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Effect of Transpiration on Plant Accumulation and Translocation of PPCP/EDCs

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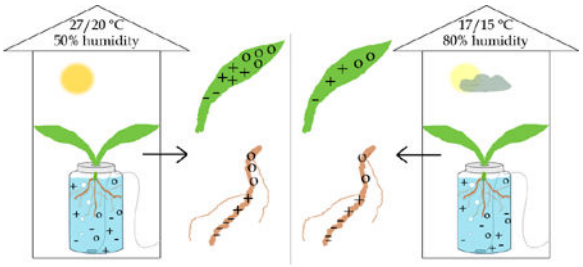
Abstract

The reuse of treated wastewater for agricultural irrigation in arid and hot climates where plant transpiration is high may affect plant accumulation of pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs). In this study, carrot, lettuce, and tomato plants were grown in solution containing 16 PPCP/EDCs in either a cool-humid or a warm-dry environment. Leaf bioconcentration factors (BCF) were positively correlated with transpiration for chemical groups of different ionized states ($p < 0.05$). However, root BCFs were correlated with transpiration only for neutral PPCP/EDCs ($p < 0.05$). Neutral and cationic PPCP/EDCs showed similar accumulation, while anionic PPCP/EDCs had significantly higher accumulation in roots and significantly lower accumulation in leaves ($p < 0.05$). Results show that plant transpiration may play a significant role in the uptake and translocation of PPCP/EDCs, which may have a pronounced effect in arid and hot climates where irrigation with treated wastewater is common.



Anionic (-), cationic (+), and neutral (o) PPCP/EDCs

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Supporting Information

Supporting information contains Tables S1–S6 and Figures S1–S2.

Keywords

water reuse; PPCPs; EDCs; plant uptake; transpiration

Introduction

Population growth, urbanization, and climate change have created unprecedented stress on water resources. The reuse of treated wastewater from wastewater treatment plants has been increasing by 15% per year to meet regional water needs (Miller, 2006). For example, in 2006, about 3.6×10^9 cubic meters of treated wastewater were reused in the U.S. for purposes including agricultural and landscape irrigation (Miller, 2006). However, numerous studies have shown that a wide range of trace organic contaminants are present in treated wastewater, including pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) (Anderson et al., 2010; Kinney et al., 2006; Suárez et al., 2008; Xia et al., 2005).

Pharmaceuticals are consumed worldwide for various human therapeutic and animal husbandry purposes. For example, 2×10^6 kg of acetaminophen and 7.7×10^5 kg of aspirin are used every year in the United Kingdom (Smith and Riddell-Black, 2007). Thousands of personal care products, including sunscreen and soap, are also used (Ternes et al., 2004). Some of these compounds have been shown to affect cell functioning and reproductive behavior in aquatic species at environmentally relevant levels (Daughton and Ternes, 1999; Luckenbach and Epel, 2005). In addition, PPCP/EDCs may have intentional or incidental hormonal properties that can disrupt endocrine functioning in non-target organisms (Lee et al., 2009).

The beneficial reuse of treated wastewater for agricultural irrigation introduces PPCP/EDCs into the soil environment, where they may be taken up by plants and cause human exposure by ingestion (Calderón-Preciado et al., 2011; Dodgen et al., 2013; Holling et al., 2012; Wu et al., 2010). While a number of studies have examined the uptake potential of PPCP/EDCs, most studies only considered a few compounds, making it difficult to discern the underlying mechanisms. On the other hand, plant uptake has been extensively investigated for many pesticides (Briggs et al., 1982; Oorschot, 1970; Sterling, 1994; Zhang et al., 2009). Studies show that systemic pesticides are passively taken up through the transpiration stream (Ryan et al., 1988), and greater transpiration leads to increased accumulation of non-ionic compounds (Collins et al., 2005). However, many PPCP/EDCs are ionizable compounds that exist partially as ions at environmentally relevant pH (Babić et al., 2007). The ionic state of a compound greatly affects the compound's interactions with plants, such as adsorption on root surfaces, interaction with the cell membrane, and sequestration into plant compartments (Trapp, 2009). In a recent study, Wu et al. (2013) examined multiple PPCP/EDCs and observed a strong correlation between plant bioconcentration of a compound and its pH-adjusted octanol-water partition coefficient (D_{ow}), but did not consider transpiration effects. Herklotz et al. (2010) and Shenker et al. (2011) suggested that movement through transpiration-driven mass flow of water was likely an important route for the uptake of carbamazepine. Carter et al. (2014) suggested that transpiration likely contributed to the

difference in uptake of carbamazepine, diclofenac, fluoxetine, and propranolol by radish and ryegrass. However, to date researchers have yet to quantitatively evaluate the dependence of plant accumulation of PPCP/EDCs on transpiration and the role of ionization in transpiration-facilitated uptake.

In this study, we measured plant accumulation and translocation of 16 PPCP/EDCs, including neutral and ionizable compounds, in 3 plant species grown hydroponically in two distinct environments. Losses of nutrient solution through transpiration were monitored throughout the 21 d incubation and the levels of PPCP/EDCs in plant tissues were measured at the end of cultivation. The effect of transpiration on bioconcentration or translocation was statistically evaluated for anionic, cationic, and neutral PPCP/EDCs. Knowledge of the interplay between transpiration and plant uptake is useful for identifying types of PPCP/EDCs, as well as climate conditions, that may enhance plant accumulation of PPCP/EDCs.

Materials and Methods

Chemicals

A total of 16 PPCP/EDCs with different physicochemical properties were considered in this study (Table 1). Surrogates were used to assess recovery and quantitatively analyze all PPCP/EDCs. Standards of caffeine, carbamazepine, diazepam, diuron, gemfibrozil, meprobamate, perfluorooctanoic acid, and trimethoprim were purchased from Sigma-Aldrich (St. Louis, MO). Standard of primidone was from Spectrum Chemical (Gardena, CA). Standard of sulfamethoxazole was from MP Biomedicals (Solon, OH). Standards of diclofenac and dilantin were from TCI America (Portland, OR). Standards of ibuprofen and naproxen were from Alfa Aesar (Ward Hill, MA). Standards of (3S,5S)-atorvastatin sodium salt, clofibric acid, clofibric-d_4 acid, and perfluorooctane sulfonate were from Santa Cruz Biotechnology (Santa Cruz, CA). Standard of diazepam-d_5 was from Cerilliant (Round Rock, TX). All other deuterated standards were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). The solvents used in this study were from Fisher (Fair Lawn, NJ) or VWR (Visalia, CA). Ultrapure water was produced using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA). Individual stock solutions of each compound were prepared in methanol and stored in an amber glass vial at −20 °C.

Plant Species and Growth Chamber Conditions

Three plant species were included in this evaluation. ‘Champion II’ tomato seedlings were purchased from Armstrong Growers (Glendora, CA) and ‘Nevada’ lettuce seedlings were purchased from Do-Right’s Plant Growers (Santa Paula, CA) at 3 weeks post-seeding through a local nursery. ‘Danvers 126’ carrot was started from seed in commercial potting soil (Master Nursery, Suisun, CA) and seedlings were used at 26 d post-seeding.

Two growth chambers (CMP 3244, Conviron, Temecula, CA) were used in this study. One chamber was programmed to simulate a cool and humid environment with a day time temperature of 17 °C, followed by a night time temperature of 15 °C, while the relative air humidity was kept at 80%. The other growth chamber was programmed to simulate a warm and dry environment with a day time temperature of 27 °C, a night time temperature of 20

°C, with relative humidity at 50%. The cool-humid and warm-dry environments were used to induce distinctively different plant transpiration patterns. Both chambers received irradiation from a mix of incandescent and fluorescent bulbs, which gradually ramped over 7 h each day to a maximum light intensity of 300 μmol/m^2s of photosynthetic active radiation that was maintained for 2 h before decreasing to darkness for a total daily photoperiod of 16 h.

Hydroponic Plant Cultivation

Hydroponic nutrient solution was made using chemicals and concentrations as in Seyfferth et al (2008). Nutrients were supplied at the following concentrations (in mM): NO_3^-, 4900; Ca, 1900; K, 1080; Mg, 500; S, 500; Cl, 191; Si, 187; NH_4^+, 100; P, 80; Fe, 20; B, 10; Zn, 8; Cu, 2; Mn, 0.6; Mo, 0.1; and Ni, 0.1. Nutrients and pH were buffered using HEDTA, HCl, NaOH, and 2-(*N*-morpholino)ethanesulfonic acid (MES). Initial solution pH averaged 5.3. Glass jars with 2 L capacity and screw-top lids were used for plant cultivation. Before use, containers were washed with soap and water, rinsed with methanol, and rinsed again with methyl *tert*-butyl ether (MTBE). Each lid had a 1.9 cm hole drilled in the middle and was fitted with a modified Horticube foam collar (Smithers-Oasis, Kent, OH) to hold the plant suspended in the nutrient solution. During cultivation, each jar was fitted with an opaque plastic cover to block light exposure to the solution.

Six days before the start of the incubation, plants were carefully removed from their growth media, rinsed with DI water, inserted through jar lids, fitted with the foam collars, and placed in 2 L glass jars filled with fresh nutrient solution, at one plant per jar. After the plants were transferred to the growth chambers, jars were attached to a small pump system to aerate the solution with ambient air. After 3 d, plants were transferred into clean jars of fresh nutrient solution to replenish nutrients and minimize microbial growth. After a total of 6 d of acclimation, 4 replicates of each plant species in each chamber were randomly selected and transferred into clean jars with 1900 mL of fresh nutrient solution that was amended with 5 mL of a working solution of PPCP/EDCs prepared in ultrapure water. The nominal concentration was 1 μg/L for each compound in the nutrient solution, a level at the higher end of concentration ranges found in treated wastewater effluents (Anderson et al., 2010). The actual chemical concentration of each compound was measured with solid-phase extraction, as described below.

Plants were grown in fortified solution for 21 d in the growth chambers. Every 1 to 3 d, based on the amount of solution transpired, all plants were transferred to clean jars containing fresh solution fortified with PPCP/EDCs. At each solution exchange, the masses of used and fresh solutions from each container were gravimetrically measured to determine the exact amount of solution transpired by each plant. The total transpired mass was defined as the cumulative mass of nutrient solution removed from a jar throughout the 21 d treatment. Evaporation from jars was negligible due to use of fitted lids. The pH in the nutrient solution was measured at each exchange, with pH paper that covered the range pH 4 to 7, which was later used to calculate the average log D_{ow} of each compound (Wu et al., 2013). At 21 d, all plants were removed from their treatment jars, rinsed with DI water, and

separated into different parts. Plant tissues were weighed, placed in self-sealing plastic bags, and then stored at −70 °C before analysis.

Nutrient Solution Extraction

To characterize the depletion of PPCP/EDCs in the nutrient solutions between solution exchanges, solution samples were analyzed for levels of PPCP/EDCs on day 8 and 10. On day 8, freshly prepared nutrient solutions were analyzed for the initial chemical concentrations of PPCP/EDCs. To determine the masses of PPCP/EDCs remaining in the solution after 2 d of plant growth, the used nutrient solution from each plant container on day 10 was analyzed. To assess the removal of PPCP/EDCs not attributable to the presence of plants, triplicate jars of fortified nutrient solution without plants were included in each growth chamber from 8 – 10 d and then similarly analyzed.

Prior to analysis, nutrient solution from each container was weighed and mixed by shaking, from which a 275 mL subsample was removed. The solution sample was extracted according to a previously published method (Vanderford and Snyder, 2006). Briefly, 100 μL of surrogate solution (200 μg/L for compounds analyzed in positive mode and 400 μg/L for compounds in negative mode) was added to each sample. A Supelco Visiprep DL solid phase extraction (SPE) manifold with disposable liners (Sigma-Aldrich, St. Louis, MO) and HLB cartridges (150 mg, 6 cc, Waters, Milford, MA) were used for extraction. Cartridges were sequentially conditioned with 5 mL each of MTBE, methanol, and water, and samples were loaded at 5 mL/min under vacuum. Sample vessels were rinsed with 200 mL of ultrapure water, and the rinsate was also passed through the cartridge. Sample cartridges were dried with nitrogen gas and then eluted with 5 mL each of 90/10 MTBE/methanol and methanol. The eluent was evaporated under a gentle stream of nitrogen at 40 °C to a volume of 400 μL and then transferred to a 2 mL glass vial. The condensing vessel was rinsed twice with 300 μL of methanol and the rinsate was added to the sample vial to make the final volume to be 1.0 mL for analysis.

Plant Tissue Extraction and Clean-Up

The extraction of plant tissue samples followed a previously published method (Wu et al., 2012). In brief, plant samples were removed from the freezer and immediately placed in a freeze-drier (Labconco, Kansas City, MO). Samples were dried for 16 h, or to dryness, and then weighed. Each plant sample was then finely ground in a stainless steel coffee grinder. The grinder was cleaned between samples using soap, water, and acetone. A 0.20 g aliquot was placed in a 50 mL polypropylene centrifuge tube and spiked with 100 μL surrogate solution. Samples were sequentially extracted with 20 mL MTBE, and then 20 mL acetonitrile, by sonication in a Fisher Scientific FS110H ultrasonic water bath for 20 min followed by centrifugation at 3000 rpm. The supernatant from each extraction step was combined in a 60 mL glass tube and evaporated at 40 °C under a gentle flow of nitrogen to a volume of 0.5 mL. The residue was re-dissolved in methanol (1 mL) and then mixed in 55 mL ultrapure water. The HLB (150 mg, 6 cc, Waters, Milford, MA) SPE cartridges were conditioned with 5 mL methanol and then 5 mL water. Samples were passed through cartridges at 5 mL/min under vacuum, and then sample tubes were rinsed with 30 mL of ultrapure water, which was also passed through the cartridge. Sample cartridges were dried

with nitrogen gas and then eluted with 7 mL methanol. The eluent was evaporated under a gentle stream of nitrogen at 40 °C to a volume of 200 μL and then transferred to a 2 mL glass vial. The condensing vessel was rinsed twice with 150 μL of methanol and the rinsate was added to the sample in the vial to create a final volume of 0.5 mL.

Chromatographic Separation and Analysis

The final sample extracts from the solution and plant tissue samples were injected into an ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) equipped with an ACQUITY BEH C18 column (2.1 mm × 100 mm, 1.7 urn particle size, Waters) at 40 °C. Mobile phase A was 95/5 water/methanol with 0.001% formic acid and mobile phase B was methanol. The following mobile phase program, run at 0.2 mL/min flow rate, was used: 0 – 0.5 mm, 5 – 50% B; 0.5 – 12 mm, 50 – 100% B; 12 – 13 mm, 100% B; 13 – 16 mm, 5% B. Analysis was performed with a Waters Micromass triple quadrupole detector (MS/MS) equipped with an electrospray ionization (ESI) source in the positive or negative mode. Parameters of MS/MS were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 3.0 kV; cone voltage, 20 V; desolvation gas flow, 600 L/h; cone gas flow, 50 L/h. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode. All data were processed using MassLynx 4.1 software (Waters, Milford, MA).

Calculation of Predicted Plant Concentrations

The extent of PPCP/EDC accumulation into leaf tissues was predicted using the assumptions that accumulation would occur in a passive manner through the transpiration stream and that no loss would occur in the system via processes such as metabolism or foliar release. Predicted concentrations (PC) for each compound were calculated as below:

$$PC = \frac{transpired\ \ solution\ (kg) * \frac{\left(initial\ \ solution\ \ concentration\left(\frac{\mu g}{kg}\right) + final\ \ solution\ \ concentration\ \left(\frac{\mu g}{kg}\right)\right)}{2}}{dry - weight\ \ leaf\ \ mass\ \ (kg)} \quad (1)$$

where the initial solution concentration is the concentration of each compound in the fresh solution and the final solution concentration is the measured concentration in the used solution from day 10.

QA/QC and Data Analysis

All plant treatments were created in quadruplicate and solution treatments were created in triplicate. Containers with plants grown in non-spiked nutrient solution were included as blank controls. Laboratory blanks were included with each sample extraction and pure methanol was analyzed in each UPLC/MS/MS run to check potential contamination. Surrogates were used in all sample analyses to account for losses during extraction and matrix effects during instrumental analysis. Recovery of the surrogates was used to calculate the actual concentration of each target analyte. Recoveries of surrogates in plant tissue and nutrient solution samples are listed in Table S1 of the Supporting Information. Statistical analysis of data including ANOVA with Tukey's Honestly Significant Difference, linear regression, and t-test was performed using R (R Development Core Team, 2008). Normality was investigated with Q–Q plots and significance was assigned at $p \leq 0.05$.

Results and Discussion

Transpiration and PPCP/EDC Dissipation in Nutrient Solution

Carrot, lettuce, and tomato plants grown in both environments were found to be generally healthy, with the exception of one tomato plant from the cool-humid treatment which had yellow, stunted leaves and was excluded from analysis. No significant difference in biomass was detected between treatment and control plants. For the same plant species, the weight of a plant part was not significantly different in the warm-dry environment compared to the cool-humid environment ($p < 0.05$). The nutrient solution was found to average pH 5.2 for carrot, pH 5.3 for lettuce, and pH 6.0 for tomato during the study, which was used to calculate the neutral fraction and the pH-adjusted octanol-water partition coefficient (log D_{ow}) for the different PPCP/EDCs, as described in Wu et al. (2013) (Table 1). Based on the primary ionic state in the nutrient solution, the selected PPCP/EDCs were placed into anionic, cationic, and neutral chemical groups (Table 1).

The transpired mass for each plant was measured at every solution exchange and the mean daily transpiration rates were estimated (Figure S1). The mean transpired masses in the cool-humid and warm-dry treatments during the 21 d of growth were, respectively, 65.50 ± 19.36 and 194.33 ± 30.72 g/d for lettuce, 127.04 ± 15.52 and 503.38 ± 59.76 g/d for tomato, and 16.82 ± 8.05 and 55.31 ± 26.41 g/d for carrots. Therefore, for the same plant type, the warm-dry environment induced a 3-4-fold increase in plant transpiration as compared to the cool-humid environment.

The dissipation of PPCP/EDCs from nutrient solution during the hydroponic growth of plants may be attributed to plant uptake and microbial degradation in the rhizosphere (Chaudhry et al., 2005). In the spiked nutrient solutions without plants, most PPCP/EDCs showed limited dissipation from the solution after a 2 d incubation (≤ 15%), suggesting that these compounds were mostly stable in the nutrient solution (Table S2). The only exception was atorvastatin, where 49.0% and 61.7% were not recovered from the solution from the cool-humid and warm-dry treatments, respectively (Table S2). In the presence of plants, levels of PPCP/EDCs in the solution significantly decreased compared to the plant-free control. For example, after exposure to a tomato plant, 38.8% of the initially spiked diclofenac was not recovered from the solution for the cool-humid treatment and 75.6% was not recovered for the warm-dry treatment, while there was essentially no chemical loss in the plant-free control (Table 2).

The transpired mass over the 2 d interval between day 8 and day 10 was compared to the measured removal of the anionic, cationic, or neutral PPCP/EDCs over the same period to assess the effect of plant transpiration on the removal of PPCP/EDCs from the nutrient solution. A significantly positive ($p < 0.0001$, $r^2 = 0.244 – 0.488$) relationship was found for each group of compounds (Figure 1), across the different plant species and environments. The separation of PPCP/EDCs by ionic state in the regression analysis decreased the model residuals for both the cationic and neutral groups, as compared to a linear regression with all compounds grouped together ($r^2 = 0.257$), showing that consideration of ionic state better describes the removal of PPCP/EDC from solution when compared to transpiration. It is likely that PPCP/EDCs were also microbially transformed in the solution, as is known to

occur for other organic compounds (Alsanius and Jung, 2004), and that degradation of PPCP/EDCs in solution may vary with rhizosphere community of different plant species. These variations further complicate the relationship between removal in the nutrient solution and plant transpiration of PPCP/EDCs. In general, removal trends clearly suggest that increased plant transpiration contributed to enhanced PPCP/EDC dissipation in the nutrient solution (Figure 1).

Bioconcentration of PPCP/EDCs in Plant Tissues

To facilitate comparisons of PPCP/EDC accumulation among different compounds and between different treatments, a bioconcentration factor (BCF) was calculated by dividing the concentration of a compound in a plant tissue (μg/kg) after the 21 d cultivation by the concentration in fresh solution (μg/L) (Tables S3 – S5). In this study, atorvastatin, diclofenac, and clofibric acid were the least accumulated (BCF = 0.0 – 69.3), while perfluorooctanoic acid, diazepam, and diuron were the most accumulated compounds (BCF = 4.5 – 718.6). After averaging across all compounds and plant types, BCF values for root tissues (BCF_{root}) were found to be significantly greater ($p < 0.001$) than those for leaves (BCF_{leaf}), with the respective mean BCF values of 51.3 and 21.0. An exception to this trend is the accumulation of carbamazepine and dilantin, where BCF_{leaf} values were generally similar or greater than BCF_{root}. These BCF values suggest that many PPCP/EDCs have the ability to accumulate in plant tissues, and accumulation into roots exceeds that into leaves for many compounds. In addition, some PPCP/EDCs may be accumulated to relatively high levels.

In general, BCF_{leaf} values followed the order cationic ≥ neutral > anionic and BCF_{root} values were in the order anionic > neutral ≥ cationic. Anionic compounds were accumulated significantly less ($p < 0.05$) than cationic or neutral compounds in leaves and significantly more ($p < 0.05$) in roots. For anionic PPCP/EDCs, accumulation in root was significantly more than in leaf ($p < 0.001$), with mean BCF_{root} of 72.8 and mean BCF_{leaf} of 3.3. In comparison, accumulation into leaf and root tissues was similar for cationic or neutral compounds (BCF = 28.3 – 52.2, $p > 0.88$). Overall, these results suggest that root tissues may accumulate high levels of anionic compounds, while in leaf tissues, cationic and neutral compounds may be more prevalent.

A few other studies have considered some of the same PPCP/EDCs under hydroponic conditions, but often used higher chemical concentrations. Herkltoz et al. (2010) investigated the growth of cabbage in solution spiked with carbamazepine, sulfamethoxazole, and trimethoprim at 232.5 μg/L and found BCF values of 0.045 – 0.081 in leaf tissues and 7.04 – 10.92 in root tissues, values similar to this study for sulfamethoxazole (below detection – 12.9) and carbamazepine in root (8.0 – 16.2), but lower than carbamazepine accumulation in leaves (36.4 – 150.5) or trimethoprim accumulation (4.8 – 79.2). In another study, Zhang et al. (2013) measured the uptake of clofibric acid by *Scirpus validus* from a culture spiked at 0.5 – 2 mg/L, and observed wet-weight BCFs of 9.5 – 32.1 in leaf tissues and 6.6 – 23.2 in root tissues. These values were similar to the uptake of clofibric acid in this study (Tables S3 – S5). Wu et al. (2013) used greenhouse conditions to examine many of the same compounds at similar concentrations in nutrient solution

growing cucumber, lettuce, pepper, or spinach and observed similar BCF values in leaf and root tissues.

Effects of Plant Transpiration on Leaf Accumulation

The different environmental conditions influenced bioconcentration of the PPCP/EDCs in the test plants. The mean BCF in the warm-dry treatment was 33.7, which was greater than that in the cool-humid treatment (25.6), although the difference was not statistically significant ($p = 0.105$), likely due to the large differences in plant biomass and the wide range of chemicals used in this study. When BCF_{leaf} for anionic, cationic, or neutral compound groups was correlated to the transpired mass during the 21 d of plant growth, a positive correlation was observed ($p < 0.05$) (Figure 2). This finding suggests that the mass flow of water caused by plant transpiration influenced the accumulation of PPCP/EDCs in leaves. Transpiration had the greatest impact on the leaf bioconcentration of cationic and neutral PPCP/EDCs (slope = 0.0067 and 0.0041, respectively), however anionic PPCP/EDCs were significantly less affected (slope = 0.00056, $p < 0.01$).

The measured concentrations in leaf tissues were compared to PC values. In this study, measured concentrations ranged from 0 – 432 and PC values ranged from 22 – 2575. Of the 16 compounds, 3 (carbamazepine, diazepam, dilantin) had measured/predicted concentrations ratios greater than 0.2 and 7 had ratios greater than 0.05 in the cool-humid treatment, while 2 (diazepam and dilantin) compounds and 5 compounds in the warm-dry treatment were above those respective thresholds. In addition, the cool-humid treatment generally had higher measured/predicted concentrations ratios than the warm-dry treatment, which may be attributed to increased metabolism of PPCP/EDCs within plants and/or in nutrient solution exposed to the warmer environment (Table 2) (Loveys et al., 2003). For meprobamate, primidone, and trimethoprim the ratio was significantly larger in cool-humid treatments. Overall, measurable levels of PPCP/EDCs in plant tissues were consistently lower than that might be theoretically expected from plant transpiration-facilitated transport, perhaps due to metabolism in plants after uptake (Bokern and Harms, 1997; Macherius et al., 2012), binding or conjugation to plant tissues (Dodgen et al., 2013), and degradation in the nutrient solution prior to plant uptake (Table S2). Thus, the actual accumulation of PPCP/EDCs into plants may be substantially greater than that observed in this and other studies that only consider parent compounds in the extractable form. A recent study using ^{14}C labeling of two compounds considered in this study, diclofenac and naproxen, found that these compounds were almost wholly not extractable from plant tissues (Dodgen et al., 2013).

Effects of Compound Properties on Root Accumulation

In contrast to the relationship found between BCF_{leaf} and transpiration, a relationship between BCF_{root} and transpired mass was only observed for the neutral chemical group (Figure S2). For anionic compounds, it is known that the negatively charged molecules may experience repulsion from a root cell membrane, due to the membrane's negative electrical potential, and that plant accumulation of anions may be mainly due to diffusion of the neutral fraction through the membrane and ion trap effects.(Trapp, 2009) A comparison of BCF values of anionic compounds across all plants with their respective log D_{ow} showed a

negative correlation for BCF_{leaf} ($p = 0.03$) or BCF_{root} ($p = 0.01$), suggesting that anionic compounds with lower effective hydrophobicity had higher accumulation in the leaf or root tissues (lettuce in cool-humid treatments shown in Figure 3). Across all plants, this effect was greater for root tissues (slope = −36.4) and significantly smaller for leaf tissues (slope = −2.66, $p < 0.001$), suggesting that hydrophobicity may have a large impact on root accumulation.

The cationic fraction of a compound may slowly diffuse through plant membranes due to electrical attraction between the positively charged molecules and the negative potential of the cell membrane, while the neutral fraction may diffuse with preference to compounds of moderate hydrophobicity.(Trapp, 2009) In this study, a positive correlation was observed between BCF_{leaf} and log D_{ow} ($p < 0.005$) for cationic PPCP/EDCs in all plants (lettuce in cool-humid treatments shown in Figure 3), suggesting that more hydrophobic cationic PPCP/EDCs have a higher accumulation potential in leaf tissues. Further, this effect was significantly stronger ($p < 0.01$) for cationic compounds (slope = 38.9 across all plants) compared to neutral (slope = 25.1) or anionic compounds (slope = −2.66). In comparison, no significant correlation was observed between BCF_{root} and log D_{ow} for cationic compounds (Figure 3), suggesting that other factors (e.g., electrical attraction) contributed to the accumulation of cationic compounds in roots. However, this result is based on a limited pool of cationic compounds used in this study and the conclusion merits further validation.

The mechanisms for plant accumulation of neutral organic compounds have been well studied for pesticides, but relatively little work has been reported for PPCP/EDCs. Neutral compounds are thought to be taken up by passive diffusion through the root cell membrane, which is hampered by strong polarity or hydrophobicity (Trapp, 2004). For neutral PPCP/EDCs in this study, a positive linear correlation with log D_{ow} was observed for BCF_{leaf} ($p < 0.05$) or BCF_{root} ($p < 0.001$). The effect of hydrophobicity was significantly greater ($p < 0.05$) for root tissues (slope = 40.5 across all plants) compared to leaf tissues (slope = 25.1), likely due to the contribution of adsorption to the accumulation in root tissues. Other studies have suggested that the optimum log K_{ow} value for plant uptake is around 1 – 3.5.(Boxall et al., 2006; Briggs et al., 1982; Pilon-Smits, 2005) In this study, diazepam, with a log D_{ow} value of 2.82, exhibited the largest BCF values among the neutral compounds considered.

Translocation of PPCP/EDCs from Root to Leaf Tissues

Translocation of compounds from root to aerial tissues may lead to their accumulation in edible leaves or fruits. A translocation factor (TF), the concentration in leaf tissue divided by that in root tissue, was calculated for PPCP/EDCs in each treatment (Table S6). In this study, atorvastatin, ibuprofen, and sulfamethoxazole were the least translocated (TF = 0), while carbamazepine, meprobamate, and dilantin were the most translocated (TF = 0.99 – 18.40). The mean TF value was the highest for tomato at 2.90, with a range of 0 – 18.40, followed by carrot at 1.47, with a range of 0 – 13.58, while lettuce showed the least translocation with an average TF of 0.84 and a range of 0 – 5.50. To assess the effect of transpiration on TFs of anionic, cationic, and neutral PPCP/EDCs, the TF values in each treatment were compared to the mass of nutrient solution transpired in that treatment (Figure 4). For cationic and neutral PPCP/EDCs, a significant positive correlation ($p \leq 0.05$) was

observed between TF values and the transpired masses (Figure 4), suggesting that translocation of cationic and neutral compounds from root to leaves was influenced by transpiration. The impact of transpiration on TF was similar for both cationic and neutral compounds (slope = 0.00055 and 0.00049, respectively; $p < 0.72$) (Figure 4). In contrast, a similar relationship was not found for anionic PPCP/EDCs ($p = 0.107$). Cationic compounds had significantly greater TF values (mean TF = 3.89) than neutral compounds (mean TF = 1.65) or anionic compounds (mean TF = 0.79) ($p < 0.01$), which suggests that cationic compounds were more likely than other compounds to translocate from root to leaf tissues, perhaps due to the partitioning behavior of cation molecules (Trapp, 2009).

In this study, the mass of solution transpired by plants was manipulated to investigate the effect of transpiration on accumulation and translocation of various PPCP/EDCs in vegetable plants. Many PPCP/EDCs were detected in the leaves and roots of the test plants, and increased accumulation in leaves was positively related to transpiration for all groups. Overall, neutral and cationic PPCP/EDCs showed a similar potential to accumulate in leaf and root tissues, while anionic PPCP/EDCs preferentially accumulated in root tissues. The influence of transpiration on accumulation of PPCP/EDCs in aerial tissues has practical implications. For instance, the use of treated wastewater for irrigation is critical in arid or semi-arid regions. However, in these locations PPCP/EDCs may have a greater tendency to accumulate into edible leaves and fruits due to the generally high plant transpiration rates. In addition, it appears that transpiration affects uptake of PPCP/EDCs differently with respect to chemical species. These results are an important foundation for future modelling and risk assessment. Further work targeting the edible portions of crops grown in soil systems with natural radiation should be done in order to understand how these results on transpiration effects translate to a field situation. Eventually, this information may be used to identify “priority” PPCP/EDCs and crop types that may exhibit the most significant accumulation. For these “priority” compounds, a focused effort may be developed that includes field validations and risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Leaf accumulation of PPCP/EDCs is dependent on plant transpiration.
- Cationic and neutral PPCP/EDCs have similar leaf and root accumulation.
- Anionic PPCP/EDCs have greater root accumulation and lesser leaf accumulation.
- PPCP/EDCs are extensively metabolized in plant tissue and hydroponic solution.

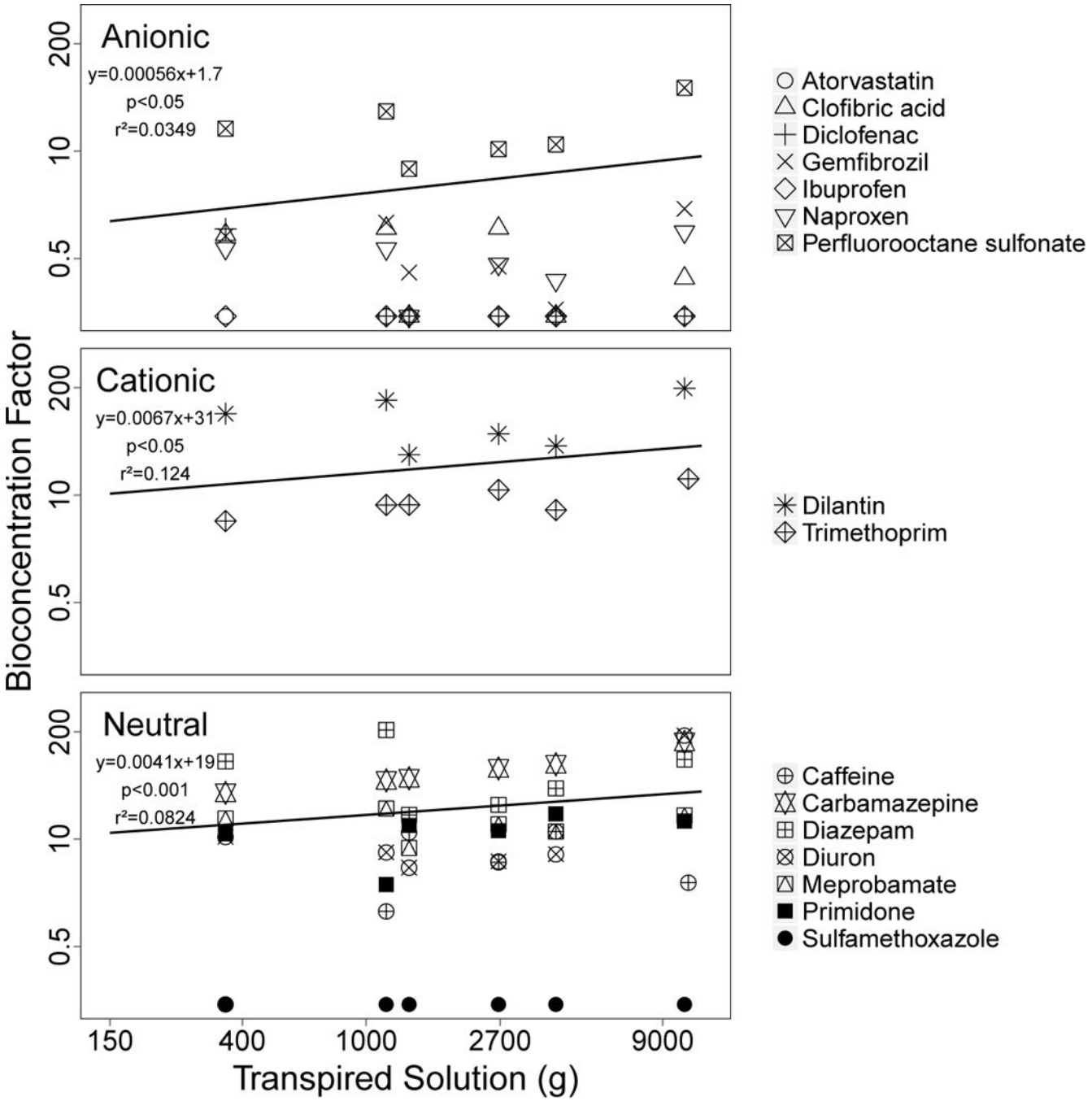

Figure 1.

Removal of anionic, cationic, and neutral PPCP/EDCs from nutrient solution after a 2 d growth period (from study day 8 – 10) with carrot, lettuce, or tomato plants in a cool-humid or warm-dry environment. Plot shows mean percent removed of initial compound mass compared to the mass of nutrient solution transpired by each plant treatment during that period (n = 3).

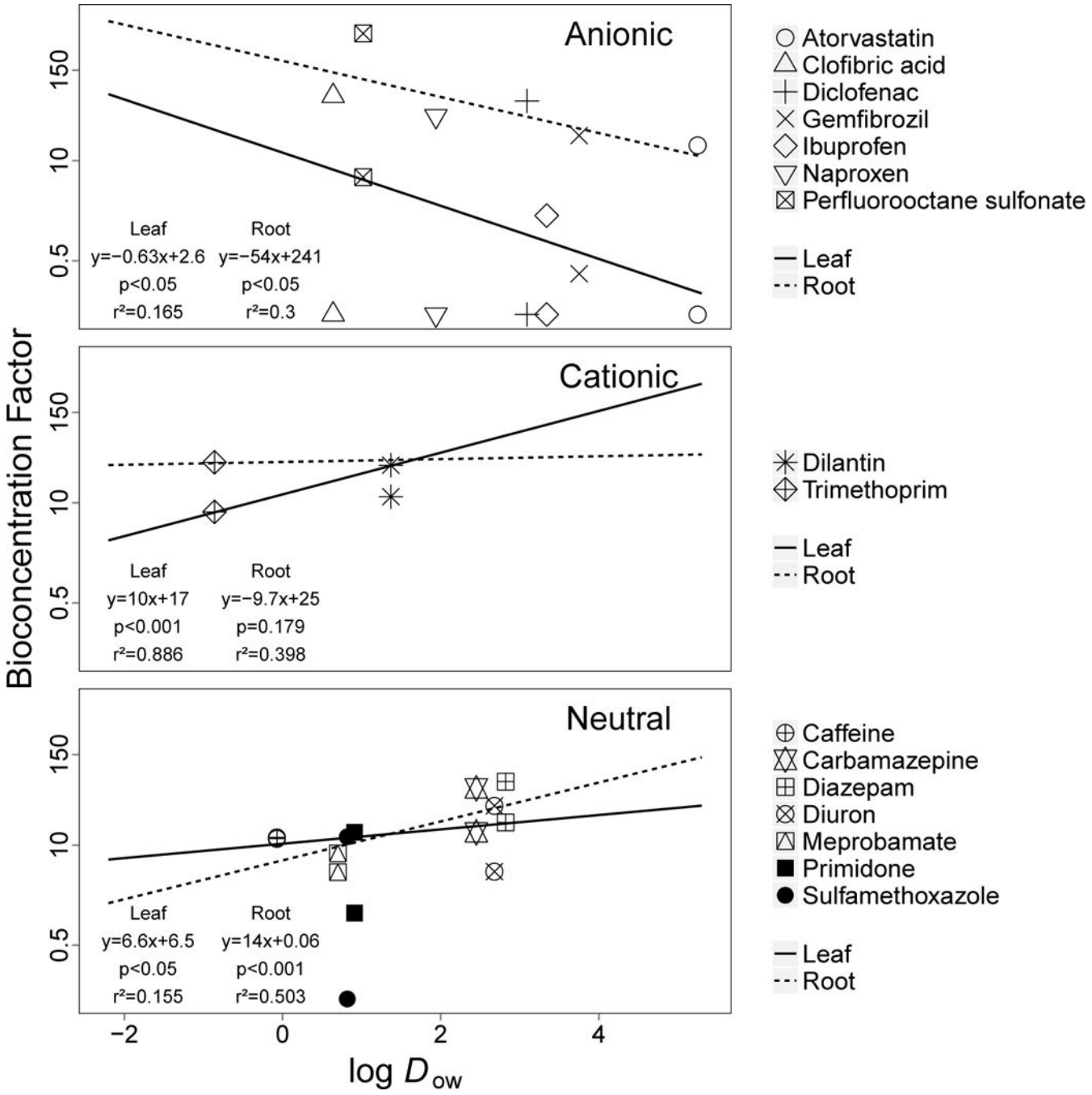

Figure 2.

Bioconcentration factors (BCF) of PPCP/EDCs in leaf tissues of carrot, lettuce, or tomato plants grown in spiked nutrient solution for 21 d in a cool-humid or warm-dry environment. Plot shows mean BCF, calculated as concentration in plant leaves divided by concentration in fresh solution, compared to mass of nutrient solution transpired by the plant treatment during the 21 d ($n \geq 3$).

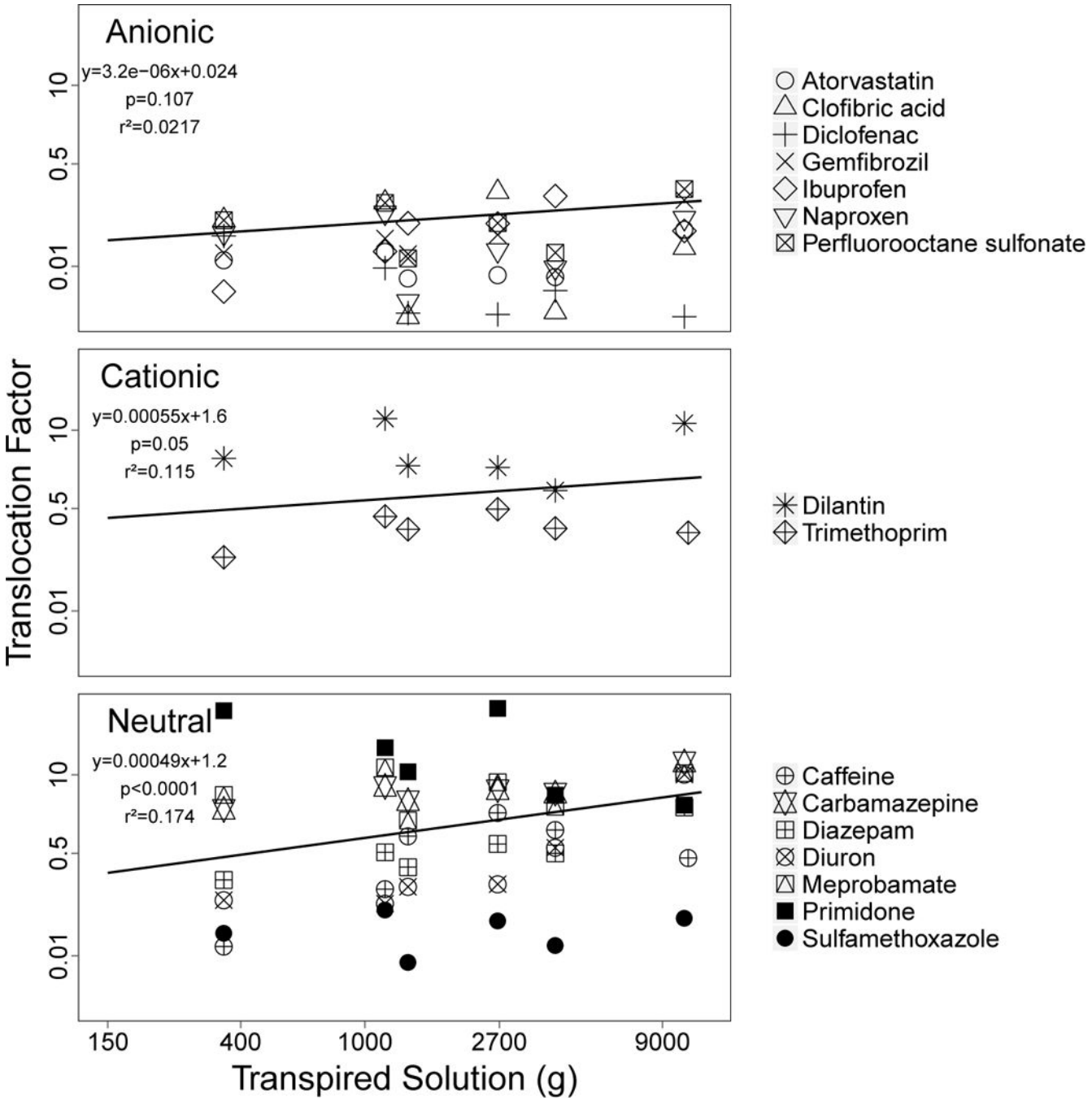

Figure 3.

Bioconcentration factors (BCF) of PPCP/EDCs in leaf or root tissues of lettuce plants grown in spiked nutrient solution for 21 d in a cool-humid environment. Plot shows mean BCF, calculated as concentration in plant tissue divided by concentration in fresh solution, compared to the log D_{ow} for the plant treatment ($n \geq 3$).

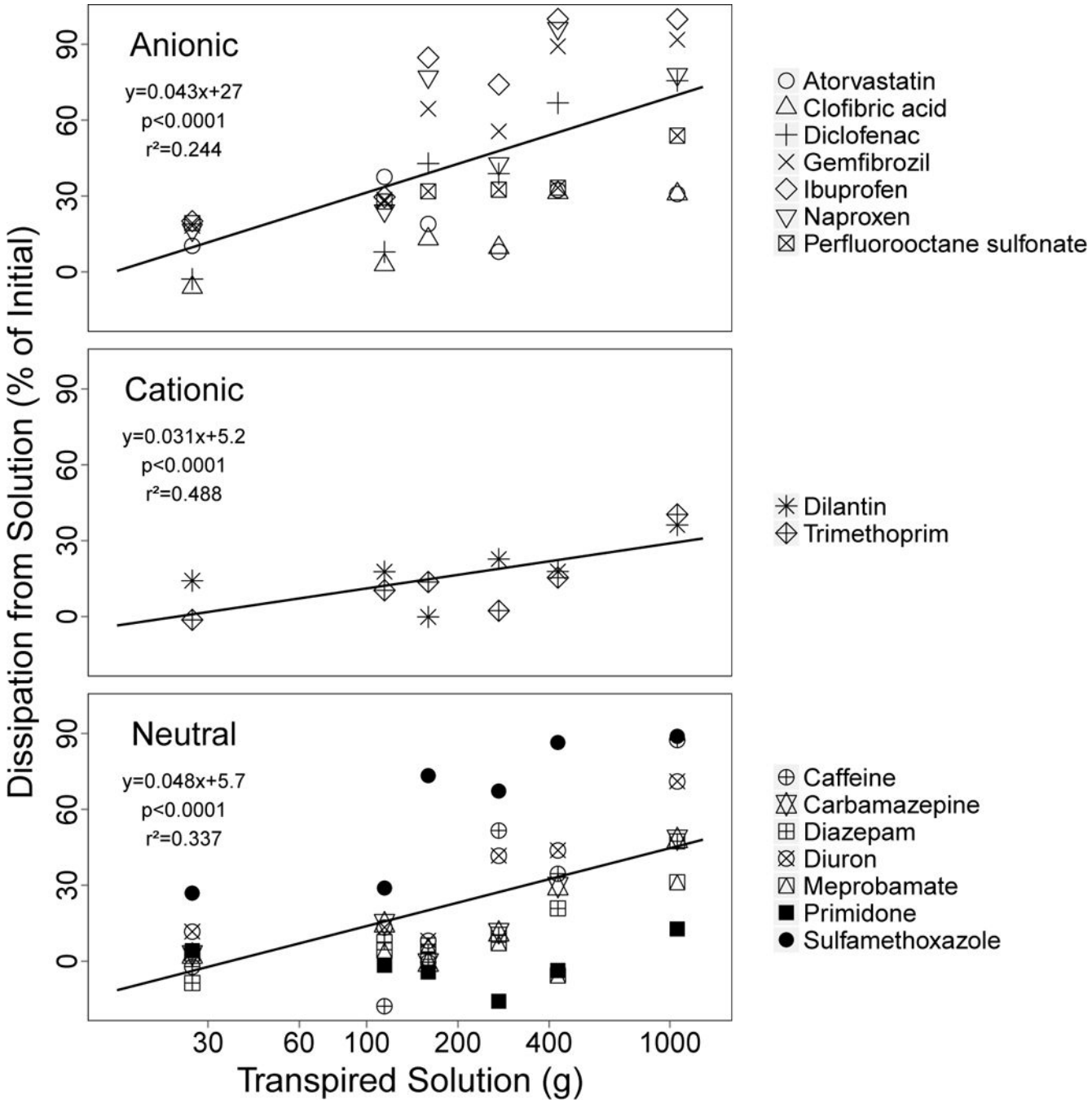

Figure 4.

Translocation factors (TF) from root to leaf tissues of PPCP/EDCs in a carrot, lettuce, or tomato plant grown in spiked nutrient solution for 21 d in a cool-humid or warm-dry environment. Plot shows mean TF, calculated as concentration in leaves divided by concentration in roots, compared to total mass of nutrient solution transpired by the plant treatment ($n \geq 3$).

Table 1

Properties of compounds used in the study, namely the $\log_{10}$ transform of the octanol-water partition coefficient (log K_{ow}), the $\log_{10}$ transform of the acid dissociation constant (pK_a), the fraction of chemical in a neutral state as averaged across all treatments (Neutral Fraction), the predominant ionic state of a chemical across all treatments (Primary Form), and the $\log_{10}$ transform of the pH-adjusted octanol-water partition coefficient averaged across all treatments (log D_{ow}).

Compound	log K_{ow}[a]	pK_a[b]	Neutral Fraction	Primary Form	log D_{ow}
Atorvastatin	6.36	4.33	0.0804	Anionic	5.14
Caffeine	−0.07	1.22[d]	0.9999	Neutral	−0.07
Carbamazepine	2.45	2.3,13.9[f]	1.0000	Neutral	2.45
Clofibric acid	2.84	3.2[c]	0.0066	Anionic	0.52
Diazepam	2.82	2.92	0.9965	Neutral	2.82
Diclofenac	4.51	4.0	0.0399	Anionic	2.98
Dilantin	2.47	6.46	0.1379	Cationic	1.46
Diuron	2.68	NA[e]	1.0000	Neutral	2.68
Gemfibrozil	4.77	4.42	0.0966	Anionic	3.63
Ibuprofen	3.97	4.88	0.2252	Anionic	3.22
Meprobamate	0.7	15.17	1.0000	Neutral	0.70
Naproxen	3.18	4.19	0.0601	Anionic	1.83
Perfluorooctane sulfonate	6.28	0.14[a]	0.0000	Anionic	0.90
Primidone	0.91	11.50	1.0000	Neutral	0.91
Sulfamethoxazole	0.89	6.16	0.7780	Neutral	0.77
Trimethoprim	0.91	7.16	0.0355	Cationic	−0.75

NA – Not applicable (does not dissociate);

[a] – Syracuse Research Corporation: http://esc.syrres.com/fatepointer/search.asp.;

[b] – (Stevens-Garmon et al., 2011);

[c] – (Scheytt et al., 2005);

[d] – (Prankerd, 2007);

[e] – IUPAC http://sitem.herts.ac.uk/aeru/iupac/;

f_{-} (Bui and Choi, 2010)

Table 2

Removal of PPCP/EDCs from nutrient solution after a 2 d period with one carrot, lettuce, or tomato plant in a cool-humid or warm-dry environment (from study day 8 – 10). Data shows mean percent removed ± standard deviation of initial spiked mass (n = 3).

Compound	Carrot (% ± SD)		Lettuce (% ± SD)		Tomato (% ± SD)	
	Cool-Humid	Warm-Dry	Cool-Humid	Warm-Dry	Cool-Humid	Warm-Dry
Atorvastatin	10.4 ± 31.3	37.6 ± 37.4	19.0 ± 25.0	32.5 ± 7.7	8.1 ± 13.2	30.8 ± 20.8
Caffeine	−2.2 ± 7.3	−17.7 ± 14.6	−0.3 ± 4.7	34.6 ± 4.0	51.7 ± 41.6	87.5 ± 7.7
Carbamazepine	2.7 ± 4.4	15.0 ± 7.6	−0.6 ± 19.1	29.6 ± 6.3	11.4 ± 4.4	48.4 ± 15.2
Clofibric acid	−6.1 ± 0.4	2.8 ± 3.3	13.0 ± 6.8	31.3 ± 6.2	9.6 ± 4.6	30.8 ± 21.7
Diazepam	−8.6 ± 13.2	7.5 ± 7.9	3.7 ± 14.7	20.9 ± 11.6	10.4 ± 8.5	47.4 ± 10.9
Diclofenac	−2.8 ± 4.6	7.9 ± 3.6	42.8 ± 33.0	66.8 ± 1.4	38.8 ± 21.3	75.6 ± 12.8
Dilantin	14.2 ± 10.2	17.7 ± 28.3	−0.1 ± 7.7	17.8 ± 16.5	22.7 ± 4.3	36.2 ± 24.9
Diuron	11.7 ± 11.4	13.4 ± 9.1	8.1 ± 5.2	43.8 ± 7.2	41.7 ± 35.9	71.1 ± 18.9
Gemfibrozil	18.2 ± 3.2	28.6 ± 0.6	64.5 ± 28.3	89.2 ± 6.2	55.6 ± 21.5	91.8 ± 7.3
Ibuprofen	20.1 ± 21.1	29.6 ± 11.2	84.8 ± 26.4	100.0 ± 0.0	74.1 ± 28.0	99.9 ± 0.2
Meprobamate	3.0 ± 2.8	4.2 ± 3.2	6.2 ± 3.9	−5.7 ± 7.8	7.1 ± 1.6	31.2 ± 17.5
Naproxen	16.6 ± 18.9	24.2 ± 7.2	77.1 ± 29.8	96.3 ± 0.4	42.8 ± 40.3	78.0 ± 4.2
Perfluorooctane sulfonate	19.2 ± 4.6	27.8 ± 13.1	31.9 ± 6.1	33.3 ± 10.7	32.5 ± 6.9	53.9 ± 12.4
Primidone	4.1 ± 14.3	−1.6 ± 8.4	−4.3 ± 11.5	−3.7 ± 9.4	−15.8 ± 27.1	12.7 ± 23.8
Sulfamethoxazole	27.0 ± 14.1	29.0 ± 16.0	73.4 ± 26.9	86.5 ± 3.9	67.3 ± 25.9	88.9 ± 13.8
Trimethoprim	−1.3 ± 0.7	10.4 ± 5.6	13.7 ± 5.5	15.4 ± 4.1	2.3 ± 18.0	40.4 ± 7.5