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Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water

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ABSTRACT

The dissemination of pharmaceuticals in agroecosystems originating from land application of animal manure/sewage sludge and irrigation with treated wastewater in agricultural production has raised concern about the accumulation of pharmaceuticals in food products. The pathways of pharmaceutical entries via plant roots, transport to upper fractions, and the factors influencing these processes have yet been systematically elucidated, thus impeding the development of effective measures to mitigate pharmaceutical contamination in food crops. In this study, lettuce uptake of thirteen commonly used pharmaceuticals was investigated using a hydroponic experimental setting. Pharmaceutical sorption by lettuce roots was measured in order to evaluate the influence on pharmaceutical transport from roots to shoots. Small-sized pharmaceuticals e.g., caffeine and carbamazepine with molecular weight (MW) $< 300\,g\,mol^{-1}$ and a low affinity to lettuce roots (sorption coefficient $Kp < 0.05\,L\,g^{-1}$) manifested substantial transport to shoots. Small-sized molecules lamotrigine and trimethoprim had a relatively strong affinity to lettuce roots ($Kp > 12.0\,L\,g^{-1}$) and demonstrated a reduced transport to shoots. Large-sized pharmaceuticals (e.g. $MW > 400\,g\,mol^{-1}$) including lincomycin, monensin sodium, and tylosin could be excluded from cell membranes, resulting in the predominant accumulation in lettuce roots. Large-sized oxytetracycline existed as zwitterionic species that could slowly enter lettuce roots; however, the relatively strong interaction with lettuce roots limits its transport to shoots. The mass balance analysis revealed that acetaminophen, β-estradiol, carbadox, estrone and triclosan were readily metabolized in lettuce with > 90% loss during 144-h exposure period. A scheme was proposed to describe pharmaceutical uptake and transport in plant, which could reasonably elucidate many literature-reported results. Molecular size, reactivity and ionic speciation of pharmaceuticals, as well as plant physiology, collectively determine their uptake, transport and accumulation in plants.

1. Introduction

Pharmaceuticals ubiquitously present in the environment have been considered as chemicals of environmental concern. They are released into agroecosystems through livestock excretion of administered veterinary pharmaceuticals, land application of biosolids, and irrigation with treated wastewater (effluents) from wastewater treatment plants (WWTPs) (Aga et al., 2005; Franklin et al., 2016; Ternes, 1998). To alleviate water scarcity in arid and semi-arid regions, treated wastewater has been increasingly used in agricultural irrigation (Goldstein et al., 2014; Williams and McLain, 2012). This practice results in the dissemination of pharmaceuticals in soils some of which could be taken up by crops/vegetables (Calderon-Preciado et al., 2011; Carter et al., 2014; Wu et al., 2010). Biosolids and animal manures are applied to agricultural lands as a convenient approach of waste disposal while providing fertilizer values and improving soil structures. As a result, many pharmaceuticals have been found to accumulate in vegetables and agricultural products at μg kg^{-1} levels (Calderon-Preciado et al., 2011; Shenker et al., 2011). These levels of pharmaceuticals are far below the suggested dosage for the therapeutic purpose, thereby posing limited risks to human health (FDA, 2009; Malchi et al., 2014; Sabourin et al., 2012; Wu et al., 2013). However, pharmaceuticals can induce the changes of plant hormone levels and cause detrimental impacts to plant health (Carter et al., 2015; Macherius et al., 2012b; Shargil et al.,

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Table 1
Physicochemical properties of the pharmaceuticals studied.

Pharmaceuticals	Molecular weight ($g\,mol^{-1}$)	Molecular volume[a] (nm^3)	Water solubility[b] ($mg\,L^{-1}$)	pK_a[b]	Predominant species (pH at 5.8)	log K_{ow}[b]	log D_{ow}[c] (pH at 5.8)
Acetaminophen	151.16	0.201	14,000	9.63[d]	Neutral: 100%	0.46	0.46
Caffeine	194.19	0.221	21,600	1.22 (conjugate acid)[d]	Neutral: 100%	−0.07	−0.07
Carbamazepine	236.27	0.311	18	2.3 (conjugate acid)[e], 13.9	Neutral: 100%	2.45	2.45
Lamotrigine	256.1	0.271	170	5.7 (conjugate acid)[f]	Cationic: 44% Neutral: 56%	2.57	2.32
Carbadox	262.22	0.301	58	1.8 (conjugate acid)[g], 10.5[g]	Neutral: 100%	0.13	0.13
Estrone	270.37	0.385	30	10.77[d]	Neutral: 100%	3.13	3.13
β-Estradiol	272.38	0.387	3.9	10.71[d]	Neutral: 100%	4.01	4.01
Triclosan	289.54	0.322	10	7.9	Neutral: 99% Anionic: 1%	4.76	4.76
Trimethoprim	290.32	0.385	400	7.12 (conjugate acid)[d]	Cationic: 95% Neutral: 5%	0.91	−0.43
Lincomycin	406.54	0.520	927	7.6 (conjugate acid)[d]	Cationic: 98% Neutral: 2%	0.2	−1.61
Oxytetracycline	460.43	0.445	313	3.27[d], 7.32[d], 9.11 (conjugate acid)[d]	Zwitterionic: 97% Anionic: 3%	−0.9	−0.91
Monensin sodium	692.87	0.919	3×10^{-3}[h]	4.2[i]	Neutral: 2% Anionic: 98%	5.43[h]	3.82
Tylosin	916.10	1.221	5000	7.73 (conjugate acid)[d]	Cationic: 99% Neutral: 1%	1.63	−0.31

[a] Calculated from molar volume acquired from USEPA website: https://comptox.epa.gov/dashboard/.
[b] From TOXNET database: https://toxnet.nlm.nih.gov/.
[c] For neutral compounds: log D_{ow} = log K_{ow}; for acidic compounds: log D_{ow} = log K_{ow} + log $\frac{1}{1+10^{(pH-pKa)}}$; for basic compounds: log D_{ow} = log K_{ow} + log $\frac{1}{1+10^{(pka-pH)}}$(Carballa et al., 2008).
[d] Prankerd (2007).
[e] Nghiem et al. (2005).
[f] Nason et al. (2018).
[g] Song et al. (2010).
[h] https://pubchem.ncbi.nlm.nih.gov/.
[i] Bohn et al. (2013).

2015). Low chemical doses also can change the growth of smallest and largest individuals within a population, even though the mean response remains unchanged (Belz et al., 2018). Moreover, synergistic or antagonistic effects of mixtures of pharmaceuticals under long-term exposure are currently unclear, which impedes the development of appropriate risk assessment framework (Boxall et al., 2006; Chitescu et al., 2013; Marsoni et al., 2014).

Pharmaceutical accumulation in agricultural products is a key component in the risk assessment to human health. Bioaccumulation factor is commonly referred to as the ratio of pharmaceutical concentration in plant to that in surrounding media, e.g., accumulation in roots is commonly referred as root concentration factor (RCF) (Briggs et al., 1982; Eggen et al., 2011; Trapp, 2000). Pharmaceutical movement within plant is presented as translocation factor (TF) which is defined as the ratio of pharmaceutical concentration in shoots to that in roots (Miller et al., 2016; Wu et al., 2015). Several previous studies reported a positive relationship between log RCF and log D_{ow} (octanol-water partitioning coefficient adjusted to the neutral fraction of pharmaceutical in water), and a negative relationship between log TF and log D_{ow} (Macherius et al., 2012b; Tanoue et al., 2012; Wu et al., 2010; Wu et al., 2013). However, the results from different experimental settings such as plant species, hydroponic vs. pot experiments, applied pharmaceutical concentration and exposure periods revealed that for a given pharmaceutical, RCF or TF values could vary up to two orders of magnitude, and no apparent relationship was found between log RCF (or TF) and log D_{ow} (Miller et al., 2016; Wu et al., 2015). Generally, plant uptake of neutrally charged pharmaceuticals is greater than ionic species, because anionic pharmaceuticals are repelled by cell membranes with negative electrical potential, and cationic species are attracted to the cell membranes thus limiting their movement into plants (Goldstein et al., 2014; Trapp, 2000; Wu et al., 2013). Some pharmaceutical species could be altered in plant organs, and consequently trapped in plant cells in response to pH variation in different organs, e.g., 5.5 in intercellular space, 7.2 in cytosol, and 5.5 in vacuoles (Goldstein et al., 2014; Taiz et al., 2015). For instance, neutral form of lamotrigine (pK_a 5.7) could pass through cell membranes and tonoplasts by passive diffusion. Once in vacuole, the neutral lamotrigine converts to cationic species that is limited to cross lipid bilayer hence being trapped in plant cell vacuoles (Goldstein et al., 2014; Miller et al., 2016; Wu et al., 2015).

Most previous research efforts have been dedicated to investigating the impact of pharmaceutical speciation and lipophilicity on their accumulation in plants, while less attention was paid to the influence of water flow on their uptake and transport (Dodgen et al., 2015; Goldstein et al., 2014; Malchi et al., 2014; Wu et al., 2013). Water flow enters plant roots via apoplast and symplast pathways (Cooper, 2000; Miller et al., 2016; Raven et al., 1999; Taiz et al., 2015). The apoplast pathway refers to the movement of water molecules through cell walls and between intercellular spaces. In the cortex, the space for apoplast water movement accounts for 8 to 25% of root volume (Trapp, 2000). The symplast pathway refers to the process of water molecules crossing cell membranes and moving between cells. Water flow is believed to be the primary carrier for uptake and transport of pharmaceuticals in plants (Malchi et al., 2014; Tanoue et al., 2012; Wu et al., 2013). At endodermis, the highly hydrophobic Casparian strip might block the transport of pharmaceuticals carried with water flow via the apoplast pathway. Only those pharmaceuticals that cross cell membranes (symplast pathway) and/or diffuse through cells surrounded by Casparian strip could move to xylem and then transport upwards to plant shoots (Miller et al., 2016; Taiz et al., 2015; Tanoue et al., 2012).

In this study, we hypothesized that water flow is the primary carrier for pharmaceuticals to enter plants and facilitates their distribution in different plant parts. Interaction of pharmaceuticals with roots could retard their upward transport to shoots. To test these hypotheses, uptake and transport of a range of pharmaceuticals in lettuce were measured using a hydroponic experimental setting. Pharmaceutical sorption

by lettuce roots was determined to evaluate its influence on pharmaceutical transport from roots to shoots. This study, together with some literature results, provides innovative mechanistic insights to pharmaceutical uptake, accumulation and transport in plant.

2. Materials and methods

2.1. Chemicals and materials

Pharmaceuticals including acetaminophen, β-estradiol, caffeine, carbadox, carbamazepine, estrone, lincomycin, monensin sodium, oxytetracycline, trimethoprim and tylosin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lamotrigine was obtained from Toronto Research Chemicals, Inc. (Toronto, ON, Canada), and triclosan from AK Scientific, Inc. (Union City, CA, USA). Physicochemical properties of the studied pharmaceuticals are listed in Table 1. Simeton, used as an internal standard, was supplied by Absolute Standards, Inc. (Hamden, CT, USA). Acetonitrile, ammonium hydroxide (NH_4OH) and anhydrous sodium sulfate (Na_2SO_4) were obtained from EMD Chemicals (Gibbstown, NJ, USA). HPLC-grade methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Disodium ethylenediaminetetraacetate (Na_2EDTA), formic acid, and sodium chloride (NaCl) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Waters Oasis hydrophilic–lipophilic balance (HLB) cartridge was acquired from Waters Corporation (Milford, MA, USA). Ceramic homogenizers, C18, and primary secondary amine (PSA) powders were purchased from Agilent Technologies (Santa Clara, CA, USA). Ultrapure water was generated from a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Lettuce uptake of pharmaceuticals

Black Simpson lettuce seeds (*Lactuca sativa*) were placed on moistened paper tissues until they began to sprout (2 to 3 days). The seedlings were cultured in hydroponic nutrient solution containing 9.375 g of MaxiGro plant food (10–5-14) (General Hydroponics, Sevastopol, CA, USA) in 15 L of deionic water. The lighting period was set as 16 h per day with light intensity of 150 μmol/m^2/s using an Apollo Horticulture Full Spectrum 300 W LED Light source (Rowland Heights, CA, USA). The nutrient solution was continuously aerated using a fusion air pump at a flow rate of ~10 L per hour. The lettuce seedlings grew for approximately 22 days at 18 °C, and reached 25–30 cm in height and 8.0–10.5 g (fresh weight) of biomass with well-developed roots.

Two lettuce plants were transferred to an Erlenmeyer flask containing 210 mL of nutrient solution spiked with thirteen pharmaceuticals at the initial concentration of 50 ng mL^{-1} for each pharmaceutical. The nutrient solution was also continuously aerated with air flow at a rate of ~0.2 l per hour. All flasks were fully wrapped with aluminum foil to minimize the potential photodegradation of pharmaceuticals. During the exposure period, the loss of water from the flasks (due to transpiration by lettuce) was determined gravimetrically every day, and fresh nutrient solution free of pharmaceuticals was replenished to reach the initial volume. Solution was checked every 12 h to keep pH at 5.8 and electrical conductivity (EC) at 0.8 mS/cm. Solution pH and EC decreased slightly to ~5.4 and 0.7 mS/cm within 12-h interval, and a diluted potassium bicarbonate ($KHCO_3$) (Earth Juice Natural Up, Hydrofarm, Petaluma, CA, USA) and a nutrient solution prepared from MaxiGro plant food (EC at 4.0 mS/cm) were used to adjust pH and EC to 5.8 and 0.8 mS/cm, respectively. At 12, 24, 48, 72, 105 and 144-h sampling time, three flasks were sacrificed to collect lettuce samples for extraction and analysis of pharmaceuticals. The experimental controls included lettuce plants without pharmaceuticals, and nutrient solution containing pharmaceuticals without lettuce. The sampled lettuce was rinsed with deionic water, blotted dry with paper tissue, separated into roots and shoots, immediately freeze-dried to obtain powders, and stored at −20 °C prior to extraction.

2.3. Pharmaceutical extraction and analysis

Pharmaceuticals in the nutrient solution were extracted using solid phase extraction (SPE). Twenty mL of solution was mixed with 1.0 mL of 3.0 g L^{-1} of Na_2EDTA, and passed through a HLB SPE cartridge. The cartridges were eluted with 5.0 mL of methanol for analysis. Pharmaceuticals in lettuce roots and shoots were extracted using a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) method described in our previous study (Chuang et al., 2015). Dried lettuce samples (250 mg of shoots or 100 mg of roots) were mixed with 1.0 mL of 300 mg L^{-1} of Na_2EDTA for 1 min, then two ceramic homogenizers and 1.75 mL of methanol were added, and vortexed for 1.5 min. Afterwards, 2.0 g of Na_2SO_4 and 0.5 g of NaCl were added, and shaken with the mixture to enhance the extraction efficiency. The extracts were centrifuged at 5050*g* for 10 min, and the supernatants were collected. The residues were then extracted with 3.25 mL of acetonitrile, and centrifuged at 5050*g* for 10 min. The two extracts were combined, and cleaned up using disperse SPE (d-SPE) sorbents. The d-SPE sorbents were the mixture of 12.5 mg C18, 12.5 mg PSA and 225 mg Na_2SO_4. The combined extracts (1.2 mL) and the d-SPE sorbents were mixed vigorously for 1 min, centrifuged at 9240*g* for 10 min, and the supernatant was transferred to a clean glass vial for the analysis of pharmaceuticals.

Pharmaceuticals in the extracts were analyzed using a Shimadzu Prominence high-performance liquid chromatography (Columbia, MD, USA) coupled to a Sciex 4500 QTrap mass spectrometer (LC-MS/MS) (Foster City, CA, USA) under either positive or negative ionization mode. An Agilent Eclipse Plus C18 column (50 mm × 2.1 mm, particle size 5 μm, Santa Clara, CA, USA) was used to separate the thirteen pharmaceuticals. Under the positive ionization mode, the binary mobile phase consisted of water (Phase A) and a mixture of acetonitrile and methanol (65/35, v/v) (Phase B), and both contained 0.3% of formic acid. After 2 min of pre-equilibration with 100% of Phase A, the gradient program was set as follows: Phase B increased to 40% during 0.1–1.0 min, then to 70% during 1.0–2.0 min, to 80% during 2.0–3.0 min, to 100% during 3.0–3.5 min, and held at 100% of Phase B until 7.2 min. Under the negative ionization mode, Phase A was 0.005% NH_4OH aqueous solution, and Phase B was a mixture of acetonitrile and methanol (90/10, v/v). After 2 min pre-equilibration with 100% of Phase A, Phase B increased to 5% during 0.1–2.0 min, to 100% during 2.0–10.0 min, and held it until 12.0 min. The flow rate was 0.35 mL/min, and the sample injection volume was 10 μL. In the tandem mass spectrometer, multiple reaction monitoring (MRM) mode was set up for precursor and product ion transitions. The quantification and qualification parameters for each pharmaceutical are listed in Table S1. The ionspray voltage, temperature, curtain gas pressure and entrance potential were 5000 V, 700 °C, 20 psi and 10 V for the positive ionization mode, and −4500 V, 700 °C, 40 psi, and −10 V for the negative ionization mode. The pharmaceuticals were quantified using matrix-matched standard curves. The extraction efficiencies ranged within 85.1–116.3% for the pharmaceuticals in nutrient solution (with spiked concentration of 100 ng mL^{-1}), within 87.5–106.9% for the pharmaceuticals in shoots and within 85.2–129.6% for the pharmaceuticals in roots. The spiked concentration was 250 ng g^{-1} to lettuce roots and shoots in methanol, and the solvent was fully evaporated after 6 h in fume hood prior to extraction (Table S2).

2.4. Lincomycin uptake by whole lettuce and lettuce shoots

The comparison of lincomycin uptake by whole lettuce plants from roots versus shoots only (after the removal of roots) was conducted using the same hydroponic setting as described above. Two whole lettuce plants and lettuce shoots were transferred to Erlenmeyer flasks containing 210 mL of nutrient solution spiked with 50 ng mL^{-1} of lincomycin. For the uptake by whole lettuce, the roots were fully immersed in the nutrient solution. For the lettuce shoots, approximately

1 cm of the shoots (incised bottom) was immersed in the nutrient solution. After 48 h of exposure, lettuce samples were collected, rinsed with deionic water, blotted dry with paper tissue, separated into roots and shoots for whole lettuce samples and immediately freeze-dried to obtain powders followed by extraction and analysis of lincomycin by LC-MS/MS.

2.5. Sorption by lettuce roots

Pharmaceutical sorption by lettuce roots from nutrient solution was measured using a batch equilibration method (Card et al., 2012). Freeze-dried lettuce root powder (25 mg) was mixed with 20 mL of nutrient solution containing the thirteen pharmaceuticals at concentrations of 0, 10, 20, 30, 40 or 50 ng mL^{-1} for each pharmaceutical. The experimental controls consisted of the nutrient solution with pharmaceuticals devoid of lettuce root powders. The tubes were shaken on an Innova 2300 platform shaker (New Brunswick Scientific, Edison, NJ, USA) at 150 rpm for 24 h in dark, and centrifuged at 1460*g* for 15 min. The supernatant was collected and analyzed using LC-MS/MS. The mass loss of each pharmaceutical between the initial and final nutrient solution was assumed to be sorbed by lettuce roots.

2.6. Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY, USA). For all experimental treatments, the assumptions of homogeneity of variances and normality were tenable as assessed by Levene and Shapiro-Wilk tests, respectively (p-value > 0.05). Significant difference between experimental treatments was analyzed using one-way analysis of variance (ANOVA) (p-value < 0.05).

3. Results

3.1. Pharmaceuticals uptake by lettuce

During 144 h of exposure period, pharmaceuticals in lettuce-free nutrient solution remained relatively stable, indicating that photolysis and abiotic transformation of pharmaceuticals in solution could be negligible (Fig. S1). Lettuce exposure to pharmaceuticals in solution did not render adverse visible impacts to plant growth, or significant difference in biomass compared to those pharmaceutical-free controls (t-test, $p > 0.05$) (Fig. S2). The mean transpired rate measured gravimetrically was approximately 0.06 g/g lettuce (fresh weight)/hour, suggesting that the lettuce grew well during the experiment.

In the presence of lettuce, pharmaceutical concentration in nutrient solution decreased with time (left scale in Fig. 1). Caffeine, carbadox, carbamazepine, lamotrigine, monensin sodium and trimethoprim solution concentration gradually decreased to > 50% of the initial concentration during 144 h of exposure. Lincomycin, oxytetracycline and tylosin concentration decreased to a less extent, with > 80% of the applied pharmaceuticals remaining in the nutrient solution. In contrast, acetaminophen, β-estradiol, estrone and triclosan concentration decreased dramatically to < 10 ng mL^{-1} (i.e., < 20% of the initially added pharmaceuticals) during the first 24 h of exposure. At the same time, most pharmaceuticals were found to accumulate in lettuce with concentration up to 565.8 ng g^{-1} for monensin sodium in roots and up to 258.2 ng g^{-1} for carbamazepine in shoots (right scale in Fig. 1). Relatively high concentration in lettuce roots was found for lincomycin (80.5–189.3 ng g^{-1}), monensin sodium (209.5–565.8 ng g^{-1}), oxytetracycline (171.9–294.4 ng g^{-1}), trimethoprim (185.4–365.5 ng g^{-1}) and tylosin (207.0–362.6 ng g^{-1}). Caffeine, carbadox, carbamazepine and lamotrigine concentration in roots was < 136.9 ng g^{-1}. The accumulation of acetaminophen, β-estradiol and triclosan was < 10 ng g^{-1} after 48 h in lettuce roots. The averaged pharmaceutical accumulation in roots (during the 48 and 144 h of uptake experiment) ranked in the order of monensin sodium > trimethoprim ≈ tylosin > oxytetracycline > lincomycin > carbamazepine > lamotrigine > caffeine ≈ carbadox > estrone > triclosan > acetaminophen ≈ β-estradiol. Pharmaceuticals accumulated in lettuce roots could be transported upwards to shoots with water. Caffeine, carbamazepine, lamotrigine and trimethoprim concentration in shoots reached up to 258.2 ng g^{-1}, whereas acetaminophen, carbadox, lincomycin, monensin sodium and oxytetracycline were found in shoots at much lower concentration e.g., < 20 ng g^{-1}. It is noted that after 48 h of exposure, the concentration of caffeine or carbamazepine in shoots was greater than that in roots, indicating that these two pharmaceuticals could be readily transported to the upper portions of lettuce. This could be attributed to their molecular size and weak affinity to lettuce roots (discussed below). It has been demonstrated that the metabolism of caffeine occurred primarily in lettuce shoots, rather than in roots (Chuang et al., 2018), further supporting the transport from roots to shoots. Estrone, β-estradiol and triclosan were found to accumulate primarily in lettuce roots. These results are consistent with the previous hydroponic studies (Card et al., 2012; Wu et al., 2013). However, several studies reported that triclosan could enter the shoots of soybean, cabbage, ryegrass and radish from soils in pot experiments (Carter et al., 2014; Holling et al., 2012; Wu et al., 2010).

During 144-h exposure period, lettuce root and shoot biomass each increased approximately 1.5 and 1.9 times (Fig. S2), which could dilute pharmaceutical concentration in lettuce. Therefore, the accumulated mass of pharmaceuticals was used to calculate their distribution in nutrient solution, lettuce roots and shoots (Fig. 2). Among the tested pharmaceuticals, the sum of mass recoveries of nutrient solution, lettuce roots and shoots were > 60% for carbamazepine, lincomycin, monensin sodium, oxytetracycline, trimethoprim and tylosin after the 144 h of exposure (Fig. 2c, i to m). Acetaminophen, β-estradiol, estrone and triclosan could be readily metabolized with the mass recoveries of < 25% during the first 48 h of exposure (Fig. 2a, f, g and h). Many pharmaceuticals and personal care products in plants can undergo enzyme-mediated phase I reactions and/or phase II conjugations (Carter et al., 2018; Goldstein et al., 2014; Li et al., 2018; Sandermann Jr, 1994; Schroder and Collins, 2002). For example, acetaminophen and triclosan could conjugate with glucoside, glutathione and other plant components in vegetables (Bartha et al., 2014; Huber et al., 2009; Macherius et al., 2012a). Caffeine underwent phase I demethylation and oxidation reactions (Chuang et al., 2018). These results indicate the mass balance analysis provides useful information to evaluate the potential metabolism of pharmaceuticals in plant. Carbadox, lamotrigine, lincomycin, monensin sodium, oxytetracycline, trimethoprim and tylosin demonstrated more mass accumulation in roots than that in shoots (Fig. 2d, e, and i to m). For caffeine and carbamazepine, the accumulation in shoots was significantly more than that in roots after 48 h of exposure (Fig. 2b and c). The difference in pharmaceutical distribution in roots and shoots could result from multiple factors such as transport pathway of pharmaceuticals in lettuce, physicochemical properties of the pharmaceuticals, their affinity to roots and exposure time, which will be discussed below.

3.2. Root concentration factor and translocation factor

RCF calculated on the basis of fresh lettuce weight at different sampling time fell within a relatively narrow range during 48 to 144 h of exposure. Therefore, the RCF values were averaged with the standard deviations presented as error bars in Fig. 3A. For the readily metabolized pharmaceuticals acetaminophen, β-estradiol and triclosan, the RCF values were calculated using the data obtained at 12 h of exposure. The calculated RCF values follow the order of triclosan (229.2 mL g^{-1}) > estrone (64.0 mL g^{-1}) > acetaminophen (28.6 mL g^{-1}) > monensin sodium (13.1 mL g^{-1}) > trimethoprim (7.4 mL g^{-1}) > β-estradiol (6.2 mL g^{-1}) > tylosin (5.2 mL g^{-1}) > oxytetracycline (4.9 mL g^{-1}) > carbadox (4.1 mL g^{-1}) > lincomycin (2.8 mL g^{-1}) > lamotrigine (2.3 mL g^{-1})

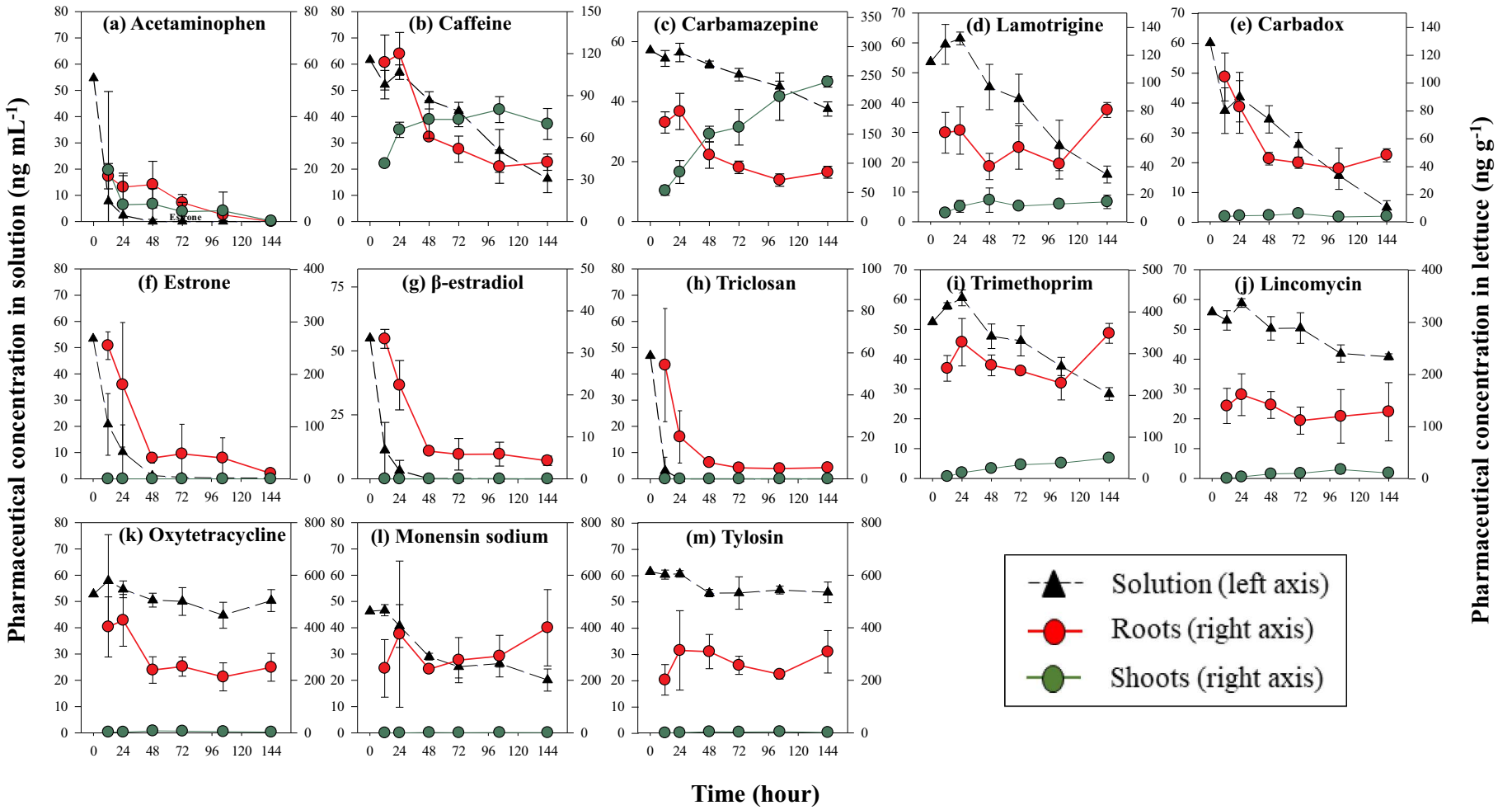

Fig. 1. Pharmaceutical concentration in solution (left y-axis), lettuce roots and shoots (right y-axis) as a function of exposure time. The thirteen pharmaceuticals from (a) to (m) are arranged in the order of increasing molecular weight.

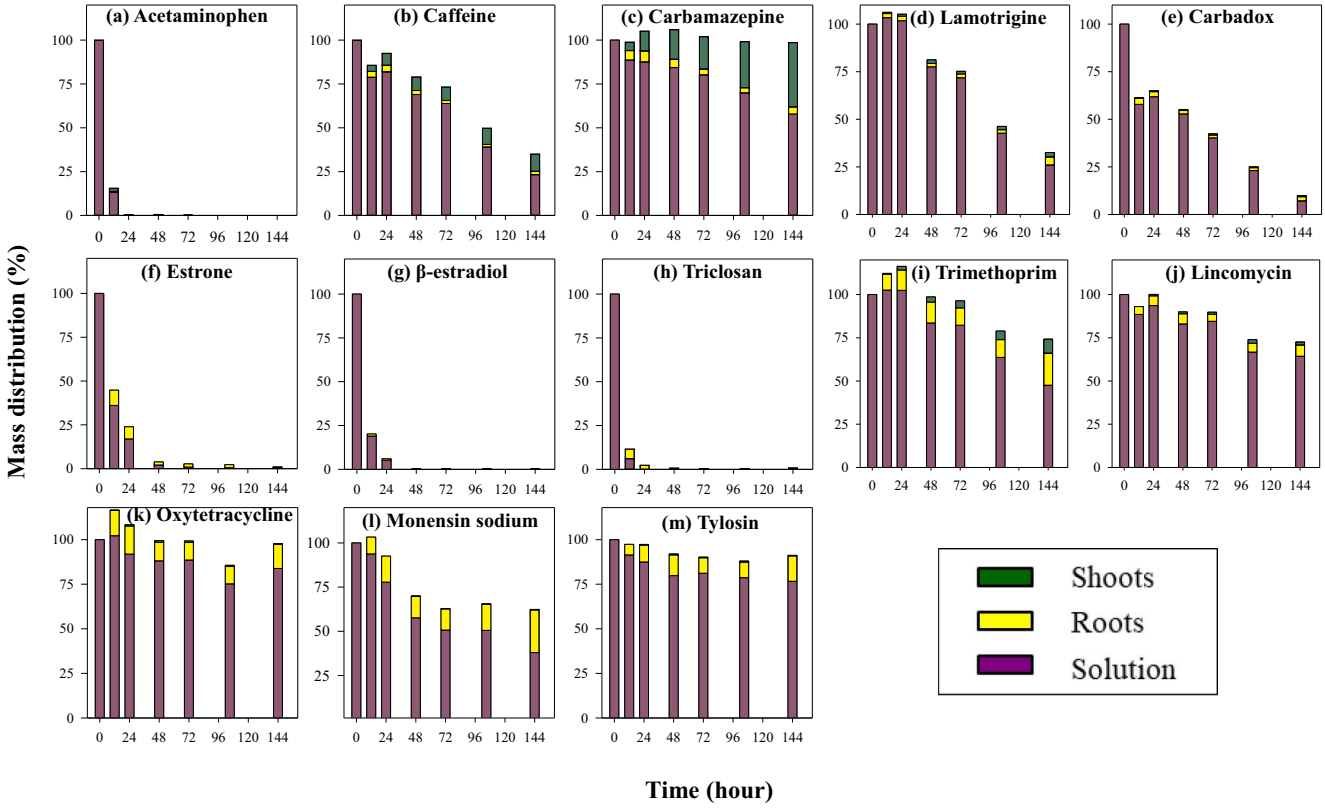

Fig. 2. Mass distribution (%) of pharmaceuticals in nutrient solution, lettuce roots and shoots as a function of exposure time. The thirteen pharmaceuticals from (a) to (m) are arranged in the order of increasing molecular weight.

> carbamazepine (2.0 mL g^{-1}) > caffeine (1.7 mL g^{-1}). Statistical analysis revealed no significant difference (one-way ANOVA, $p > 0.05$) in the RCF values at different exposure periods for carbamazepine, lincomycin, monensin sodium, oxytetracycline and tylosin, indicating that lettuce root uptake of these pharmaceuticals approached a quasi-equilibrium after 48 h. However, a significant difference (one-way ANOVA, $p < 0.05$) in the RCF values was found for caffeine, lamotrigine, carbadox and trimethoprim, and the RCF values increased with the exposure period. In this study, the thirteen pharmaceuticals exit as neutral, cationic, anionic or zwitterionic species in nutrient solution (Table 1), log D_{ow} values (nutrient solution pH 5.8) ranged from −1.61 to 4.76, and the corresponding log RCF from 0.2 to 2.4 mL g^{-1}. To evaluate the effect of pharmaceutical hydrophobicity on their accumulation in roots, the correlation between log RCF and log D_{ow} was analyzed for carbamazepine, estrone, lincomycin,

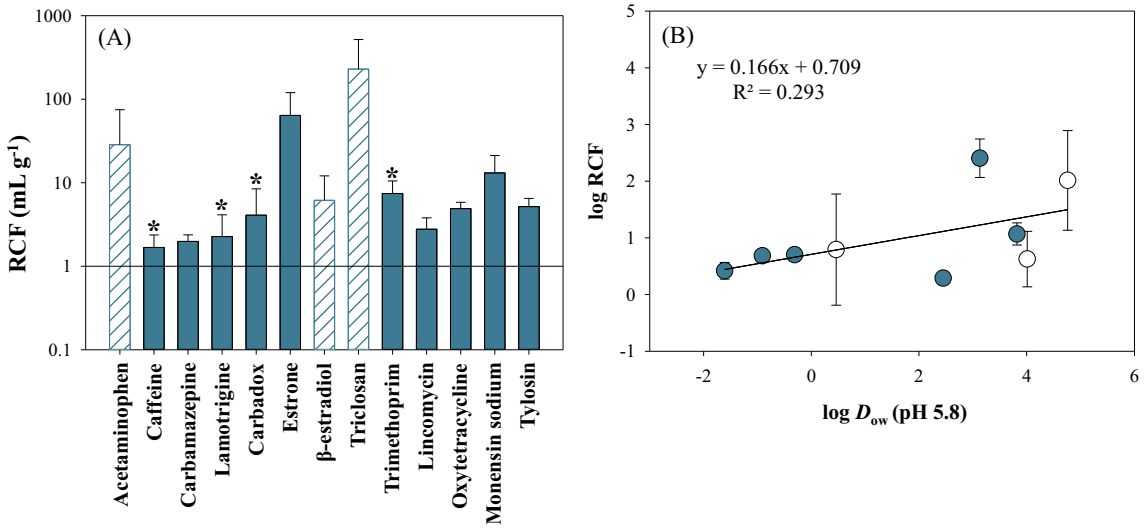

Fig. 3. (A) Root concentration factors (RCF) of the tested pharmaceuticals. Solid bar represents the average value during 48 to 144 h of pharmaceutical exposure, and the open bar represents the average values of 12 h of exposure. Asterisk * indicates the significant difference ($p < 0.05$) among the RCF values at different exposure time. (B) Relationship between log RCF and log D_{ow} at pH 5.8. Solid circles represent the average values from 48 to 144 h of exposure for carbamazepine, estrone, lincomycin, monensin sodium, oxytetracycline, and tylosin (solid circles), and open circles represent the values at the 12 h of exposure for acetaminophen, β-estradiol, and triclosan.

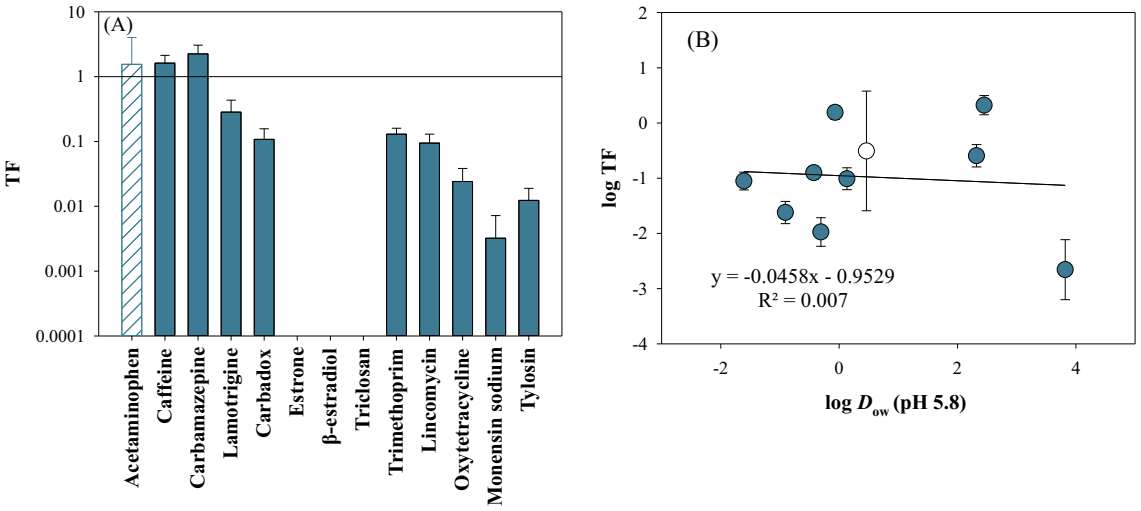

Fig. 4. (A) Translocation factors (TF) of pharmaceuticals in lettuce on fresh weight basis. Solid bar represents the average value during 48 to 144 h of pharmaceutical exposure, and the open bar represents the average values of 12 h of exposure. (B) Relationship between log TF and log D_{ow} at pH 5.8. Solid circles represent the average values from 48 to 144 h of exposure, and the open circle represents the values at 12 h of exposure.

monensin sodium, oxytetracycline, and tylosin (the average RCF values during 48 to 144 h), as well as for acetaminophen, β-estradiol, and triclosan whose RCF values were obtained at 12 h of exposure. The linear regression between log RCF and log D_{ow} demonstrated a poor relationship with $R^2 = 0.293$ (Fig. 3B). This result suggests that the uptake of pharmaceuticals by lettuce roots is different to hydrophobic compounds that are governed primarily by partitioning into plant tissues (Briggs et al., 1982; Chiou et al., 2001).

The magnitude of pharmaceutical transport from roots to shoots could be evaluated using TF values, and the averaged TF values (during 48 to 144 h of uptake) are presented in Fig. 4A. The TF value for acetaminophen was calculated during 12 h of exposure. The TF values could not be obtained for β-estradiol, estrone and triclosan due to their substantial metabolism in lettuce and the minimal amount detected in shoots. The TF values for caffeine and carbamazepine were 1.6 and 2.2, indicating these two pharmaceuticals were readily transported to shoots. As a result, the accumulation of caffeine and carbamazepine in shoots was greater than that in roots. For other pharmaceuticals, the TF values ranked as lamotrigine (0.3) > carbadox (0.1) ≈ trimethoprim (0.1) ≈ lincomycin (9.5×10^{-2}) > oxytetracycline (2.4×10^{-2}) > tylosin (1.2×10^{-2}) > monensin sodium (3.2×10^{-3}), which were all below 1.0. Lettuce roots could be considered as the major domain for accumulation of these pharmaceuticals. Although no apparent relationship was observed between log TF and log D_{ow} (Fig. 4B), the TF value was found to generally decrease with increasing molecular weight (MW) (Fig. 4A and Table 1). Among the tested pharmaceuticals, the MW of acetaminophen, caffeine, and carbamazepine was < 240 g mol^{-1}, and their TF values were > 1.0. The TFs ranged from 0.1 to 0.3 for carbadox, lamotrigine and trimethoprim, and their MW are between 250 and 300 g mol^{-1}. For those pharmaceuticals with MW > 400 g mol^{-1}, their corresponding TF values were < 0.1. These findings suggested that pharmaceutical MW, more specifically molecular size, could notably influence their transport from lettuce roots to shoots.

3.3. Pharmaceutical sorption to lettuce roots

Sorption by dry plant tissues has been used to evaluate the uptake and accumulation of organic chemicals in living plants. Strong sorption by plant roots could diminish chemical transport to plant shoots, and root lipid content plays an important role in the retention of neutral hydrophobic compounds (Li et al., 2005). The similar method could potentially provide clues to better elucidate accumulation and transport of pharmaceuticals in plant because the chemical compositions in dry and living fresh plant tissues are similar. However, some plant cell structures e.g., membranes could be disrupted during the freeze-dried process, which impacts the diffusion of pharmaceuticals into cells.

Sorption isotherm of pharmaceuticals by lettuce roots was fit well with the linear sorption model, from which sorption coefficient (K_p) was estimated from the slope of sorption isotherm. Pharmaceutical sorption could be apparently grouped into three categories: weak sorption (caffeine, carbadox, carbamazepine and lincomycin) with K_p < 0.05 L g^{-1}, intermediate sorption (lamotrigine, monensin sodium, oxytetracycline, trimethoprim and tylosin) with K_p between 0.38 and 2.22 L g^{-1}, and strong sorption (acetaminophen, β-estradiol, estrone and triclosan) with K_p > 12.0 L g^{-1} (Fig. S3). The root-sorbed concentration was calculated by the difference between the initial and equilibration aqueous concentration. The strong sorption of acetaminophen, β-estradiol, estrone and triclosan could be plausibly attributed to conjugated reactions. Plant tissues contain many polyphenolic components (e.g., quercetin in lettuce) (Llorach et al., 2008), which easily transform to quinone. Quinone could further react with the chemicals containing –NH_2 or –OH functional groups by forming covalent bindings (Taiz et al., 2015). These four pharmaceuticals all contain –NH_2 or –OH functional groups, and are potentially coupled to plant tissues (i.e., polyphenols), as evidenced by the fact that they all demonstrated much low mass recovery in the uptake experiment (Fig. 2).

3.4. Influence of pharmaceutical characteristics on uptake and transport

Molecular volume of pharmaceuticals used in this study demonstrates a strong positive linear relationship with their MW ($R^2 = 0.97$) (Fig. S4). Therefore, MW is clearly an appropriate index to the molecular size. At pH 5.8, caffeine (MW = 194 g mol^{-1}, 100% neutral), carbamazepine (MW = 236 g mol^{-1}, 100% neutral), lamotrigine (MW = 256 g mol^{-1}, 44% cationic + 56% neutral) and trimethoprim (MW = 290 g mol^{-1}, 95% cationic + 5% neutral) apparently entered lettuce roots, and were transported to shoots (Fig. 2b, c, d and i). Carbadox (MW = 262 g mol^{-1}, 100% neutral) was considerably metabolized in lettuce; at the 144 h only 7.2% of the initially applied amount remained in nutrient solution, and 2.2% in roots and 0.6% in shoots (Fig. 2e). Lincomycin (MW = 406 g mol^{-1}, 98% cationic + 2% neutral), monensin sodium (MW = 693 g mol^{-1}, 2% neutral + 98% anionic) and tylosin (MW = 916 g mol^{-1}, 99% cationic + 1% neutral) showed minimal accumulation in lettuce shoots (< 10 ng g^{-1}). Oxytetracycline (MW = 460 g mol^{-1}, 3% cationic + 97% zwitterionic) has a moderate molecular size and existed predominantly as zwitterionic

species. These results, along with pharmaceutical accumulation in lettuce, reveal that pharmaceuticals with MW < 300 g mol^{-1} have smaller molecular size, and could move up to lettuce shoots, while the large-sized pharmaceuticals (e.g. MW > 400 g mol^{-1}) accumulated primarily in roots. The charged speciation could also affect the uptake of pharmaceuticals (to be discussed below).

4. Discussion

This study revealed that many pharmaceuticals could enter lettuce and distribute in lettuce roots and shoots to varying degrees. The amount of pharmaceutical transport from roots to shoots decreased with increasing MW or molecular size. Poor relationship between log RCF-log D_{ow} or log TF-log D_{ow} indicates that the accumulation and transport of most pharmaceuticals in lettuce is not governed by their lipophilic characteristics, instead by water movement because of the relatively high water solubility of pharmaceuticals (Table 1). In general, pharmaceuticals enter lettuce via root uptake and transport to shoots with transpiration flow. During these processes, the molecular size of pharmaceuticals could determine their diffusion rate through root cell membranes, and pharmaceutical affinity to lettuce roots could influence their transport to shoots.

The diffusion of pharmaceuticals across cell membranes, especially the cells at endodermis which are impregnated in Casparian strip, is the key process influencing their long-distance transport and distribution in lettuce. In general, water and small-sized organic molecules could readily diffuse through plant cell membranes. Water molecules can enter cells by osmosis or through aquaporin (Chaumont and Tyerman, 2014; Tornroth-Horsefield et al., 2006), while organic compounds (with net neutral charge and MW < 450 g mol^{-1}) could enter the cells by passive diffusion across lipid bilayer membranes (Kumar and Gupta, 2016; Trapp and Farlane, 1995). Ionic pharmaceutical species might enter cells via integral proteins on cell membranes, which is driven primarily by concentration gradient (Di et al., 2012; Dobson and Kell, 2008; Raven et al., 1999; Sugano et al., 2010; Taiz et al., 2015). However, large-sized molecules experience a slow diffusion rate through plant cell membranes hence retarding their accumulation in shoots (Di et al., 2012; Dobson and Kell, 2008; Kumar and Gupta, 2016; Trapp and Farlane, 1995).

The results of pharmaceutical uptake experiments suggest that small-sized pharmaceuticals (MW < 300 g mol^{-1}) such as caffeine, carbamazepine, lamotrigine, and trimethoprim could readily enter lettuce roots and move upwards to shoots. It has been documented that ionic pharmaceuticals might be restricted from passing through plant cell walls or membranes (Goldstein et al., 2014; Wu et al., 2015). In this study, trimethoprim existed primarily in cationic form in solution, and a certain amount of trimethoprim still passed through root cell membranes and transported to shoots. The substantial metabolism of carbadox (Fig. 2) renders unfeasible to evaluate its accumulation and transport in lettuce. The relatively large-sized molecules (MW > 400 g mol^{-1}) lincomycin, monensin sodium, and tylosin might enter lettuce roots possibly through apoplast pathway, and be retarded to enter plant cells as evidenced by their limited upward movement to shoots (Camenisch et al., 1998; Kumar and Gupta, 2016). However, some large-sized pharmaceuticals could still enter plant leaves after a relatively long time of exposure (~5 to 7 weeks) (Li et al., 2019). The zwitterionic oxytetracycline might pass through cell membranes via passive diffusion but at a relatively slow rate (Boonsaner and Hawker, 2012; Borghi and Palma, 2014). The relatively high affinity of oxytetracycline to lettuce roots could contribute to the reduced transport to lettuce shoots (Fig. 1k and 2k).

Once they enter lettuce roots, pharmaceuticals interact with root constituents. The strong affinity to roots retards pharmaceutical transport to shoots, and leads to a great accumulation in roots. Caffeine and carbamazepine were in neutral species, weakly sorbed by lettuce roots, and MW < 300 g mol^{-1} (Fig. S3 and Table 1). As expected, they could easily enter xylem and transport to shoots with minimal accumulation in roots i.e. TF > 1 (Fig. 4). Similar results were found for these two pharmaceuticals in carrots and sweet potatoes with concentration in leaves greater than that in roots (Malchi et al., 2014). Lamotrigine and trimethoprim exhibited moderate affinity to lettuce roots (Fig. S3). Although their MW are < 300 g mol^{-1}, the moderate sorption by lettuce roots could result in more accumulation of lamotrigine and trimethoprim than those in shoots (Figs. 1d, i, 2d and i). Lincomycin existed predominantly as cation (> 98%) in nutrient solution. The relatively large molecular size and positively charged species might restrict a large amount of lincomycin into plant cells from the symplast pathway. As a result, lincomycin accumulated mainly in lettuce roots, and a small amount transported to shoots (Figs. 1j, 2j and 3). To further elucidate the role of Casparian strip in the inhibition of pharmaceutical movement in lettuce, the uptake and transport of lincomycin were compared between lettuce shoots only and whole lettuce plants. The results reveled that lincomycin could be taken up and reach to 37.5 ng g^{-1} in lettuce shoots (without roots), whereas minimal amount of lincomycin was detected in the shoots of whole lettuce plants. These results indicate that cationic lincomycin could enter lettuce roots predominantly via apoplast pathway, and the Casparian strip functions as a barrier to inhibit or retard the diffusion to xylem for the upward movement. This explanation could be also applied to other large-sized pharmaceuticals e.g., monensin sodium and tylosin; the large-sized molecules limit their diffusion to xylem resulting in the dominant accumulation in roots. In addition, the stronger affinity of oxytetracycline, monensin sodium, and tylosin to roots could contribute to the greater accumulation in roots compared to lincomycin (Figs. 2j to 2m and S3).

Based on the elucidation of the influence of pharmaceutical affinity to lettuce roots and molecular size on diffusion and movement of pharmaceuticals in lettuce, we propose a scheme to describe pharmaceutical uptake and accumulation in plants (Fig. 5). Small-sized pharmaceuticals e.g., MW < 300 g mol^{-1} readily enter lettuce roots with water flow via symplast pathway in which neutral pharmaceuticals might enter cells by passive diffusion, and ionic species via integral transport proteins into root cells. Water flow is the carrier to distribute pharmaceuticals in roots and shoots. The small-sized pharmaceuticals carried by water flow in symplast pathway could pass through the Casparian strip to xylem, followed by the upward transport to shoots. Pharmaceutical sorption by lettuce roots reduces the amount and rate of pharmaceutical transport to shoots. These processes collectively influence the accumulation of small-sized pharmaceuticals such as caffeine, carbamazepine, trimethoprim and lamotrigine in lettuce roots and shoots. The TF values of trimethoprim (0.1) and lamotrigine (0.3) were less than that of caffeine (1.6) and carbamazepine (2.2) due to their stronger affinity to lettuce roots. In addition, cationic trimethoprim and lamotrigine in root cell vacuole (pH 5.5) might develop electrostatic interaction with tonoplast membranes (Goldstein et al., 2014; Taiz et al., 2015), limiting their transport from roots to shoots. Relatively large-sized pharmaceuticals, e.g. MW > 400 g mol^{-1} for lincomycin, oxytetracycline, monensin sodium and tylosin might enter lettuce roots mainly via apoplast pathway, and their diffusion might be limited to lettuce xylem and the subsequent transport to shoots. Recently, Lamshoeft et al. (2018) found that the transport to vegetable upper parts decreased with increasing MW, the transpiration stream concentration factor decreased to < 0.1 when MW was > 394 g mol^{-1}.

To validate the proposed conceptual scheme (Fig. 5), we collected the literature results from eighteen studies which included the total of 366 TF values for 55 pharmaceuticals and personal care products in 20 types of plants (Table S3). The results showed that for small-sized pharmaceuticals defined as MW < 300 g mol^{-1}, 141 out of 321 TF values were > 1 accounting for > 44% of the literature data, 222 TF values > 0.1 accounting for > 69% of the literature data. The TF values within the range of 0.1 and 1 could be due possibly to the intermediate/strong sorption by plant roots and/or ionic speciation. The present

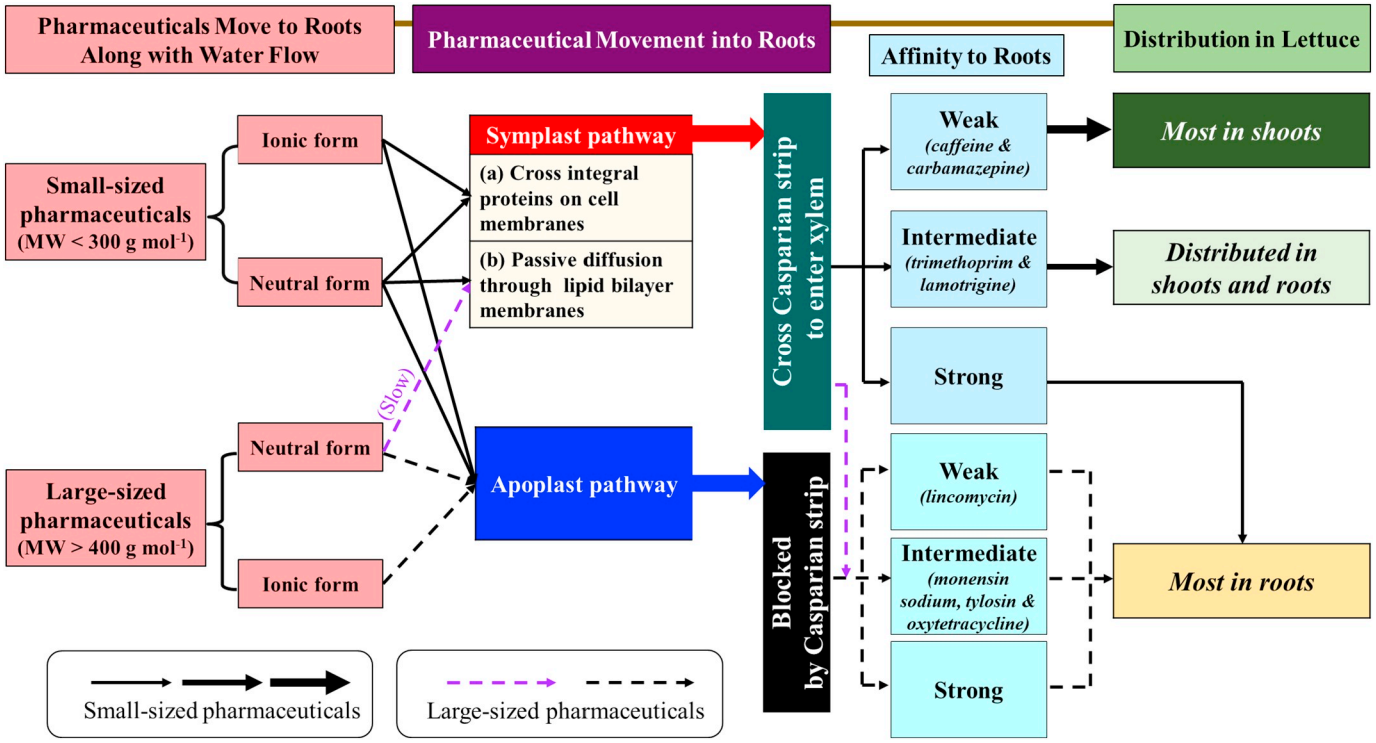

Fig. 5. Scheme of pharmaceutical movement into lettuce roots, affinity to roots, and distribution in lettuce.

study showed lettuce roots had intermediate sorption for lamotrigine and trimethoprim (MW < 300 g mol^{-1}) and cationic species in solution, and their TF values were between 0.1 and 1.0. Unfortunately, most reported studies did not measure pharmaceutical sorption by roots, which limits the evaluation on their transport. For the pharmaceuticals with MW between 300 and 400 g mol^{-1}, 7 out of 22 TF values are > 1, indicating that some pharmaceuticals with MW between 300 and 400 g mol^{-1} could move up to shoots. As for the large-sized pharmaceuticals (MW > 400 g mol^{-1}), the TF value was < 1.0 for 21 chemicals out of 23. The study with 2 TF values > 1 could be due to the extremely long experiment period (~100 days) during which pharmaceuticals might have sufficient time to passively diffuse through cell membranes (Malchi et al., 2014). Overall, the proposed scheme could reasonably elucidate 45% of reported studies involving small and large-sized pharmaceuticals. In addition to pharmaceutical physicochemical characteristics (MW and speciation) and sorption by roots, pharmaceutical uptake and transport in plant could also be affected by enzyme-mediated metabolism in plants, microbial degradation (e.g., by endophytes), plant species, and bioavailability of pharmaceuticals in soil and water. Therefore, further evaluation via intergrating these physical, chemical and biological processes is needed for better understanding of uptake, accumulation and transport of pharmaceuticals in plants.

5. Conclusions

This study evaluates the role of pharmaceutical molecular size, speciation and affinity to lettuce roots in their uptake and transport in lettuce. Small-sized pharmaceuticals with MW < 300 g mol^{-1} manifested substantial transport from roots to shoots, and this transport decreased with increasing sorption by plant roots and/or ionic fractions. Large-sized pharmaceuticals MW > 400 g mol^{-1} predominantly accumulated in lettuce roots. No apparent relationship between log RCF-log D_{ow} or log TF-log D_{ow} indicates that uptake and distribution of most pharmaceuticals in lettuce is not governed by the lipophilic characteristics of pharmaceuticals, but plausibly by water mass flow which carries highly soluble pharmaceuticals to plants. Casparian strip in plant roots could function as a barrier to inhibit the diffusion of pharmaceutical into xylem and retard the subsequent upward movement. These findings help develop a scheme to elucidate uptake and transport of pharmaceuticals in plants from water.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.envint.2019.104976.

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