STUDY ON ACCUMULATION OF PERFLUORINATED COMPOUNDS ON VEGETATION AND SEDIMENT IN THE AI RIVER BASIN, JAPAN AND IN THE CHAO PHRAYA RIVER BASIN, THAILAND

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Introduction

Over the past 60 years, perfluorinated compounds (PFCs) have been used in a broad range of applications such as surfactants, refrigerants and polymers, and also as components of pharmaceuticals, fire retardants, lubricants, adhesives, paints, cosmetics, food packaging and so on. Recently, PFCs have been detected globally in most of the water environment and biota[1,2,3]. PFCs have raised global attention since researches have reported on their bioaccumulative potential[4] and various adverse effects on human and wildlife such as hepatotoxicity, immunotoxicity and developmental toxicity[5]. It was reported that PFCs are not removed effectively by conventional treatment processes in wastewater treatment plants (WWTPs) and levels of PFCs in the effluents are often found higher than those in the influents[6]. Thus, bioaccumulation of PFCs on biota in the downstream of WWTPs is of great concern. Up to 2013, more than 100 literatures have shown PFCs concentrations in various types of biota including invertebrates, fishes, amphibians, reptiles, birds, mammals and humans and most of them have reported on organisms within aquatic food webs[7]. It was suggested that levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in water supplies were correlated to those levels in the blood of residents living in some of the most heavily contaminated areas[8]. Moreover, consumption of well water and home grown fruits and vegetables was found to be correlated to levels of PFOA in human blood in a contaminated area[9]. However, there are few studies on occurrences of PFCs in vegetation and little information is available on the bioaccumulation of PFCs on vegetation. Main objective of this study was to examine accumulation of PFCs from water to vegetation and sediment at effluent discharging points of WWTPs where high levels of PFCs were suspected.

Materials and methods

Sampling: Details of survey campaign are described in **Table 1**. In Japan, water, sediment and reed plant (*Phragmites australis*) samples were collected at an effluent discharging point of a WWTP in the Ai river basin on 7 July 2012 (W1). At this time, sediment samples were collected by acrylic core tubes (ø64 × 1,000 mm (H)) and approximately 30 cm sediment cores (n=2) were obtained. Similarly, in Thailand, water, sediment and water plantain plant (*Alisma* spp.) samples were collected in the Chao Phraya river basin on 24 September 2012 (W2), 28 September 2012 (W3), and 2 October 2012 (W4). Collected samples were carried to laboratory and stored at 4 °C in refrigerator. Water samples were filtered by GF/B glass fiber filter (Whatman™) to separate filtrates (dissolved-phase) and suspended solids (particulate-phase). Sediment in core sample was separated every 3 cm. Reed and water plantain plants were separated into leaves and stems. Sediment and plant samples were dried at 105 °C. Sediment samples were crushed into powder using mortar and pestle, and were sieved through 106 μm testing sieve (Iida manufacturing). Plant samples were crushed into powder by Wonder Crush/Mill (WDL-1, Osaka Chemical).

Table 1 Sampling campaign

Target River basin	Survey at WWTPs				
	Site ID	Survey Date	Plant	Water	Sediment
			n		
The Ai river basin	W1	27, Jul. 2012	2 (*Phragmites australis*)	2	2
The Chao Phraya river basin	W2	24, Sep. 2012	1 (*Alisma* spp.)	2	2
	W3	28, Sep. 2012	1 (*Alisma* spp.)	2	2
	W4	2, Oct. 2012	2 (*Alisma* spp.)	2	2

Sample Pre-treatment: PFCs in dissolved-phase of water sample (500 mL) were extracted by solid phase extraction (SPE) passing through a Presep®C-Agri cartridge (Wako) connected inline to an Oasis® HLB cartridge (Waters). After drying, PFCs were eluted with 2 mL methanol (LC/MS grade, Wako) followed by 2 mL acetonitrile (LC/MS grade, Wako). Samples

were evaporated with nitrogen gas and reconstituted into a final volume of 1 mL with 40% acetonitrile with 60% *Milli-Q* water (*Milli-Q* Advantage A10®, Millipore) (v:v). PFCs in particulate-phase of water (filtered volume: 500 mL), sediment (1.0 g-dry wt.) and plant (0.5 g-dry wt.) samples were extracted by shaking with methanol. Sample was put into 15 mL tube with 5 mL methanol, mixed by vortex and shaken for 30 min. Then, extracted sample was centrifuged at 3,000 rpm for 15 min. and supernatant was separated. Extraction procedure was repeated again. Supernatant was mixed and passed through 0.2 μm syringe filter (Whatman®) and ENVI™-carb filter (Supelco) to eliminate matrix substances followed by evaporation and reconstitution as explained above.

Table 2 Target chemicals and analytical parameters by HPLC-ESI-MS-MS

Compound	Abbreviation	Molecular structure	Precursor ion (*m/z*)	Daughter ion (*m/z*)	CE* (eV)	*IDL*** (ng/mL)	*IQL**** (ng/mL)
Perfluorohexanoic acid	PFHxA	$CF_3(CF_2)_4CO_2^-$	313	269	5	0.02	0.06
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6CO_2^-$	413	369	5	0.00	0.02
Perfluorooctane sulfonate	PFOS	$CF_3(CF_2)_7SO_3^-$	499	80	55	0.01	0.05
Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	$^{13}C_2$-PFHxA	$CF_3(CF_2)_3{}^{13}CF_2{}^{13}CO_2^-$	317	271	5	0.01	0.03
Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	$^{13}C_4$-PFOA	$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}CO$	417	373	5	0.02	0.08
Perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulfonate	$^{13}C_4$-PFOS	$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}SO_3$	503	80	5	0.02	0.06

*CE = Collision Energy, **IDL = Instrument Detection Limit, ***IQL = Instrument Quanitification Limit

Instrumental Analysis and Quantification: Analytical parameters of each PFC are shown in **Table 2**. PFCs were separated by using high-performance liquid chromatography (HPLC), (1200SL, Agilent). The HPLC was interfaced with a tandem mass spectrometer (6400 Triple Quadrupole, Agilent) operated with electrospray ionization negative mode. For quantification, calibration curves were obtained by six points covering 0.01 to 10 ng/mL and generally provided linearity with determination coefficients (*R*) more than 0.995 in every compound. Instrumental quantification limit (*IQL*) was used for quantifying analyte, which was defined by S/N 10:1 (Table 2). Recovery rates were calculated by spiking 10 ng of mass-labeled PFCs ($^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS, Wellington Laboratories) into each sample prior to pre-treatment. Recovery of $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were ranged between 41% to 115%, 39% to 111% and 109% to 180%, respectively.

Results and discussion

Concentrations of PFHxA, PFOA and PFOS measured in plant, sediment and water at effluent discharging points of WWTPs in two river basins are shown in **Fig 1**. Concentrations in water were expressed by total values of dissolved and particulate phase and concentrations of sediment were shown as average values of core samples. At W1 in the Ai river basin, PFHxA was predominant and detected at 21,400 ng/L in water, 13,600 ng/kg-dry in reed plant and 14,100 ng/kg-dry in sediment. PFOA was also detected at high levels at W1, 157

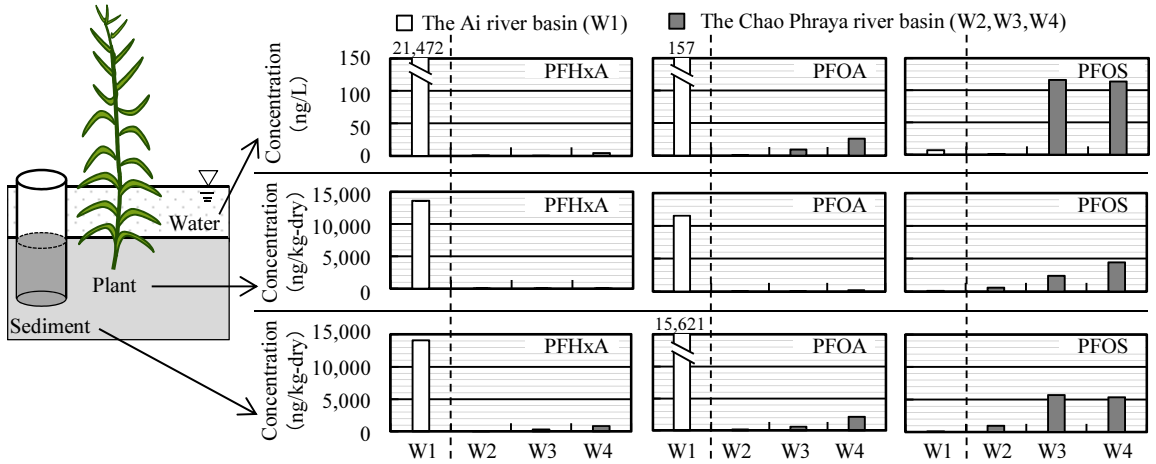

Figure 1 PFHxA, PFOA and PFOS concentration at effluent discharging points of WWTPs (W1,W2,W3,W4)

ng/L in water, 11,600 ng/kg-dry in reed plant and 15,600 ng/kg-dry in sediment. At W2, W3 and W4 in the Chao Phraya river basin, PFOS was predominant and detected at 1 – 116 ng/L in water, 600 – 4,400 ng/kg-dry in water plantain plant and 1,000 – 5,700 ng/kg-dry in sediment.

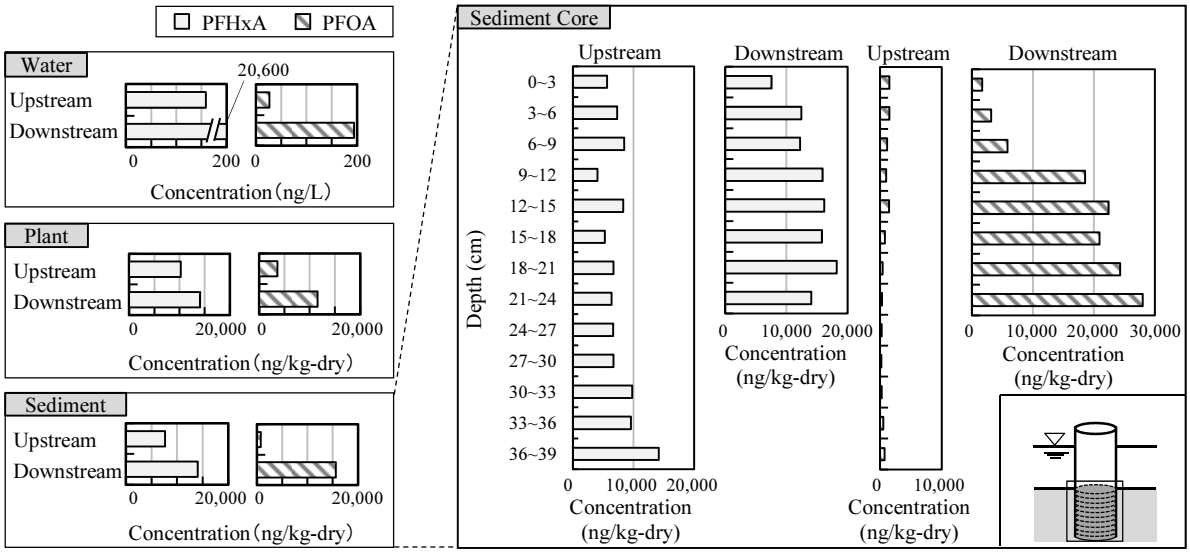

Figure 2 PFCs concentration at upstream and downstream of the effluent discharging point of WWTP (Water, plant sediment, sediment core at W1 in the Ai river basin, Japan)

Concentrations of PFHxA and PFOA at upstream and downstream of the effluent discharging point of the WWTP at W1 in the Ai river basin are shown in **Fig 2**. At the downstream, concentrations in water were 20,600 ng/L for PFHxA and 194 ng/L for PFOA which were higher than those at the upstream (PFHxA: 158 ng/L, PFOA: 27 ng/L). Similarly, PFHxA and PFOA concentration in reed plants were 14,000 and 11,600 ng/kg-dry which were higher than those at the upstream (PFHxA: 10,200 ng/kg-dry, PFOA: 3,600 ng/kg-dry). Average concentration of PFHxA and PFOA in sediment core at the downstream (PFHxA: 14,000 ng/kg-dry, PFOA: 15,600 ng/kg-dry) were also higher than those at the upstream (PFHxA: 7,600 ng/kg-dry, PFOA: 800 ng/kg-dry). This result indicated that PFHxA and PFOA concentrations in the effluent of WWTP affected to PFHxA and PFOA concentration in vegetation and sediment at the downstream. Concentration of PFHxA and PFOA for every 3 cm in sedement core at W1 is also shown in Fig 2. At the downstream, PFOA concentration was higher in the deeper core samples at 9~24 cm depth (18,600 – 28,000 ng/kg-dry) than those in the shallower core at 0~9 cm depth (1,700 – 5,900 ng/kg-dry) while there were no remarkable differences in PFHxA concentrations among different depths of the sediment core. Historical changes of PFHxA and PFOA concentrations in water at W4 are shown in Fig 3. Previous surveys conducted by our research group demonstrated that PFOA concentration in water had been decreased from 8,000 ng/L (2005) to 160 ng/L (2012)[10] while PFHxA concentration started to increase since 2009 (1,600 ng/L). Thus, historical changes of PFOA concentration in water corresponded to the concentration pattern in the sediment core. This explains that PFOA discharged in the past years was possibly remained in the deeper layers of the sediment and PFOA concentration in the vegetation might be influenced not only by water but also by carryover in the sediment as suggested by a literature [11].

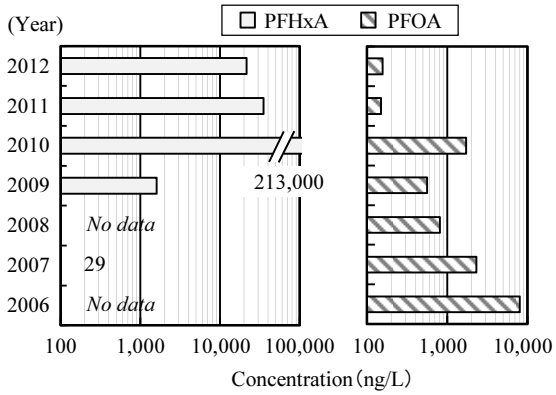

Figure 3 PFHxA and PFOA concentration in water at the effluent discharging point of WWTP (W1) in the Ai river basin, Japan (2006-2012)

Figure 4 shows relationship among PFCs concentration in plant, sediment and water at the same location of effluent discharging points. Overall, PFHxA, PFOA and PFOS concentrations in plants tended to be similar with those in sediment. In contrast, PFHxA, PFOA and PFOS in vegetation and sediment tended to be higher than those in water. Therefore, accumulation of PFHxA, PFOA and PFOS from water to vegetation and sediment was suspected. At downstream of W1 in the Ai river basin, PFHxA concentration in water was remarkably high (20,600 ng/L) so that PFHxA concentrations in plants (7,400－12,700 ng/kg-dry) and in sediment (141,00 ng/kg-dry) were probably saturated in the environment and the relationship was not in the similar way with other plots.

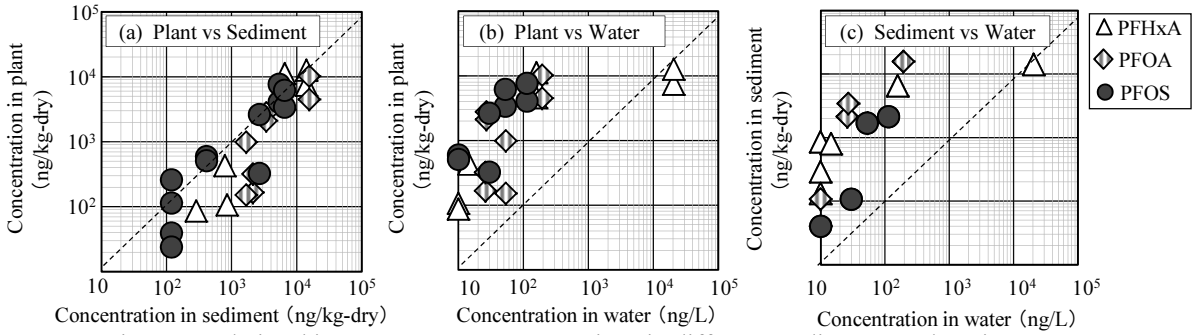

Figure 4 Relationship among PFCs concentrations in different media at same locations
(a) in plant and sediment (b) in plant and water (c) in sediment and water (W1, W2, W3, W4)

Bioaccumulation factor (*BAF*) of PFCs on vegetation was calculated for samples in W1, W2, W3 and W4 according to formula as follows:

$$BAF_{\text{water}\rightarrow\text{vegetation}}\,(\text{L/kg-wet}) = C_{\text{veg}}\,(\text{ng/kg-wet}) / C_{\text{water}}\,(\text{ng/L})$$

where C_{veg} is PFCs concentration in plant and C_{water} is PFCs concentration in water.

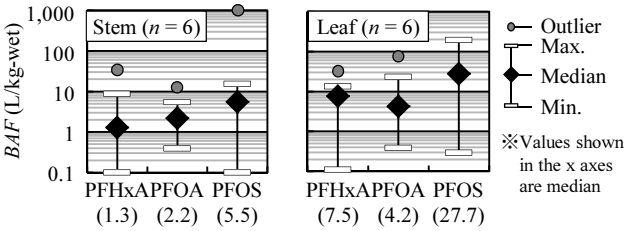

Figure 5 *BAF* values of PFHxA, PFOA and PFOS on vegetation at WW1, WW2, WW3 and WW4

Calculated *BAF* values on vegetation are shown in **Fig 5**. Median values of *BAF* were 1.3 (PFHxA), 2.2 (PFOA) and 5.5 (PFOS) in case of stems and 7.5 (PFHxA), 4.2 (PFOA) and 27.7 (PFOS) in case of leaves. *BAF* values tended to be higher in leaves than those in stems. *BAF* values of PFOS with sulfo group were higher than those of PFOA with carboxyl group (carbon number of PFOS and PFOA is 8). *BAF* values of PFHxA (carbon number is 6) were comparable to those of PFOA.

Acknowledgements
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References

1. Giesy JP, Kannan K. (2001); *Environ. Sci. Technol.*, **35**, 1339–1345.
2. Saito N, Harada K, Inoue K, Sasaki K, Yoshinaga T, Koizumi A. (2004); *J. Occup. Health*., **46**, 49–59.
3. Yamashita N, Kannan K, Taniyasu S, Horii Y, Petrick G, Gamo T. (2005); *Marine Poll. Bull*., **51**, 658-668.
4. Conder JM, Hoke RA, De Wolf W, Russell MH, Buck RC. (2008); *Environ. Sci. Technol.*, **42**, 995-1003.
5. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. (2007); *Toxicol. Sci*., **99**, 366–94.
6. Tanaka S, Fujii S, Lien NPH, Nozoe M, Kunacheva C, Kimura K, Shivakoti B. (2008); *J. JSWE.*, **31** (11), 665-670.
7. Houde M, De Silva A, Muir DCG, Letcher RJ. (2011); *Environ. Sci. Technol.*, **45**, 7962−7973.
8. Harada K, Saito N, Inoue K, Yoshinaga T, Watanabe T, Sasaki S, Kamiyama S, Koizumi A. (2004); *J. Occup. Health*, **46**, 141-147.
9. Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. (2006); *J. Occup. Environ. Med.*, **48**, 759-770.
10. Ishikawa K. (2012); *Undergraduate School of Global Engineering, Kyoto University* (Bachelor's thesis).
11. Stahl T, Heyn J, Thiele H, Hu¨ther J, Failing K, Georgii S, Brunn H. (2009); *Arch. Environ. Contam. Toxicol*., **57**, 289–298.