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Plant Uptake of Pharmaceutical Chemicals Detected in Recycled Organic Manure and Reclaimed Wastewater

Rumi Tanoue,[†,‡] Yuri Sato,[‡] Miki Motoyama,[‡] Shuhei Nakagawa,[§] Ryota Shinohara,[‡] and Kei Nomiyama*[,†]

[†]Center for Marine Environmental Studies (CMES), Ehime University, 2-5, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan

[‡]Graduate School of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100, Tsukide, Kumamoto 862-8502, Japan

[§]Nodaichidenshi Corporation Ltd., 335, Yoyasu, Kumamoto-city 860-0823, Japan

Supporting Information

ABSTRACT: Land application of recycled manure produced from biosolids and reclaimed wastewater can transfer pharmaceutical chemicals to terrestrial environments, giving rise to potential accumulation of these residues in edible plants. In this study, the potential for plant uptake of 13 pharmaceutical chemicals, and the relation between the accumulation features within the plant and the physicochemical properties were examined by exposing pea and cucumber to an aqueous solution containing pharmaceutical chemicals. Ten of 13 compounds tested were detected in plant leaves and stems. Comparison of the plant uptake characteristics and the octanol−water partition coefficient of pharmaceutical chemicals showed that compounds with an intermediate polarity such as carbamazepine and crotamiton could be easily transported to plant shoots. Moreover, these results suggest the possibility of highly hydrophilic pharmaceutical chemicals such as trimethoprim and sulfonamides to be accumulated in plant roots owing to their low permeability in root cell membranes.

KEYWORDS: *pharmaceutical chemicals, plant uptake, pea (Pisum sativum), cucumber (Cucumis sativus)*

INTRODUCTION

Over the past decade, pharmaceutical chemicals have been recognized as emerging contaminants owing to their physiological activities, wide usage, and constant discharge to the environment. Pharmaceutical chemicals excreted by human activities are introduced into the environment through various routes.[1,2] Many studies have reported the detection of pharmaceutical chemicals at concentrations ranging from parts-per-trillion to parts-per-billion levels in aquatic environments such as surface water, groundwater, and even drinking water.[3−5]

Municipal wastewater treatment plants (WWTPs) are identified as one of the main pathways that transfer pharmaceutical chemicals found in effluents into aquatic environments.[5,6] Several studies have focused on the behavior of pharmaceutical chemicals in WWTPs, revealing that they are not completely eliminated in conventional wastewater treatment processes such as activated sludge treatment.[7,8] During wastewater treatment, many pharmaceutical chemicals have limited adsorbability and biodegradability caused by activated sludge, resulting in a considerable amount of pharmaceutical chemicals in both WWTP effluents and sewage sludge.[8−11]

Reclaimed wastewater and biosolids are commonly reused or recycled for agricultural purposes in many countries. In Japan, approximately 1.4% of effluents from WWTPs is reused as reclaimed wastewater and approximately 5.9% of reclaimed wastewater is used for irrigating agricultural lands.[12] In other countries that have semiarid or arid areas, such as the United States, China, Israel, and Spain, reclaimed wastewater is recognized as an important source of agricultural water; thus, the usage rates of reclaimed wastewater for agricultural land in these areas are much higher than those in Japan.[13−18] In addition, recycled municipal biosolids have been promoted all over the world since ocean dumping has been banned by international treaty. In Japan, approximately 2.2 million tons of municipal biosolids (dry weight) are generated annually, of which 14% is applied to agricultural lands as a fertilizer in order to improve soil quality and stimulate plant growth.[12] In the United Kingdom and the United States, several million tons (dry) of biosolids are generated every year, and about 50% is applied to agricultural lands.[10,19] In addition to biosolids, livestock wastes such as animal feces containing veterinary pharmaceutical chemicals are also recycled and applied to agricultural lands.[1] Following land application of reclaimed wastewater or recycled manure produced from biosolids or livestock waste, pharmaceutical chemicals are released into agricultural fields. Once the pharmaceutical chemicals reach the upper soil layer, they may either accumulate or be readily available for transport to surface and groundwater through leaching and surface runoff.[20−22]

There is a growing concern that residual pharmaceutical chemicals have the potential to be taken up by edible plants and then enter the food supply. Many studies have focused on evaluating phytotoxicity or plant uptake of veterinary pharmaceutical chemicals, which are associated with recycled

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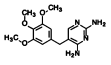
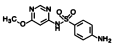
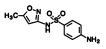
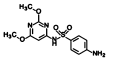
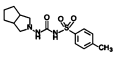
Table 1. Physicochemical Properties of Selected Pharmaceutical Chemicals

Compounds	CAS No.	Structure	Mass	Therapeutic class	Log K_{ow}[a]	pK_a[b]
Trimethoprim (TMP)	738-70-5		290	Antibacterial agent	0.73	3.23, 6.76[d]
Sulfamonomethoxine (SMMX)	1220-83-3		280	Antibacterial agent	0.20	2.0, 6.0[e]
Sulfamethoxazole (SMXZ)	723-46-6		253	Antibacterial agent	0.48	1.83, 5.60[d]
Sulfadimethoxine (SDMX)	122-11-2		310	Antibacterial agent	1.17	2.13, 6.08[d]
Crotamiton (CTM)	483-63-6		203	Anti-itch agent	2.73	0.62[f]
Gliclazide (GLZ)	21187-98-4		323	Antidiabetes agent	2.12	0.89, 5.32[f]
Carbamazepine (CBZ)	298-46-4		236	Antiepileptic agent	2.25	13.9[c]
Losartan (LS)	114798-26-4		422	Hypertensive agent	4.01	5.50
Cyclophosphamide (CPA)	50-18-0		260	Antineoplastic agent	0.97	0.50[f]
Acetaminophen (AAP)	103-90-2		151	Antipyretic agent	0.27	9.38
Ketoprofen (KP)	22071-15-4		254	NSAIDs[g]	3.00	4.45
Diclofenac (DF)	15307-86-5		295	NSAIDs	4.02	4.15
Indomethacin (IND)	53-86-1		357	NSAIDs	4.23	4.50

[a]Estimated values, from database of chemspider. Royal Society of Chemistry: http://www.chemspider.com. [b]Experimental values, from database of physicochemical properties. Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm. [c]Kasprzyk-Hordern et al.[57] [d]McClure and Wong.[58] [e]Yang et al.[59] [f]Estimated values, from SPARC on line calculator: http://archemcalc.com/sparc/test/login.cfm?CFID=555817&CFTOKEN=91846832. [g]NSAIDs, nonsteroidal anti-inflammatory drugs.

manure produced from animal wastes.[23−28] Only recently, some studies have demonstrated that plant uptake from water or soil spiked with pharmaceutical chemicals is associated with recycling of the waste products from WWTPs.[13,15,17,29−34] Recent studies have shown that several pharmaceutical chemicals such as carbamazepine (CBZ) have specific uptake ability of plants.[15,17,30] However, information on the uptake of pharmaceutical chemicals by edible plants is very limited and factors which contribute to uptake ability of plants are not clear. Demonstration of the relation between uptake ability of plants and physicochemical properties of pharmaceutical chemicals is therefore necessary to establish models which make a significant contribution to risk assessment studies. In most plant uptake models established for nonionic compounds, the octanol−water partition coefficient (log K_{ow}) is recognized as one of the main parameters.[35−38] However, many pharmaceutical chemicals are ionic compounds, and very few plant-uptake study has been performed on such chemicals. Furthermore, the previous reports were based on experiments investigating the uptake of a limited number of pharmaceutical chemicals into plants.[26,33]

In our previous study, it was clarified that various pharmaceutical chemicals remain in the many recycled manure produced from biosolids and livestock wastes in the marketplace, and their residue levels can be reduced by fermentation of the recycled manure.[39] However, low decomposition of CBZ in fermentation process was observed, suggesting the high persistency of that in agricultural land soils. In the current study, the ability of plants to uptake 13 pharmaceutical chemicals including CBZ, and the relation between the distribution features within the plant and the physicochemical properties of pharmaceutical chemicals were examined by exposing pea (*Pisum sativum*) and cucumber (*Cucumis sativus*) to an aqueous solution containing pharmaceutical chemicals. The purpose of this study is to provide experimental data for verification and improvement of plant uptake models on pharmaceutical chemicals.

MATERIALS AND METHODS

Selected Target Pharmaceutical Chemicals. Thirteen pharmaceutical chemicals (Table 1) were selected in this study because of their high usage and frequent detection in the recycled manure or reclaimed wastewater. This work focused on representative pharmaceutical chemicals intended for human use, with a wide range of physicochemical properties for examining different factors that affect plant uptake. Analytical standards of CBZ, ketoprofen (KP), diclofenac sodium (DF), and indomethacin (IND) were purchased from Sigma-Aldrich (St. Louis, MO). Trimethoprim (TMP), sulfamonomethoxine (SMMX), sulfamethoxazole (SMXZ), sulfadimethoxine (SDMX), crotamiton (CTM), gliclazide (GLZ), losartan potassium (LS), cyclophosphamide monohydrate (CPA), and *p*-acetamidophenol (AAP) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Deuterium-labeled CBZ-d_{10} was purchased from Cambridge Isotope Laboratories (Andover, MA) for use as an internal standard. Stock standard solutions were prepared by dissolving a

certain amount of a standard in methanol. Working standard solutions were prepared and diluted with methanol from the stock standard solution. Pea and cucumber seeds used for studying plant uptake were obtained from Nakahara Seed Co. Ltd. (Fukuoka, Japan).

Study of Plant Uptake Using Pea (*P. sativum*). Pea was used for investigating the distribution of pharmaceutical chemicals taken up into the plant organs (shoots, leaves and stems; underground parts, roots and cotyledons), because legumes are commonly consumed all over the world, grow at a fast rate, and have sturdy roots. A plant was precultivated in nutrient solution before it was exposed to test compounds. Information about precultivation conditions is described in the Supporting Information. A plant (8 d after seeding) with its third real leaf fully expanded was transferred to a glass container consisting 15 mL of distilled water and a single test compound or mixture of test compounds (methanol content not exceeding 0.3%). The glass container was covered with aluminum foil to prevent exposure to light. The uptake study was performed with two different treatments: (A) Pea was exposed to a mixed pharmaceuticals solution, the initial concentration of each compound was 0.25 mg L^{-1} for 24 h. (B) Pea was exposed to an individual pharmaceuticals solution, the initial concentration of each compound was 1 mg L^{-1} for 72 h. Although the concentrations of test compounds were higher than those observed in reclaimed wastewater and recycled manure,[13,14,16,18,39] they were chosen in order to examine the plant uptake capacity of these chemicals for a short period (so as to avoid biodegradation or metabolism within plants). In addition, a short period (72 h) was chosen because phytotoxicity, such as wilting or inhibition of growth, did not appear on the plants during the exposure period. In treatment A, the exposure solution was not changed during the exposure period. In treatment B, distilled water was added to the solution every 24 h to replace evaporative loss. In both the treatments, at the end of the exposure period, the plant was decapitated 3−5 cm above the base of cotyledon. The above shoot was separated into leaves and stems, and finely cut into less than 1 mm pieces. After weighting, 0.20 g (64−82% of total amount) of each leaf and stem sample was stored at −28 °C until analyses. Stems 3−5 cm above the base of cotyledons were not analyzed. Instead, the amounts of pharmaceutical chemicals in the stems were calculated by using the analyzed concentrations. During collection, plant roots and cotyledons were rinsed with distilled water and then with methanol. The distilled water used for rinsing was added to the exposure solution. The glass container used for the experiment was rinsed in the same way as the roots and cotyledons. Concentrations of residual pharmaceutical chemicals in the methanol solution used for rinsing roots and glass container were analyzed for calculation of mass attached to roots and glass container, respectively. Rinsed roots and cotyledons were wiped with Kimwipes, and homogenized by fine cutting. After weighing, 0.20 g (67−91% of total amount) of each root and cotyledon sample was stored at −28 °C until analyses. The concentrations of residual pharmaceutical chemicals in the test solutions were analyzed after the experiment. Three replicates were used in this experiment. Two types of control tests were performed: cultivating plants without exposure compounds and exposure solutions without plants.

Study of Plant Uptake Using Cucumber (*C. sativus*). A cucurbitaceous plant with a high uptake of water was selected to directly collect xylem saps. In this study, concentration ratios of the cucumber xylem saps and the test solutions (mean concentrations at the beginning and end of the test) were calculated, in order to evaluate the ability of plant shoots to uptake chemicals from roots. The plant was precultivated in nutrient solution before it was exposed to test compounds. Information about precultivation conditions is described in the Supporting Information. A plant (21 d after seeding) with its first real leaf fully expanded was transferred to a glass container consisting 20 mL of distilled water and mixture of test compounds (methanol content not exceeding 0.3%). The glass container was covered with aluminum foil to prevent exposure to light. Initial concentrations of individual pharmaceutical chemicals in the exposure solution were adjusted to the theoretical value of 0.25 mg L^{-1} and the plant was grown for 24 h. At the end of the exposure period, the plant was decapitated 5 mm above the stem base, and the xylem sap, which continued to seep out from the stem stump, was collected for up to 60 min with a Teflon pipet.[17]

The transpiration water was collected and analyzed for CTM, CBZ, and CPA, in which relatively high concentration ratios of the cucumber xylem saps and the test solutions were observed for cucumber plants. A cucumber (21 d later) was transferred to a container containing distilled water (20 mL) and a mixture of CTM, CBZ, and CPA. Initial concentrations of individual pharmaceutical chemicals in the exposure solution were adjusted to the theoretical value of 1 mg L^{-1}. After 6 h, a leaf was wrapped with a plastic sheet. Transpiration water from the leaf was collected in the plastic sheet for 24 h and analyzed. Five replicates were used in cucumber experiments, and plants were grown in distilled water without test compounds (a control test).

Analytical Procedure for Pharmaceutical Chemicals. Analytical procedures were described briefly as follows, a plant sample was extracted with acetonitrile or 0.5% (v/v) formic acid in methanol and evaporated to less than 0.2 mL, and the residue was diluted with Milli-Q water (10 mL). The solution was extracted by solid phase extraction with an Oasis HLB cartridge and eluated with methanol. CBZ-d_{10} (5 ng) was added to the eluate as an internal standard. The eluate was finally diluted with acetonitrile/Milli-Q water (1:1; v/v) up to 1 mL for liquid chromatography−tandem mass spectrometry (LC−MS/MS) analysis. Detailed information about the preparation for analysis is included in the Supporting Information. The concentrations of the target compounds in plant samples were determined by comparing the ratios of target peak areas relative to the peak area of the internal standard and were corrected by recovery ratios, which were calculated by standard spiked tests from blank samples.

Analytical Conditions of LC−MS/MS. Chromatographic analysis was performed with an Agilent 1200 Rapid Resolution LC system (Agilent Technologies). Prepared samples were separated with a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, particle size 1.8 μm, Agilent Technologies). Acetonitrile (mobile phase A) and 0.05% (v/v) formic acid in Milli-Q water (mobile phase B) were selected, and the total flow rate was 0.2 mL min^{-1} and an injection volume was 5 μL. The gradient started with 5% A, gently up to 40% A in 10 min, up to 60% in 5 min, ramped up to 80% in 2 min, up to 95% A in 1 min and kept for 3 min, returned to 5% A in 1 min, and held 8 min for re-equilibrium. The LC was coupled with an Agilent 6410 Triple Quadrupole Mass Spectrometer (MS/MS) equipped with an electrospray ion source. The mass spectrometer was operated in a positive ionization mode, and ion source parameters were as follows: capillary voltage, 4000 V; drying gas, N_2 at 10 L min^{-1}; temperature, 300 °C. The LC−MS system was controlled and data analyzed by MassHunter software (Agilent Technologies). Quantitative analysis of target pharmaceutical chemicals was performed in multiple reaction monitoring mode (MRM). Information about ion source parameters and monitor ions is presented in the Supporting Information (Table S1).

Quality Assurance and Control. Recovery ratios were calculated by the standard addition method from plant samples. Method detection limits (MDLs) and method quantification limits (MQLs) were calculated using standard deviation (SD) derived from five replicating spike tests. They were calculated using the following equation: MDL = $\{t\ (n - 1, 0.05) \times 2 \times \mathrm{SD}\}$/recovery rate and MQL = $\{10 \times \mathrm{SD}\}$/recovery rate, where $t\ (n - 1, 0.05)$ is the t value appropriate for the 95% confidence level with $n - 1$ degrees of freedom and the number of measurements. These recovery ratios, MDLs, and MQLs are presented in the Supporting Information (Table S2).

Statistical Analysis. The Student's t test at the 95% confidence interval was used to compare the concentration factors of pharmaceutical chemicals for the parts of the pea plant (leaves, stems, roots, and cotyledons) between the two treatments. All statistical data analyses were carried out using Microsoft Excel (Redmond, WA). Measured initial and final concentrations of pharmaceutical chemicals in exposure solutions were used for calculation of concentration factors (pea) and ratios of concentrations in xylem sap to the test solution (cucumber).

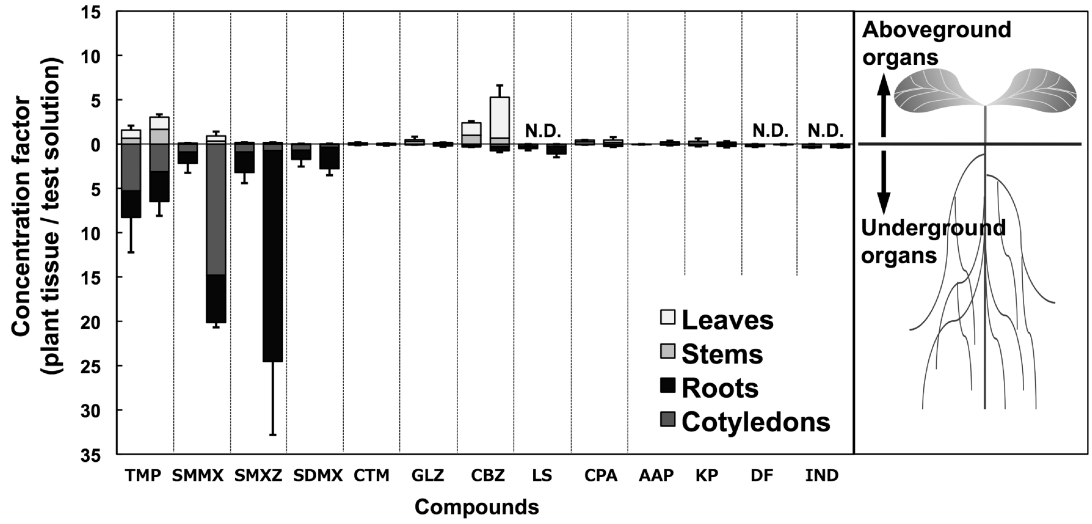


Figure 1. Concentration factors (ratios of concentrations in plant tissues to test solutions) calculated for pea leaves, stems, roots, and cotyledons (n = 3). Y-axis, ratios of the concentrations in plant tissues to test solutions; x-axis, abbreviations of test compounds. Left bars show values of treatment A, and right bars show values of treatment B. Error bars represent the standard deviations.

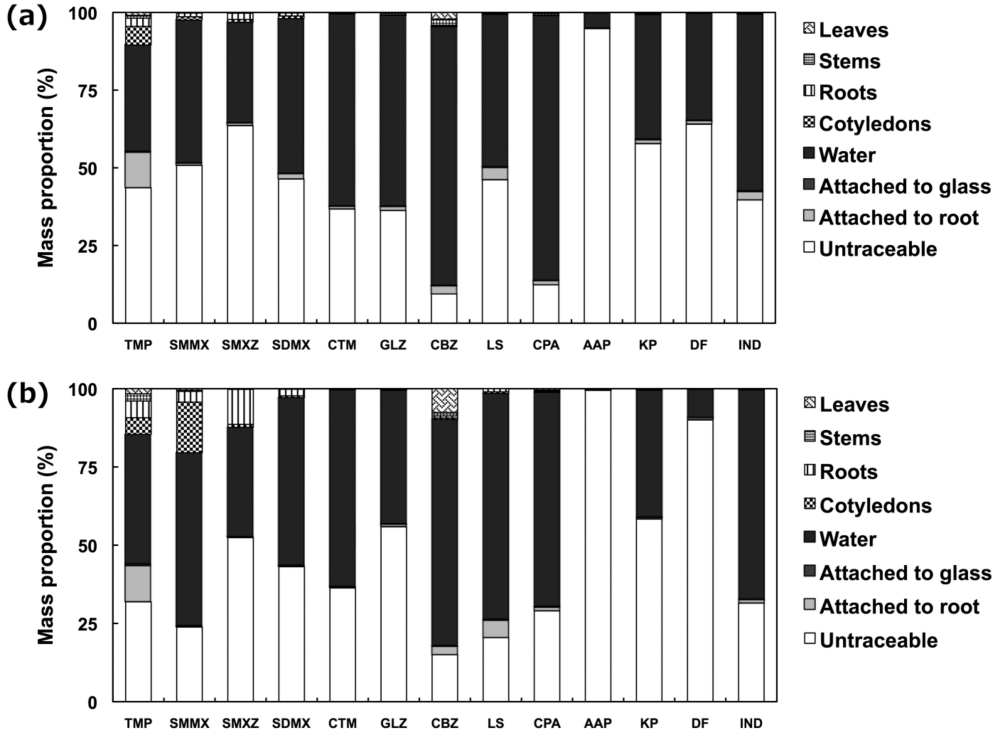


Figure 2. Mass proportion of pharmaceutical chemicals at the end of the test; (a) treatment A (mixture, 24 h, 0.25 mg L^{-1}), (b) treatment B (single, 72 h, 1 mg L^{-1}). Y-axis, mass proportion of pharmaceutical chemicals in pea after exposure; x-axis, tested pharmaceutical chemicals.

RESULTS AND DISCUSSION

Uptake of Pharmaceuticals by Pea (*P. sativum*). No pharmaceutical chemicals were detected in the control of pea plants. Decrease in the levels of pharmaceutical chemicals was not observed in the control test without plant. This indicates that photodegradation did not occur, or was negligible. Figure 1 presents the concentration factors (ratios of concentrations in plant tissues to exposure solution) calculated for leaves (LCF), stems (SCF), roots (RCF), and cotyledons (CCF). The concentrations of pharmaceutical chemicals in pea exposed to different types of treatments (A and B), and numerical data of concentration factors are presented in the Supporting Information (Table S3). These concentration factors are based on mean concentrations in exposure solution at the beginning and end of the test. There was no significant difference among mean moisture contents of plant tissues (leaves, 87%; stems, 90%; roots, 92%; and cotyledons, 89%). Except for LS, DF, and IND, 10 types of test compounds were detected in not only underground parts (roots and cotyledons) but also shoots (leaves and stems), indicating that many pharmaceutical chemicals have the potential to be taken up by shoots such as edible leaves, fruits, and seeds. Uptake of CTM, CPA, GLZ, and KP by plants has not been previously reported. Among the tested pharmaceutical chemicals, TMP, SMMX,

Table 2. Concentration Ratios of the Cucumber Xylem Sap after Exposure to Pharmaceutical Chemicals and the Test Solution (Mean Concentrations at the Beginning and End of the Test)

	TMP	SMMX	SMXZ	SDMX	CTM	GLZ	CBZ	LS	CPA	AAP	KP	DF	IND
Average	0.41	0.041	0.022	0.029	1.5	0.23	0.69	0.019	0.89	n.a.[b]	0.10	n.a.[b]	n.a.[b]
RSD[a] (%)	10	21	25	23	17	29	8.6	11	10		44		

[a]RSD, relative standard deviation. [b]n.a., not applicable due to no detection in cucumber xylem sap.

SMXZ, SDMX, and CBZ were detected at relatively high concentrations in plant tissues, whereas CTM, AAP, and DF were detected at relatively low concentrations in the tissues. This result shows that plant uptake ratios varied with pharmaceutical chemicals. LCF and SCF of CBZ exceeded unity, whereas RCF and CCF were 0.17−0.62 and 0.13−0.49, respectively, which did not exceed unity. Moreover, LCF was higher than SCF in both treatments, and the ratio of LCF to SCF in treatment A (LCF/SCF: 1.4) was much lower than that in treatment B (LCF/SCF: 7.1). This result indicates that transport of CBZ from stem to leaves progressed with time, and that CBZ has high accumulation capacity in leaves. Our finding of higher accumulation of CBZ in leaves than in roots is similar to previous reports that studied soybean, cucumber, and ryegrass.[15,17,30] It should be noted that CBZ showed the highest ability to transport into pea shoots among the 13 tested compounds in the present study. RCF and CCF values for TMP and sulfonamides (SMMX, SMXZ, SDMX) were relatively high, up to 8.4 (TMP), 15 (SMMX), 32 (SMXZ), and 3.1 (SDMX). The high accumulation of TMP and sulfonamides found in this study agrees with other reports, such as uptake study on SDMX in millet, pea, and corn[23] and uptake studies on TMP and SMXZ in cabbage.[29] RCF values of sulfonamides for treatment B (72 h) were higher than those for treatment A (24 h) ($p < 0.05$), suggesting that they accumulate in underground parts. On the other hand, RCF values of TMP showed no statistical difference between treatments A and B ($p > 0.6$), whereas SCF values significantly increased from treatment A to B ($p < 0.01$). These results indicate that transport of TMP from root to stem progressed with time; thus, it can be concluded that TMP is more easily transported to plant shoots than sulfonamides.

Various studies reported that roots have a high potential to accumulate lipophilic organic compounds such as PAHs, PCBs, polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) owing to their adsorption on lipophilic root solids,[40−42] and root uptake of nonionic compounds is considered to be enhanced with increase of log K_{ow}.[35] However, accumulation of hydrophobic pharmaceutical chemicals (LS, KP, DF, and IND) in roots was not observed in this study. This might be a result of the dominant anionic forms of these chemicals (pK_a = 4.15−4.5), which reduces their hydrophobicity in the test solution (pH = 6.1−7.1).

Mass proportions (quantity balance) of pharmaceutical chemicals in the experiment are presented in Figure 2, and the numerical data can be found in the Supporting Information (Table S4). Mean fresh weights of plant samples (g) were 0.24 (leaves), 0.31 (stems), 0.22 (roots), and 0.30 (cotyledons), at the end of exposure period. In more than half of tested pharmaceutical chemicals, the percentage of mass attached to roots were higher than those in pea tissues. Total mass distributions of pharmaceutical chemicals in shoots, underground parts, solution, parts attached to underground, and glass were not 100%. Metabolism and biotransformation of these compounds by pea plants presumably involved in the input−output discrepancy. Plants have xenobiotic detoxification and biotransformation systems, and the detoxification cascades are similar to the mammalian liver functions.[43,44] The structural alteration of pharmaceutical chemicals associated with biotransformation can sometimes lead to the generation of pharmacologically active metabolites. Dordio et al.[45] reported that a CBZ active metabolite (10,11-dihydro-10,11-epoxycarbamazepine) was detected in the leaves of the macrophyte *Typha* spp. In the present study, possible metabolites of pharmaceutical chemicals in plant tissues were not quantified. In future, studies of generation and the fate of active metabolites in plants are needed. Despite its low concentration in pea shoots, AAP significantly decreased in the test solution. This might be because of the degradation by bacteria on the root surface, since the experiment was conducted under nonsterile conditions. AAP is well-known as easily biodegradable compounds.[46,47]

Uptake of Pharmaceuticals by Cucumber (*C. sativus*): Calculated Concentration Ratios (Xylem Saps/Test Solutions). The concentration ratios of the cucumber xylem sap after exposure to pharmaceuticals and the test solution were calculated, and these results are presented in Table 2. No pharmaceutical chemicals were detected in the xylem sap of the cucumber control. AAP, DF, and IND were not detected in xylem sap. The concentration ratios (xylem saps/test solutions) of sulfonamides, LS, and KP were less than or equal to 0.1, indicating low uptake of these compounds in shoots. The concentration ratio (xylem saps/test solutions) of CTM (1.5) was highest value, followed by CPA (0.89), CBZ (0.69), TMP (0.41), and GLZ (0.23). This result indicates that CTM, CPA and CBZ have relatively high transport ability into cucumber shoots among tested pharmaceutical chemicals. Moreover, this result supports the fact that TMP and sulfonamides were detected at relatively high concentrations in pea roots and cotyledons, and in contrast, CBZ was detected at relatively high concentrations in pea leaves. Shenker et al.[17] previously reported statistically comparable concentrations of CBZ in xylem sap and the test solution after exposure to CBZ, suggesting that transport of CBZ from roots to shoots is not restricted. However, in our study, CBZ concentrations in xylem sap (148 ± 12 $\mu g\ L^{-1}$) and the exposure solution (214 ± 3.8 $\mu g\ L^{-1}$) were statistically different ($p < 0.01$), unlike in the previous study. Although high abilities for uptake of CTM and CPA were not observed for pea shoots, their concentration ratios (xylem saps/test solutions) for cucumber were relatively high among the tested pharmaceutical chemicals. This result implies interspecies differences between the leguminous and cucurbitaceous plants relating to uptake characteristics and metabolism.

Uptake of Pharmaceuticals by Cucumber (*C. sativus*): Transpiration Water Analysis. Since CTM, CPA, and CBZ were observed to have relatively high concentration ratios (xylem saps/test solutions) for cucumber, the transpiration water was collected and analyzed. No pharmaceutical chemicals were detected in the transpiration water of the cucumber control. Interestingly, CTM and CBZ were detected in

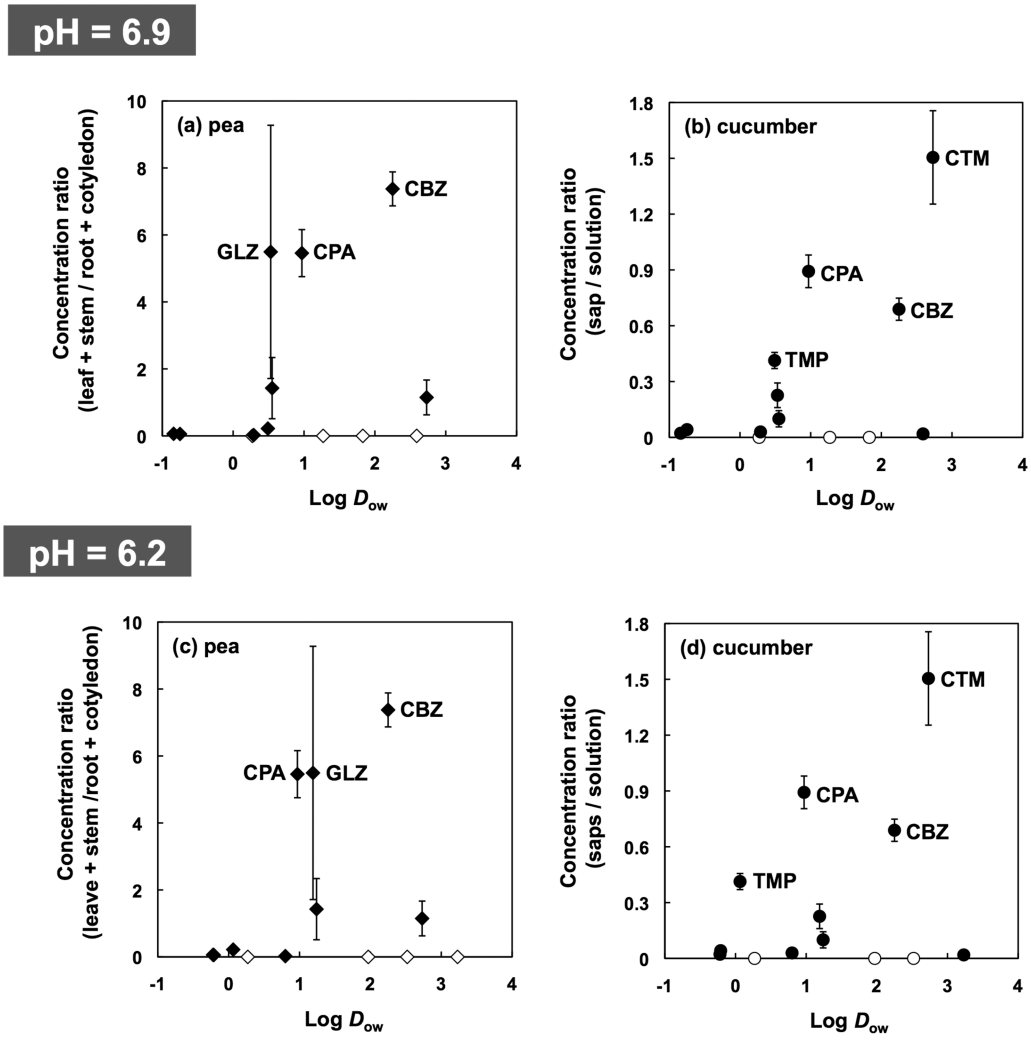


Figure 3. Comparison between the ability to transport pharmaceutical chemicals from roots to shoots and log D_{ow} for test compounds. (a) *Y*-axis, ratios of concentrations in pea leaves and stems to roots and cotyledons; *x*-axis, log D_{ow} of test compounds based on pH = 6.9 (mean pH at the beginning of the test). (b) *Y*-axis, concentration ratios of cucumber xylem saps to test solutions; *x*-axis, log D_{ow} (pH = 6.9). (c) *Y*-axis, ratios of concentrations in pea leaves and stems to roots and cotyledons; *x*-axis, log D_{ow} based on pH = 6.1 (mean pH at the end of the test). (d) *Y*-axis, concentration ratios of cucumber xylem saps to test solutions; *x*-axis, log D_{ow} (pH = 6.1). Open diamonds and open circles indicate not detectable in pea shoots and cucumber xylem saps, respectively.

transpiration water, but CPA was not (data not shown in figure or table). It has not been previously reported that CTM and CBZ are released by transpiration from leaves. However, the concentrations of CTM and CBZ in transpiration water were 2.6 and 1.3 μg L^{-1}, respectively, and these values were much lower than the initial concentrations of CTM and CBZ in test solutions (938 and 1050 μg L^{-1}, respectively). These results indicate that CTM and CBZ are mainly transported with transpiration streams within xylem, and subsequently accumulate in leaves owing to their low release from leaves.

Comparison of Ability of Shoots To Uptake Pharmaceutical Chemicals with Log K_{ow} or Log D_{ow}. The solute absorbed by the root hair from the soil passed through the root epidermis, cortex, and endodermis to reach the root xylem. Plants have an impervious layer called the casparian strip surrounding the cells of the root endodermis (Figure S1).[48] The casparian strip prevents solute from passing through the endodermis via apoplastic flow; thus, solute must pass at least one selectively permeable membrane in the endodermis (symplastic route) in order to be carried to the xylem. Therefore, membrane permeability of the solute involves uptake ability of plant shoots. The permeability of the biomembrane for nonionic organic compounds is positively correlated to chemical lipophilicity. The transpiration stream concentration factor (TSCF) is represented as the concentration ratio between the xylem sap and a test solution when it reaches the equilibrium with the root tissues. As it is difficult to directly collect xylem sap, the TSCF is very often experimentally determined from the mass transported to the shoots. Briggs et al.[35] derived the relation between TSCF and log K_{ow} of the test compounds, using barley plants. It was found that the TSCF was at a maximum in the case of compounds having log K_{ow} from 1.5 to 2.0. Hsu et al.[36] and Burgen and Schnoor[38] found a similar relation, showing that TSCF was at a maximum for compounds having log K_{ow} = 3 in soybean plants and log K_{ow} = 2.5 in hybrid poplar. These results indicated that highly hydrophilic compounds are less able to cross hydrophobic membranes, while highly lipophilic compounds are less able to be transferred to shoots because they are retained in the root lipids.[40−42] In addition, the optimal log K_{ow} that shows the

highest TSCF value differs for various plant species. For ionizable organic compounds, ionization can reduce their uptake to the shoots owing to a decrease in their membrane permeability.[49,50] In this study, the pH values of the exposure solutions at the beginning and the end of the experiment were approximately 6.5−7.1 and 6.1−6.5, respectively. Some of the tested pharmaceuticals exist predominantly as ionic forms in the exposure solutions. For example, Sulfonamides—SMXZ (pK_{a1}, 2.0; pK_{a2}, 6.0), SMXZ (pK_{a1}, 1.83; pK_{a2}, 5.60), and SDMX (pK_{a1}, 2.13; pK_{a2}, 6.08)—contain two functional moieties on both sides of the sulfonamide linkage (−$NHSO_2$-). Consequently, sulfonamides show two pK_a, one involving the protonation of the primary aromatic amine (−NH_2) and other corresponding to the deprotonation of the amide (−NH−). At pH values above their pK_{a2}, sulfonamides exist predominantly as anionic forms, while at pH values below their pK_{a1}, they are positively charged. TMP contains pK_{a1} = 3.23 (amine group) and pK_{a2} = 6.76 (imine group), and thus, at pH values below its pK_{a2}, it exists predominantly as a cationic form. For ionizable pharmaceutical chemicals, pH-dependent octanol−water partition coefficients (log D_{ow}) were calculated by the following equations.[51,52]

For neutral functional groups (CTM, CBZ, and CPA)

$$\log D_{ow} = \log K_{ow}$$

For acidic functional groups (sulfonamides, GLZ, LS, AAP, KP, DF, and IND)

$$\log D_{ow} = \log K_{ow} + \log \frac{1}{1 + 10^{pH-pKa}}$$

For basic functional groups (TMP)

$$\log D_{ow} = \log K_{ow} + \log \frac{1}{1 + 10^{pKa-pH}}$$

The comparison between the abilities to transport pharmaceutical chemicals from roots to shoots and log D_{ow} for test compounds is presented in Figure 3. This graph indicates that compounds with an intermediate quality between hydrophilicity and lipophilicity could be easily transported to shoots, which reflects the same relation as previously reported.[35−38] The result of this study suggests that log D_{ow} could be an useful determinant of accumulation features of pharmaceutical chemicals within the food crops. However, DF and IND showed different behavior. This might have been caused by biodegradation in the test solutions or high metabolic rates within plant tissues, because untraceable percentage of DF and IND were higher than that of CBZ and CPA in the pea experiment (Figure 2). However, present studies were conducted under near-neutral pH condition for 24 of 72 h. In future, investigation on the relation between log D_{ow} and plant uptake abilities over several pH ranges, and kinetic study on the uptake and dissipation are needed.

For CBZ with pK_a = 13.9 (amine group), existence as a neutral form at near-neutral pH has been shown in many reports[51,52] Tadkaew et al.[53] investigated the elimination efficiency of pharmaceutical chemicals in a membrane bioreactor over several pH ranges, and found that ionizable pharmaceutical chemicals showed pH-dependent variation of elimination efficiency, while CBZ showed stable elimination efficiency owing to pH-independent log K_{ow}. In the present study, the trasnport ability of CBZ into shoots was much greater than that of other tested pharmaceutical chemicals. This can be attributed to the pH-independent and optimal log K_{ow} of CBZ for transport to plant shoots.

Environmental Relevance. Sulfonamides and TMP tend to accumulate in plant roots; they were detected in recycled manure produced from livestock wastes (N.D.−210 $\mu g\ kg^{-1}$) in Japan.[39] Therefore, investigations on their accumulation in various root crops should be conducted in future. CTM and CBZ showed high abilities to transport into plant shoots in the present study have been found in effluent from WWTPs, reclaimed wastewater, and biosolids at relatively high concentrations among pharmaceutical chemicals. In our previous study, their maximum concentrations in recycled manure produced from biosolids were 35 $\mu g\ kg^{-1}$ (CTM) and 46 $\mu g\ kg^{-1}$ (CBZ).[39] CTM is widely used to relieve itching skin and treat scabies infections. Because pharmaceutical chemicals for external applications are discharged by washing or bathing without being metabolized, CTM was detected in the effluent of WWTPs at high concentrations (245−968 ng L^{-1}) compared with oral or injected pharmaceutical chemicals.[7] Low removal of CBZ (−67−35%) during biological wastewater treatment has been shown in many reports,[7,9] and the concentrations in WWTP effluents were summarized 111.2−290 ng L^{-1}.[5] The high persistence of CBZ was previously reported in degradation experiments with water/sediment systems, archived biosolids, and microcosm (i.e., pond water).[54−56] Moreover, low decomposition of CBZ by fermentation of the recycled manure samples was presented in our previous paper.[39] In actual agricultural lands, once pharmaceutical chemicals enter soils, they are subjected to transport and biodegradation. However, CBZ has the potential to remain in the agricultural land soils for extended period of time. In addition, highly hydrophobic pharmaceutical chemicals with a strong soil adsorption may have relatively low bioavailability for being taken up by plants from soils, whereas pharmaceutical chemicals with intermediate polarity such as CTM and CBZ may have relatively high bioavailability because of their weak soil adsorption characteristics. Consequently, there is the potential accumulation of these compounds in edible plants at environmentally relevant concentrations, and investigation on actual agricultural fields is needed.

Pharmaceutical chemicals in the pea seeds and cucumber fruits consumed by human beings were not determined in this study. On the basis of our data, it is therefore difficult to evaluate the potential risks of human exposure to residual pharmaceutical chemicals in food crops. However, our plant uptake study revealed that many pharmaceutical chemicals have the potential to be taken up by shoots of edible plants. In addition, it was revealed that pharmaceuticals with an intermediate polarity and pH-independent log K_{ow} could be easily transported to plant shoots. In contrast, highly hydrophilic pharmaceuticals tend to accumulate in roots. Various pharmaceutical chemicals are constantly prescribed all over the world, and they have wide-ranging physicochemical properties. Therefore, screening surveys based on combinations of distribution features of pharmaceuticals and edible parts of various crops (i.e., compounds with intermediate polarity and leaf crops, highly hydrophilic or highly hydrophobic compounds and root crops) are needed to evaluate the human health risk of the pharmaceuticals chemicals intake through the food supply.

■ ASSOCIATED CONTENT

Supporting Information

A detailed description of plant precultivation conditions, analytical procedure of pharmaceutical chemicals, monitor ions and ionization parameters (Table S1), details of quality assurance and control (Table S2), concentrations of pharmaceutical chemicals in pea (Table S3), mass proportion of pharmaceutical chemicals in pea (Table S4), ratios of concentrations in pea shoots (leaves and stems) to underground parts (roots and cotyledons) (Table 5), and pathways (apoplast, symplast) from root exodermis to enter a vascular system (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Address: Center for Marine Environmental Studies (CMES), Ehime University, 2-5, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan. Tel/Fax: +81-89-927-8171. E-mail: keinomi@agr.ehime-u.ac.jp.

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Notes

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■ ABBREVIATIONS USED

TMP, trimethoprim; SMMX, sulfamonomethoxine; SMXZ, sulfamethoxazole; SDMX, sulfadimethoxine; CTM, crotamiton; GLZ, gliclazide; CBZ, carbamazepine; LS, losartan; CPA, cyclophosphamide; AAP, acetoaminophen; KP, ketoprofen; DF, diclofenac; IND, indometacin

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