TRANSFER OF PERFLUORINATED COMPOUNDS AND THEIR FORMATION POTENTIALS FROM TREATED WASTEWATER AND SEWAGE SLUDGE TO CROP PLANTS

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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. PFCs have raised global attention since researches have reported on their bioaccumulative potential[1] and various adverse effects on human and wildlife such as hepatotoxicity, immunotoxicity and developmental toxicity[2]. Recently, PFCs have been detected globally in most of the water environment and biota[3,4,5]. One of the major exposure pathway was considered to be through oral route, mainly diet[6]. Klenow *et al*. conducted diet exposure survey and reported that vegetables were identified to be the most important food category for exposure to PFHxA and PFOA, with up to 69% of the total exposure[7]. Moreover, Suzuki *et al.* reported that PFCs contents in vegetation might be influenced by water as well as by carryover in the sediment where contamination in water continued for several years[8]. Plant growth experiment also indicated transfer of PFHxA, PFOA and PFOS to edible parts of crop plants from soil through water, in which mass balance between input and output was assessed[9].

Recent research has demonstrated that biosolids from wastewater treatment plant can contain PFCs at high concentraiton[10]. Furthermore, pathways and sources of PFCs in the environment are reported not only to be direct discharge from industries but also the release and subsequent degradation of their precursors[11,12]. However, the occurrences of PFCs precursor in treated wastewater and sewage sludge are not well known and their transfer to soil and vegetation are little studied due to the difficulty of analysis. According to Houtz and Sedlak[13], our research group have developed a method to evaluate comprehensive formation potentials of PFCs from their precursors in wastewater samples. Main objective of this study was to examine transfer of PFCs and their formation potentials from treated wastewater and sewage sludge to crop plants under plant growth experiment.

Materials and methods

Experiments: Plant growth experiments were designed as shown in **Fig 1**. Two different agricultural crop plants, komatsuna (*Brassica rapa var. perviridis*) and radish (*Raphanus sativus*) were selected. A wastewater treatment plant located in Kinki-region in Japan was surveyed on Oct. 2014 and treated wastewater, raw sludge and concentrated activated sludge were collected. Samples were immidiately stored at 4□ after carried to the laboratory. Sludges were centrifuged at 3,000 rpm and the precipitates were mixed together with 1:1 ratio (v:v) and freeze dried by FDU-2200 (EYELA) for creating bioslid as fertilizer from sewage sludge. An experiment (System 1) was conducted using treated wastewater for irrigaqtion and potting soil from market (PFCs-free) and another experiment (System 2) was done using tap water (PFCs-removed) for irrigation and biosolid from sewage sludge with potting soil from market. Each experiment system contained eight replicated growing pots and 70 g (wet wt.) of soil was put in each. In case of system 2, 0.7 g-dry wt. of sewage sludge (biosolid) were

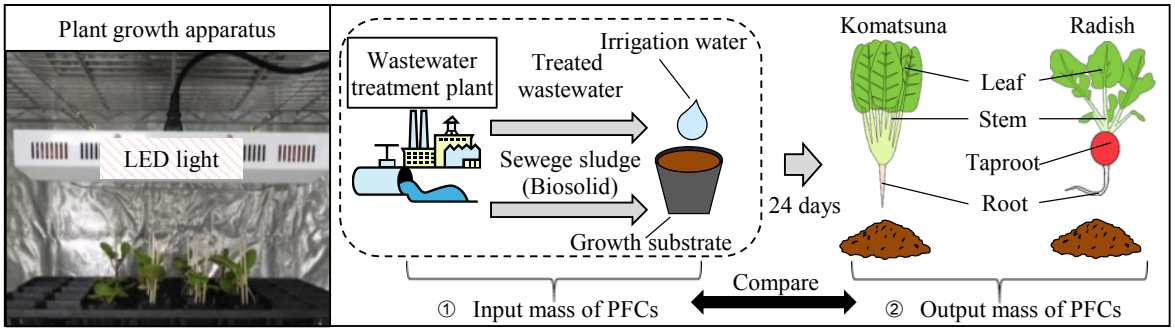

Figure 1. Ovewview of this study (plant growth experiments)

added to soil. Plants were pre-grown in the pots until the seed leaves were developed. Plants were then grown for 24 days under irradiation of LED lights for 18h/day. For irrigation, 10 mL of water was applied to each pot per day. The characteristics of irrigation water and growth substrate are shown in **Table 1**. Treated wastewater and sewege sludge were considered suitable for experiments because of its neutral pH and medium nutrient content. Temperature and humidity were controlled at 20±5°C and 45±10% respectively throughout the experiments. After 24 days past, Harvested komatsuna was separated into leaves, stems, roots and soil, and radish was separated into leaves, stems, taproots, roots and soil. Each part of grown crops and soil were freeze dried and crushed into powder by ULTRA-TURRAX (IKA) or by Wonder Crush/Mill (WDL-1, Osaka Chemical).

Table 1. General characteristcs of irrigation water and growth substrate

	Irrigation water		Growth substrate	
	Treated wastewater	Tap water	Sewege sludge	Potting soil from market
pH	7.1	6.9	7.7	6.7
EC (μS/cm)	460	130	1,420	350
OC (%)	-	-	39.8	3.6
Nutrients	(mg/L)		(mg/g-dry)	
N	2.9	1.1	56.3	10.5
P	0.1	n.a.	141.5	21.4
K	3.2	1.2	4.1	2.3
Ca	4.2	10.1	6.3	1.7
Mg	0.8	3.7	1.2	1.6
Fe	0.1	n.a.	2.4	4.1

Sample Pre-treatment: PFCs in treated wastewater (250 mL) were extracted by solid phase extraction (SPE) passing through an Oasis® WAX cartridge (Waters). After drying the cartridges via centrifugation at 3,000 rpm for 4 min., PFCs were eluted with 1 mL methanol (LC/MS grade, Wako) followed by 1 mL methanol with 0.1% ammonium. Samples were reconstituted into a final volume of 2 mL. PFCs in dried sewage sludge (0.5 g-dry wt.), plant (0.5 g-dry wt.) and soil (1.0 g-dry wt.) were extracted by ion-pair extraction method. Sample was mixed with 1 mL of tetrabutyl ammonium hydrogen sulfate and 2 mL of 0.25 M sodium carbonate. 5 mL of tert-butyl methyl ether was then added to the solution and the mixture was vigorously shaken for 5 min. After centrifugation at 3,000 rpm for 15min., organic layer of extract was taken from the solution. This extraction procedure was repeated again. Extracts were mixed and passed through 0.2 μm syringe filter (Whatman®) and ENVI™-carb cartridge (Supelco) to eliminate matrix substances. Samples were then concentrated with nitrogen gas and reconstituted into a final volume of 2 mL with 100% methanol. For the analysis of formation potentials, extracts from solid type samples were replicated and diluted with 250 mL of *Milli-Q* water (PFCs-free). All aqueous samples were then treated with $K_2S_2O_8$ (60 mM) and NaOH (150 mM) and heated for 24 hours under 95 □. After this, samples were pretreated in the same way of PFCs analysis explained above.

Instrumental Analysis and Quantification: Analytical parameters of each target PFC are shown in **Table 2**. HPLC-ESI-MS-MS was used for analysis of PFCs. Details of separation and quantification are shown in a previous literature[8]. 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. Recoveries of $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were ranged between 46% to 75%, 61% to 92% and 66% to 112% respectively and relative standard deviation of recovery values for vegetable samples were less than 20%.

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	2	0.02	0.06
Perfluoroheptanoic Acid	PFHpA	$CF_3(CF_2)_5CO_2^-$	363	319	2	0.02	0.06
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6CO_2^-$	413	419	5	0.00	0.02
Perfluorononanoic Acid	PFNA	$CF_3(CF_2)_7CO_2^-$	463	469	5	0.01	0.03
Perfluorobutane sulfonate	PFBuS	$CF_3(CF_2)_3SO_3^-$	299	80	55	0.01	0.05
Perfluorohexane sulfonate	PFHxS	$CF_3(CF_2)_5SO_3^-$	399	80	55	0.01	0.05
Perfluorooctane sulfonate	PFOS	$CF_3(CF_2)_7SO_3^-$	499	80	55	0.01	0.05
Perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulfonate	$^{13}C_2$-PFHxA	$CF_3(CF_2)_3{}^{13}CF_2{}^{13}CO_2^-$	315	271	5	0.01	0.04
Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	$^{13}C_4$-PFOA	$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}CO_2^-$	417	373	5	0.01	0.05
Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	$^{13}C_4$-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

Results and discussion

Input mass of PFCs and their formation potentials to plant growth experiment systems are shown in **Fig 2**. In system 1, the highest input among seven types of PFCs were PFHxA (26 ng) followed by PFNA (25 ng) and PFOA (23 ng). Input masses of PFCs formation potential were 52 ng for PFHxA, 36 ng for PFHpA, 17 ng for PFBuS and 14 ng for PFHxS and these were higher than PFCs themselves. In system 2, in contrast, highest input among seven types of PFCs were PFOS (12 ng) followed by PFBuS (8 ng) and PFNA (8 ng). Input masses of PFCs formation potential were higher than PFCs themselves in all target PFCs in this study. These results indicated that treated wastewater and in particular, sewage sludge can contain significant amount of PFCs formation potential and they are emitted to the surrounding environment. Hereafter, PFHxA, PFOA and PFOS are mainly discussed as their inputs are relatively high among targeted PFCs and PFOA and PFOS are the concerned representative PFCs.

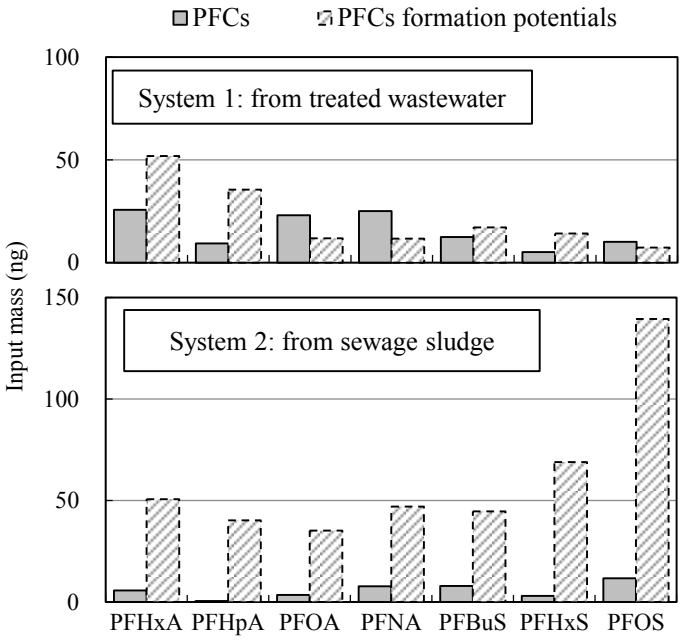

Figure 2. Input mass of PFCs and their formation potentials

Mass distribution of PFHxA, PFOA and PFOS in each part of harvested crop plant and soil at the end of the experiment with system 2 (PFCs-removed tap water as Irrigation water and sewage sludge as growth substrate) are shown in **Fig 3**. PFCs in planting pots were less than 1% of total mass, so it was negligible in these experiments. High proportion of PFCs mass were remained in soil, 82% for PFHxA, 85% for PFOA and 92% for PFOS (Komatsuna experiment) and 80% for PFHxA, 83% for PFOA and 92% for PFOS (Radish experiment). Among each part of harvested komatsuna, proportion of PFCs (of total mass in whole plant and soil) in leaf was the highest for PFHxA (13%) while that in root was the highest for PFOA (9%) and PFOS (6%). Similarly, among each part of harvested radish, proportion of PFCs (of total mass in whole plant and soil) in leaf was the highest for PFHxA (15%) while that in root was the highest for PFOA (8%) and PFOS (6%). In addition, taproot of radish contained 1% of PFOS, 2% of PFOA. These results indicated that more hydrophobic compounds preferably remained in soil, root and taproot and less hydrophobic compounds tended to be translocated from root to leaf . (Hydrophobicity: PFOS >PFOA>PFHxA). These trends in this study were in accordance with the behavior observed by a previous study [9] which were treated with standard PFCs soultion. Thus, the transfer of PFHxA to edible parts of crop plants, especially leafy vegetables, can be great concern as human exposure pathway. Continuous PFCs exposure to the experiment systems are required to be further studied for simulating actual environment. Mass distribution of PFHxA, PFOS and PFOA before and after

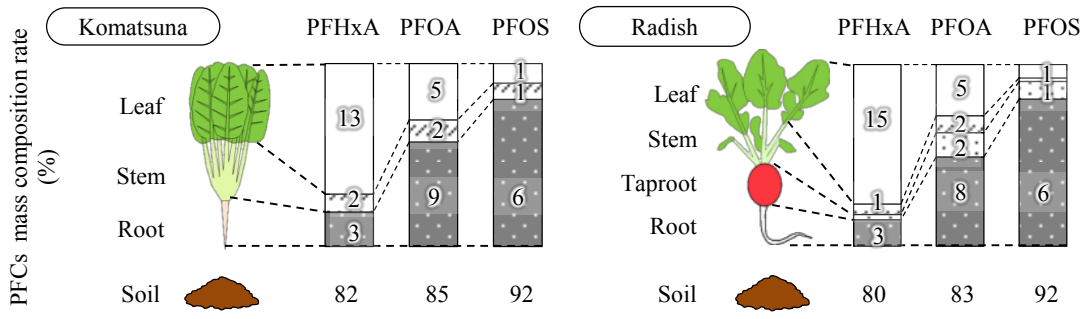

Figure 3. Mass distribution of PFHxA, PFOS and PFOA in each part of plants and soil after experiments (System 2: PFCs-removed tap water as irrigation water, sewage sludge as growth substrate)

experiments (system 2) are shown in **Fig 4**. Sum of each PFC input mass and that of formation potential was set to be 100% as the standard for calculating mass distribution. In Komatsuna and its growth substrate after experiment, as a whole, masses of PFHxA, PFOA and PFOS were 10.2, 25.6 and 14.0 times higher than input masses of those PFCs themselves. However, those masses were relatively balanced if input masses of their formation potentials were included. Masses of PFHxA, PFOA and PFOS after experiment were only 1.04, 2.34 and 1.08 times higher than the input masses including each formation potential. Therfore, it was indicated that PFHxA, PFOA and PFOS were formed from their precursors (formation potentials) during 24 days although mass of PFOA was not as well balanced as PFHxA and PFOS. These behaviors were similar in radish experiment. Thus, it was suggested that there is a high possibility of exposure to PFCs which were formed from precursors in sewage sludge. Occurence data of PFCs formation potentials is considered to play a important role for assessing risks for PFCs contamination where biosolids made from sewage sludge are applied to crop fields.

Acknowledgements: This study was supported by JSPS KAKENHI Grant Number 25289169 and Grant-in-Aid for JSPS Fellows 24-7853.

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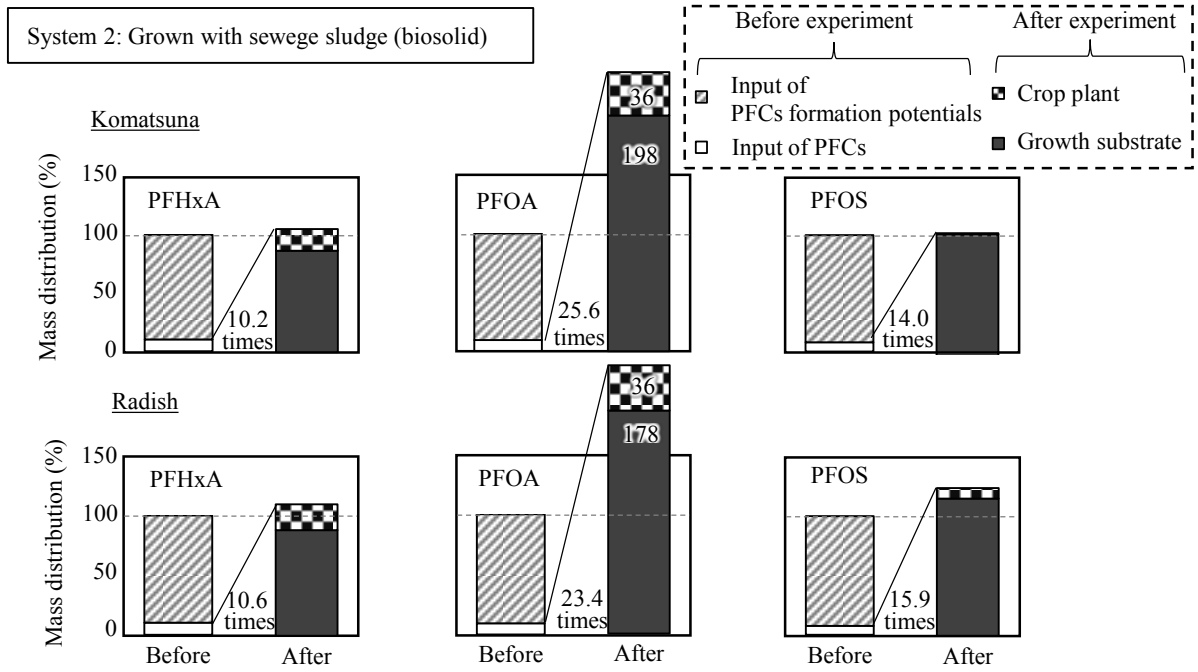

Figure 4. Mass distribution of PFHxA, PFOS and PFOA before and after experiments (System 2: PFCs-removed tap water as irrigation water, sewage sludge as growth substrate)