
Sulfapyridine	PPCP	0.008	11	0.04	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Testosterone	PPCP	0.08	4	n.d.	n.d.	0.22	n.d.	n.d.	n.d.	n.d.	n.d.
Theophylline	PPCP	0.4	11	2.9	1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tilicosin	PPCP	0.08	4	n.d.	n.d.	0.50	n.d.	n.d.	n.d.	n.d.	n.d.
Acephate	Agri.	0.02	18	n.d.	n.d.	n.d.	n.d.	0.21	0.05	n.d.	n.d.
Acetamiprid	Agri.	0.008	14	0.040	n.d.	0.029	n.d.	0.035	n.d.	n.d.	n.d.
Alachlor	Agri.	0.04	4	0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Azoxystrobin	Agri.	0.008	4	n.d.	n.d.	0.20	n.d.	n.d.	n.d.	n.d.	n.d.
Bensulfuron-methyl	Agri.	0.08	4	0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Boscalid	Agri.	0.04	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	n.d.
Carbendazim	Agri.	0.008	57	0.20	0.14	0.21	0.10	n.d.	n.d.	n.d.	n.d.
Cyprodinil	Agri.	0.04	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	n.d.
Diuron	Agri.	0.008	50	0.092	0.055	0.14	0.031	0.12	n.d.	n.d.	n.d.
Ethoxyquin	Agri.	0.04	11	n.d.	n.d.	0.12	n.d.	n.d.	n.d.	n.d.	n.d.
Fenobucarb	Agri.	0.008	61	0.053	n.d.	0.043	0.033	0.22	n.d.	0.033	0.018
Tricyclazole	Agri.	0.008	61	0.009	n.d.	0.098	0.056	0.036	0.026	0.034	n.d.
Triphenylphosphate	IC	0.02	61	0.06	0.03	0.08	0.03	0.02	n.d.	n.d.	n.d.

LOQ: the lowest concentration accepted in range of calculation curve; "n.d.": not detected or lower than LOQ

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