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List of Abbreviations

ASE	Accelerated solvent extraction
BAF	Bioaccumulation factor
CAF	Caffeine
CAFO	Concentrated animal feeding operation
CBZ	Carbamazepine
CDC	Centers for Disease Control
DI	Deionized
DW	Dry weight
E1	Estrone
EDC	Endocrine-disrupting hormone
EE2	Ethinyl estradiol
ESI	Electrospray ionization
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GEM	Gemfibrozil
HLB	Hydrophilic-Lipophilic balance
HPLC	High-performance liquid chromatography
IBU	Ibuprofen
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOD	Limits of detection
Log K_{ow}	Octanol-water coefficient
MRM	Multiple reaction monitoring
MTBE	Methyl tert-butyl ether
NAP	Naproxen
PPCP	Pharmaceutical and personal care products
pK_a	Acid dissociation constant
RPM	Revolutions per minute
SMO	Sulfamethoxazole
SPE	Solid-phase extraction
STP	Sewage treatment plant
TCS	Triclosan
TF	Translocation factor
UIUC	University of Illinois at Urbana-Champaign
βE2	17β-Estradiol

Abstract

The widespread occurrence of pharmaceutical and personal care products (PPCPs) and steroid hormones in watersheds has been recognized as an emerging environmental issue. The potential uptake and accumulation of these emerging contaminants by food plants that are irrigated with contaminated water could be a food safety issue. In the present project, uptake, translocation, accumulation, and depuration of seven PPCPs and three steroid hormones in lettuce and tomato plants were investigated using hydroponic cultures with compound concentrations of 0.5, 50, or 500 μg L^{-1} and several exposure scenarios. An isotopic dilution method was developed for the analysis of trace levels of PPCPs and hormones in food plants using liquid chromatography-tandem mass spectrometry (LC-MS/MS), combined with ultrasonication-shaking extraction and solid phase extraction (SPE) cleanup. For lettuce plants, all targeted PPCPs and hormones were detected in the roots. The bioaccumulation factors (BAFs) of PPCPs and hormones in lettuce roots were more than 1, indicating these emerging contaminants can be bound to or taken up by the plant roots. In lettuce leaves, only caffeine (CAF), carbamazepine (CBZ), and sulfamethoxazole (SMO) showed very high BAF values compared to other targeted PPCPs and hormones, indicating that these three compounds can easily translocate from lettuce roots to leaves and thereby accumulate in plant leaves. For tomato plants, all PPCPs and hormones were detected in the roots. By contrast, the translocation factor (TF) values of all targeted compounds except CAF and CBZ were very small in tomato plants, implying their poor translocation from roots to above-ground plant parts following uptake. The BAFs of all targeted hormones in tomato fruits were much less than 1, suggesting that hormone contamination of tomato fruits after irrigation with contaminated water could be negligible. In addition, exposure study showed that accumulation of PPCPs and hormones may rapidly reach a steady level (< 1 week) in lettuce plants with exposure through contaminated water. Lettuce plants also appear to have a potential to metabolize accumulated PPCPs, with the exception of triclosan (TCS) in roots and sulfamethoxazole (SMO) in leaves. Hormones did not exhibit any tendency to depurate. Comparing protective estimates of human exposure in lettuce leaves and acceptable daily intake (ADI) values suggests that CBZ and ethinylestradiol (EE2) could exceed their ADIs under some circumstances.

1. Introduction

1.1. Background

1.1.1. Food Contaminants

Although food supply safety in the U.S. is overseen by governmental agencies, foodborne illness often arises from the consumption of contaminated foods (Scallan et al., 2011). There are three main types of food contaminants: (a) harmful microorganisms including a variety of pathogenic bacteria, viruses, and parasites; (b) toxic substances such as mycotoxins and marine biotoxins; and (c) chemical contaminants including heavy metals, pesticides, or emerging contaminants such as pharmaceutical and personal care products (PPCPs) and steroid hormones. These contaminants may be introduced into the food chain at any stage while the food travels from farm to table, including growing, handling, and storage. Over the past few decades in the U.S., most foodborne illnesses have been caused by harmful pathogens (Käferstein et al., 2000; Motarjemi et al., 1995). The remaining illnesses are attributable to various biotoxins and chemical contaminants (Käferstein et al., 2000; Motarjemi et al., 1995).

Unlike foodborne pathogens, chemical contamination is only occasionally recognized as the cause of acute foodborne illness, and typically occurs due to accidental introduction or intentional adulterations. For example, the industrial chemical melamine was illegally added to infant milk formula in order to increase apparent protein content, leading to the hospitalization of over 50,000 children in China in 2008 (Ingelfinger, 2008). PPCPs and steroid hormones are emerging contaminants in the environment, known to act through chronic exposure resulting in subtle environmental effects (Daughton and Ternes, 1999). However, the extent of these emerging contaminants in food is unknown and their effects on human health from chronic exposure are not understood. Additionally, the presence of chemical contaminants in food cannot be controlled by typical food safety measures, such as thermal processing or radiation, which are frequently used to destroy pathogens (Motarjemi et al., 1995). Moreover, PPCP and hormone contaminants are increasingly introduced to the food chain due to the adoption of innovative agricultural production practices (e.g., reuse of treated wastewater) and the application of new food preparation and storage techniques. Therefore, it is critical to identify these emerging contaminants, understand their potential risk, and mitigate their presence in the food supply.

1.1.2. Environmental and Human Health Effects of PPCPs and Steroid Hormones

While PPCPs are usually detected in the environment at nanograms per liter (ng L^{-1}) levels and lower – greatly below their therapeutic dose or typical use – their frequent use continually introduces them to the environment and causes them to act as pseudo-persistent chemicals in water and soil resources. This continual exposure of wildlife and plants may imperil environmental and human health (Cleuvers, 2004; Luckenbach and Epel, 2005; Pomati et al., 2006; Rochester, 2013). Most steroid hormones and some pharmaceuticals are classified as highly potent endocrine disrupting chemicals (EDCs), which may interfere with the normal function of the endocrine systems of humans and animals (Diamanti-Kandarakis et al., 2009). As an example, these EDCs, even at ng L^{-1} levels, can adversely affect the reproduction of a variety of freshwater species (Filby et al., 2007; Jobling et al., 1998; Madsen et al., 2004; Sanchez et al., 2011; van der Linden et al., 2008). In addition to the direct effects of exposure, the presence of

antibiotic pharmaceuticals in the environment may be jeopardizing their continued therapeutic efficacy due to the emergence of bacteria resistant to antibiotics (Boxall et al., 2012; Daughton and Ternes, 1999; Kolodziej et al., 2004). For example, multiple antibiotic-resistance genes have been detected in bacteria in groundwater underlying large animal farms (Chee-Sanford et al., 2001; Koike et al., 2007). Bacteria with these antibiotic-resistance genes may transfer them to human pathogens, resulting in decreased efficacy of antibiotics during infections and diminished success in subsequent treatments (Alcaine et al., 2005), indirectly imperiling human health.

1.1.3. Environmental Occurrence of PPCPs and Steroid Hormones

Generally, PPCPs are widely utilized for therapeutic and personal use, while hormones may be endogenous or therapeutic. Their abundant use and natural production leads to the introduction of these compounds into sewage and other waste streams. PPCPs and their metabolites are incompletely removed in most conventional biological sewage treatment plants (STPs), leading to their ubiquity in effluents of STPs and their receiving waters (Baronti et al., 2000; Heberer, 2002a; Ternes et al., 1999, 2004). High concentrations of steroid hormones have also been frequently detected in manure and manure-containing wastewater derived from concentrated animal feeding operations (CAFOs), such as dairy and swine facilities (Hutchins et al., 2007; Zheng et al., 2008). Unlike sewage, CAFO wastes are not required to undergo additional treatment before land applications, indicating veterinary pharmaceuticals and animal hormones may remain in these wastes.

The discharge and land application of effluents and solids from STPs and CAFOs are major sources of PPCPs and steroid hormones to the environment (Burkholder et al., 2007; Kinney et al., 2006; Xia et al., 2005). A national survey of U.S. streams reported that organic contaminants, including PPCPs and hormones, were detected in 80% of samples (Kolpin et al., 2002), while more than 80 pharmaceuticals have been detected in water bodies worldwide (Heberer, 2002b).

1.1.4. Uptake of PPCPs and Steroid Hormones by Plants

Water supply shortages are a concern for U.S. agriculture due to increased food demands by an expanding population and more frequent droughts resulting from climate change. As water supplies dwindle, reclaimed water is becoming an increasingly important water source for crop irrigation. In the U.S., about 8% of treated wastewater is currently used for irrigation and other needs, and this reuse is growing by 15% each year (Miller, 2006). Agricultural irrigation using treated wastewater and land application of waste solids also provide nutrients and organic matter that improve plant growth; reduce fertilizer and soil amendment needs; and thereby enhance the long-term sustainability of agriculture. However, reuse of waste materials that contain PPCPs and hormones would introduce these emerging contaminants to soil, from which they may subsequently be taken up by food crops or contaminate crop surfaces (Boxall et al., 2006; Calderón-Preciado et al., 2011b; Dodgen et al., 2013; Shenker et al., 2011).

Studies indicate that PPCPs or hormones may be taken up, accumulated in, or induce phytotoxicity in beans and wetland macrophytes (Karnjanapiboonwong et al., 2011a; Matamoros et al., 2012; Wu et al., 2010). Also, some veterinary pharmaceuticals in animal manures have been shown to bioaccumulate in alfalfa, corn, lettuce, potato, and soybean (Boxall et al., 2006; Dolliver et al., 2006; Kumar et al., 2005). Overall, these emerging contaminants have the

potential to enter food supplies via the land application of reclaimed water and waste solids. The extent of their uptake, internal transfer, and accumulation in plants is likely to be associated with the compound properties, plant species and cultivar, growth substrates, compound concentrations, and plant development stages. While recent studies have begun to illuminate the extent of this process, it is still very unclear how much potential there is for plant uptake of emerging contaminants to impact human health. Moreover, the potential contamination of food crops with PPCPs and hormones is raising public concern when reclaimed water is used for irrigation. Thus, public perception and concerns need to be addressed before the widespread use of reclaimed water in agricultural fields is politically supported.

1.2. Project Objectives

The goal of this project was to investigate the uptake and accumulation potential of PPCPs and steroid hormones in food plants, determine if these emerging contaminants would be a critical food safety threat, and provide knowledge to develop the safe use of wastewater in agriculture. To achieve the goal, the following objectives were set:

1) Develop and optimize analytical methods for targeted PPCPs and steroid hormones in plant samples.
2) Conduct greenhouse experiments to elucidate the processes and mechanisms of uptake, translocation, accumulation, and depuration of PPCPs and steroid hormones in lettuce and tomato plants.
3) Evaluate potential human exposure from consumption of food plants contaminated with emerging contaminants.

2. Methodology

2.1. Chemicals and Materials

Seven PPCPs and three steroid hormones were selected for this study on the basis of their frequent occurrence in aquatic environments (Choi et al., 2008; Karnjanapiboonwong et al., 2011b) and their wide range of physicochemical properties (e.g., pK_a and log K_{ow}) (Table 1). PPCP standards for caffeine (CAF), carbamazepine (CBZ), gemfibrozil (GEM), ibuprofen (IBU), naproxen (NAP), triclosan (TCS), and sulfamethoxazole (SMO), internal standard florfenicol, and hormone standards 17β-estradiol (βE2), estrone (E1), and 17α-ethinylestradiol (EE2) were obtained from Restek (Bellefonte, PA, USA). Isotope standards including $^{13}C_3$-caffeine, D_{10}-carbamazepine, D_6-gemfibrozil, $^{13}C_3$-ibuprofen, $^{13}C_4$-naproxen, $^{13}C_{12}$-triclosan, $^{13}C_6$-sulfamethoxazole, and $^{13}C_6$-estrone were purchased from Cambridge Isotope (Andover, MA, USA). Solvents used in the study, including methanol, acetone, and acetonitrile, were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized (DI) water (>17.6 MΩ-cm) was supplied by a Labconco Water Pro Plus system (Kansas City, MO, USA). An individual stock solution of each compound was prepared in methanol and stored in an amber glass vial at -20°C.

Lettuce (*Lactuca sativa*, two cultivars: 'Green Rex Butterhead' and 'Red Lollo') and tomato (*Solanum lycopersicum*, two cultivars: 'Cherry Cascade' and 'Tiny Tim') seeds were obtained from a local nursery. Lettuce and tomato were selected for this study because they are representative of edible leaf and fruit plants, respectively. Two commercial hydroponic systems, AeroFlo2 Model 20 and Turbogarden Aero, were purchased from General Hydroponics (Sebastopol, CA) and Botanicare (Chandler, AZ), respectively.

2.2. Hydroponic Experiments

2.2.1. Cultivation of Lettuce Plants

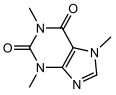
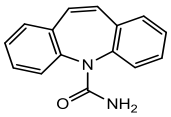
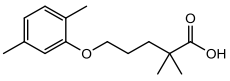
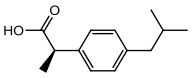
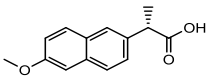
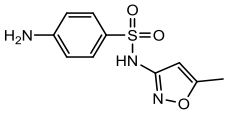
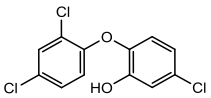
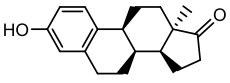
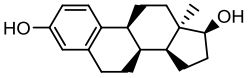
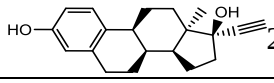
All lettuce plant uptake experiments were performed in a temperature-controlled greenhouse operated by the Plant Care Facility at the University of Illinois at Urbana-Champaign (UIUC), in collaboration with the greenhouse managers. Three lettuce studies were conducted in this project. The lettuce seeds ('Green Rex Butterhead' and 'Red Lollo') were germinated in Fafard superfine-germination mix (Agawam, MA) for two weeks (Figure 1, top-left panel). Ten seedlings of uniform size were transferred into the AeroFlo2 Model 20 hydroponic tanks (Figure 1, bottom-left panel), where they were maintained in a continuously aerated nutrient solution under a 16/8-h day/night photoperiod at 25 ± 1°C and 20 ± 1°C day/night temperature. Prior to transfer, the seedlings were thoroughly washed to remove any substrate particles attached to the plants. All lettuce plants were acclimated in nutrient solutions for one week prior to beginning experiments.

The first lettuce study aimed to determine the effect of initial PPCP and hormone concentrations on their uptake, translocation and accumulation in plants. Initial concentrations of each of the selected 7 PPCPs and 3 hormones in the nutrient solutions were 0.5, 50, or 500 μg L^{-1}, representing concentrations typical of wastewater at various levels of treatment (Anderson et al., 2010; Choi et al., 2008; Heberer, 2002a; Karnjanapiboonwong et al., 2011*b*). The stock of mixed chemicals was made in methanol and then added to the nutrient solution such that the methanol

was less than 0.1% of the total solution. Plants were also grown concurrently in 0 μg L^{-1} solutions as experimental controls. The chemical-spiked nutrient solutions were replaced twice per week to replenish nutrient levels and reduce microbial presence. After 3 weeks of treatment (Figure 1, bottom-right panel), whole lettuce plants were harvested for analysis.

The second study examined the effect of exposure time on uptake and accumulation of targeted PPCPs and hormones in lettuce plants. For this experiment, 20 lettuce plants ('Green Rex Butterhead') were cultivated for 3 weeks in nutrient solutions amended at 50 μg L^{-1} of each targeted compound. At the end of week 1, 2, and 3, lettuce plants were harvested for analysis of PPCPs and steroid hormones.

Table 1. Studied PPCPs and steroid hormones and their properties.

Compound[*]	**Abbreviation**	**Category**	**Structure**	**MW (g/mol)**	**pK_a**	**Log K_{ow}**
Caffeine	CAF	Stimulant		194.19	14.0	-0.07
Carbamazepine	CBZ	Anticonvulsant, mood stabilizer		236.27	13.9	2.45
Gemfibrozil	GEM	Lipid-lowering drug		250.33	4.5	4.77
Ibuprofen	IBU	Anti-inflammatory drug (NSAID), pain reliever		206.28	4.91	3.97
Naproxen	NAP	Anti-inflammatory drug (NSAID), pain reliever		230.26	4.15	3.18
Sulfamethoxazole	SMO	Antibacterial		253.28	1.6, 5.7	0.89
Triclosan (Irgasan)	TCS	Antibacterial, antifungal		289.54	7.9	4.76
Estrone	E1	Estrogenic hormones		270.37	N/A	3.13
β-Estradiol	βE2	Sex hormone		272.38	N/A	4.01
Ethinylestradiol	EE2	Oral contraceptive pill		296.4	N/A	3.67

[*] All values from Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov).

Figure 1. Pictures of greenhouse experiments. Top-left: Seedlings germinated in soil. Top-right: View of Aeroflo2 System including reservoir tank and gutter system. Bottom-left: Newly transplanted lettuce plants in Aeroflo2 System. Bottom-right: Fully grown lettuce plants.

For the third lettuce study, two batches of lettuce plants were cultivated in chemical-spiked nutrient solutions at different points in their life cycles to study the impact of depuration on accumulation of PPCP and hormone contaminants. Each batch was composed of 10 replicates of both two lettuce cultivars, and all nutrient solutions were changed twice per week. In the first batch, the lettuce plants were cultivated in solutions spiked with 50 µg L^{-1} of each targeted compound for 1.5 weeks and then cultivated in unspiked solution for a further 1.5 weeks. In the second batch, exposure was the opposite pattern: lettuce plants were cultivated in unspiked solution for the first 1.5 weeks followed by 1.5 weeks of cultivation in spiked solutions (50 µg L^{-1} of each compound). All lettuce plants were then harvested for sample preparation. For the second and third studies, chemicals were investigated at 50 µg L^{-1}, in which plant uptake was still expected to be measurable, so as to elucidate mechanistic processes of uptake.

2.2.2. Cultivation of Tomato Plants

Tomato experiments were also performed in a greenhouse operated by the Plant Care Facility at UIUC, using the same photoperiods and temperatures as described in section 2.2.1. As with the first lettuce study, the effect of initial PPCP and hormone concentrations on their uptake, translocation, and accumulation in tomato plants were investigated. Initial spiking concentrations for each targeted compound were 0.5, 50, or 500 μg L^{-1}, with plants grown concurrently in 0 μg L^{-1} solutions as experimental controls.

For these experiments, two tomato cultivars ('Cherry Cascade' and 'Tiny Tim') were germinated from seeds placed in soil. After 3 weeks, 6 seedlings of each cultivar were rinsed with DI water to remove soil particles and transferred to continuously aerated nutrient solution in the Turbogarden Aero System (Figure 2, top-left panel). The plants were acclimated in the nutrient solutions for 4 weeks until the first tomato fruit appeared. At this point, tomato plants were thinned to 3 plants of each cultivar to allow room for growth (Figure 2, top-right panel), and the nutrient solution was then amended with each of the targeted compounds. The chemically-spiked nutrient solutions for this study were changed once per week. After 5 weeks of cultivation in spiked solution (Figure 2, bottom panel), whole tomato plants were harvested for analysis.

2.3. Sample Preparation and Extraction

2.3.1. Homogenization of Plant Samples

After harvesting, all plants were rinsed under a stream of DI water for 5 minutes, left to drain, and then blotted dry with paper towels. Lettuce plants were separated into roots and leaves, while tomato plants were separated into roots, stems, leaves, and fruits (Figure 3, left panel). Each plant sample was weighed to determine wet-weight. Leaves and fruits were homogenized using a kitchen food processor. Roots and stems were cut into small pieces, freeze-dried (Labconco, Kansas City, MO), and then ground to powder using a mill (Glen Mills, Maywood, NJ) (Figure 3, right panel). After measuring their moisture content, all plant components were stored at -20°C until extraction.

2.3.2. Selection of Extraction Solvents

In preliminary tests, three dual-solvent (solvent A/solvent B) systems were evaluated to optimize the plant extraction method: (i) methyl tert-butyl ether (MTBE)/acetonitrile; (ii) acetonitrile/phosphate buffer; and (iii) acetonitrile/water. Briefly, uncontaminated plant samples were weighed into centrifuge tubes and spiked with 100 ng each of PPCP or hormone. After premixing, 20 mL of extraction solvent A was added to the test sample for extraction. The sample was vortexed for 1 min, sonicated for 30 min, shaken for 30 min, and then centrifuged at 10,000 rpm for 15 min. The supernatant was poured out into a turbovap tube. The solid phase was further extracted by adding 15 mL of solvent B, followed by vortexing, sonicating, shaking, and centrifuging. The aqueous layer was poured out into the same turbovap tube. The solid sample was extracted one more time using 20 mL of solvent A according to the above extraction

Figure 2. Tomatoes in the Turbogarden Aero System. Top-left: Newly transplanted tomato plants. Top-right: Tomato plants after a few weeks of growth in the system. Bottom: Tomato plants grown to harvest size.

Figure 3. Tomato plant samples. Left: Tomato plants divided into roots, stems, leaves, and fruit. Right: Freeze-dried and ground stem samples.

procedure. Extracts from each sample were combined and then mixed thoroughly. The extracts were concentrated to 1.0 mL using a closed cell concentrator (Turbo Vap@500, Hopkinton, MA) at 40°C, followed by addition of ultrapure water (49 mL at pH 2) to each sample. Based on the results from these preliminary evaluations, the best solvent system was chosen to extract all plant samples collected from the cultivation experiments.

2.3.3. Extraction and Clean Up of Plant Samples

PPCPs and hormones in plant samples were extracted and cleaned up by solid phase extraction (SPE) cartridges based on EPA Method 1694 (U.S. Environmental Protection Agency, 2007; Li et al., 2013) with some modification. To reduce interference from the plant matrix on LC-MS/MS analysis, an isotope dilution method was utilized to analyze all harvested plant samples. In brief, each plant sample was weighed into centrifuge tubes and spiked with 100 µL of 1 mg L^{-1} surrogate solution that contained a stable-labeled isotope standard for each targeted analyte. For fruits and leaves that were not freeze-dried, 10 g of sample was extracted due to the high water content (>90%) of the fruits and leaves. For roots and stems that were freeze-dried, 1.0 g of sample was extracted. All samples were extracted according to the ultrasonication-shaking procedures described in Section 2.3.2.

Extracts were cleaned up using solid phase extraction (SPE) (Oasis HLB, 500 mg, 6cc). Before loading the sample extracts, the SPE cartridges were preconditioned with 10 mL methanol, 10 mL water, and 10 mL acidified water (pH 2) in series by gravity. Sample extracts were passed through the SPE cartridges, using a vacuum to control the flow rate at 3 to 5 mL min^{-1}. For PPCP extraction, the cartridges were washed with 10 mL water after loading the sample and dried under vacuum for about 30 min. Each sample was eluted with 10 mL methanol and 6 mL acetone:methanol (1:1) by gravity. The combined sample extracts were blown down to dryness under gentle nitrogen gas and reconstituted with 1.0 mL acetonitrile:water (1:1). For hormone extraction, Oasis HLB cartridges were washed with 5 mL methanol:water (5:95) and dried under vacuum for about 30 min after loading the samples. The samples were then eluted with 6 mL of ethyl acetate:methanol (9:1). The extracts were blown down to dryness under gentle nitrogen gas and reconstituted with 1.0 mL acetonitrile:water (1:1). The concentrations of PPCPs or hormones in extracts were quantified by LC-MS/MS method described below. Analysis of each plant sample was carried out in triplicate.

2.4. Instrumental Analysis and Quantification

Concentrations of PPCPs were determined by LC-MS/MS (Waters, Quattro Macro, QA1140, Milford, MA). All targeted PPCPs were separated on a Symmetry C18 column (3.5 µm particle size, 2.1 × 150mm, Waters) by HPLC (2695 module, Waters). A gradient separation was achieved using two mobile phases: solvent A, 0.1% ammonium acetate and 0.1% acetic acid in water; and solvent B, 1:1 methanol:acetonitrile. The gradient began with 90% solvent A and 10% solvent B and was maintained for 2 min. The gradient was then ramped up to 5% solvent A and 95% solvent B linearly in 13 min and maintained for 8 min. The gradient changed back to 90% solvent A and 10% solvent B in 0.5 min and was re-equilibrated for 5.5 min. Sample extracts were spiked with 100 ng internal standard florfenicol, and 30 µL of each sample was injected.

Hormones were analyzed by the same LC-MS/MS system. Two mobile phases were applied for separation: solvent C, water with 10 mM ammonium hydroxide; and solvent D,

acetonitrile with 10 mM ammonium hydroxide. The gradient began with 90% solvent C and 10% solvent D and was maintained for 2 min. The gradient was then ramped up to 5% solvent C and 95% solvent D linearly in 13 min and maintained for 8 min. The gradient changed back to 90% solvent C and 10% solvent D in 0.5 min and was re-equilibrated for 5.5 min. Sample extracts were spiked with 100 ng internal standard florfenicol, and 30 µL of each sample was injected.

An LC system was coupled with a Quattro Macro mass spectrometer (QA1140, Waters, Milford, MA) equipped with an electrospray ionization (ESI) source. For PPCP analysis, the mass spectrometer was operated in positive and negative ESI mode simultaneously with optimized instrument conditions: desolvation gas flow rate 650 L min^{-1}; capillary voltage 3.0 kV for positive and 3.5 kV for negative mode. For hormone analysis, negative ESI mode was applied with the same desolvation gas flow and capillary voltage. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode and optimized parameters including collision energy and cone voltage for each targeted analyte are listed in Table 2. Confirmation of the analytes in plant sample extracts was based on the MRM ion transitions as well as comparing the retention time of each peak to its corresponding isotopic standard.

Table 2. LC retention times and optimized MS/MS parameters of targeted PPCPs and hormones.

Compound	RT (min)	Corresponding Isotope	ESI model	MRM ions	Isotope MRM ions	Cone (V)	Collision (V)
Caffeine	9.9	$^{13}C_3$-Caffeine	+	195.2>137.9	198.2>140.0	35	20
Carbamazepine	16.3	D_{10}-Carbamazepine	+	237.4>194.2	247.4>204.2	35	16
Gemfibrozil	21.0	D_6-Gemfibrozil	–	249.0>121.0	255.0>121.0	26	12
Ibuprofen	19.9	$^{13}C_3$-Ibuprofen	–	205.1>161.1	208.2>163.1	20	10
Naproxen	17.7	$^{13}C_4$-Naproxen	–	229.2>170.1	233.2>170.1	20	15
Sulfamethoxazole	13.1	$^{13}C_6$-Sulfamethoxazole	+	254.0>156.0	260.2>162.0	35	16
Triclosan	21.1	$^{13}C_{12}$-Triclosan	–	286.8>235.0	298.8>235.0	22	10
Estrone	16.8	$^{13}C_6$-Estrone	–	269.3>145.0	275.2>145.0	50	40
17β-Estradiol	15.8	$^{13}C_6$-Estrone	–	271.3>145.0	275.2>145.0	50	40
17α-Ethinylestradiol	16.1	$^{13}C_6$-Estrone	–	295.3>145.0	275.2>145.0	50	40

3. Results and Discussion

3.1. Optimization of Extraction Method

A series of preliminary experiments were performed to investigate the effects of extraction conditions on the recoveries of PPCPs and hormones from plant samples. It has been reported that ultrasonic extraction showed better recoveries for most targeted PPCPs than accelerated solvent extraction (ASE) (U.S. Environmental Protection Agency, 2007; Wu et al., 2012). Additionally, it has also been shown that sonication extraction using acetonitrile or methyl tert-butyl ether (MTBE) resulted in a higher extraction efficiency compared to other solvents such as methanol, acetone, and ethyl acetate (Wu et al., 2012). Method development for extraction of PPCPs from solids was based on an ultrasonic extraction with acetonitrile, according to the recommendations of EPA Method 1694 (U.S. Environmental Protection Agency, 2007).

To further improve extraction efficiency of PPCPs and hormones from plant samples, different solvent-mixture systems were examined under a two-step extraction procedure involving ultrasonication and shaking. The absolute recoveries of all of the targeted analytes in the uncontaminated tomato fruits under three solvent extraction systems are displayed in Figure 4. The absolute recovery for each analyte was calculated as the amount detected over that spiked. The recoveries ranged from 42% to 115% for acetonitrile/MTBE, 52% to 121% for acetonitrile/phosphate buffer solution, and 9% to 127% for acetonitrile/water, respectively. Using acetonitrile/phosphate buffer solution as the extraction solvent led to better recoveries for most targeted compounds (Table 3). Compared to the acetonitrile/MTBE solvent system used in a previous study for PPCP extraction (Wu et al., 2012), the acetonitrile/phosphate buffer system

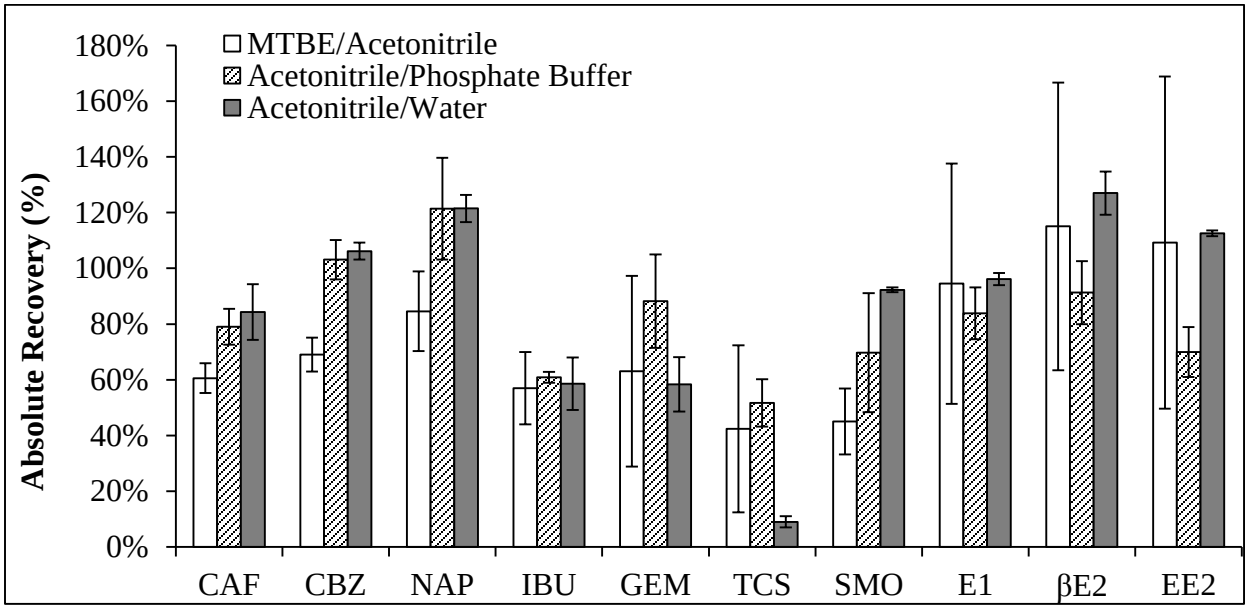


Figure 4. Effect of extraction solvents on the recoveries of PPCPs and hormones: CAF–caffeine, CBZ–carbamazepine, NAP–naproxen, IBU–ibuprofen, GEM–gemfibrozil, TCS–triclosan, SMO–sulfamethoxazole, E1–estrone, βE2–17β-estradiol, and EE2-17α-ethinylestradiol.

appeared to have less variation in recovery for most targeted compounds in this study (Figure 4). Therefore, the acetonitrile/phosphate buffer solution extraction was selected for use in this study.

3.2. Method Validation

The entire procedure used to detect and quantify the residues of PPCPs and steroid hormones in plant samples included isotope standard addition, ultrasonication-shaking extraction, SPE cartridge cleanup, and quantification by LC-MS/MS. The performance of the entire developed method was evaluated by considering response linearity, recoveries, and limits of detection (LODs) of targeted PPCPs and hormones in plant samples. For analyte quantification, six point calibration curves (1-500 μg L^{-1}) were performed for each targeted compound. For each PPCP, a Relative Response was calculated with the standards using the ratios of the integrated peak areas for each compound and its corresponding isotope surrogate (U.S. Environmental Protection Agency, 2007). For the hormones, Relative Response was calculated for each compound compared to the $^{13}C_6$-estrone surrogate, due to the structural similarity between the targeted hormones. Good linearity was achieved for standard calibration of all compounds, with squared Pearson coefficients (r^2) > 0.99.

The absolute recovery and corrected recovery for each analyte in this method are shown in Table 3. Corrected recoveries were calculated for targeted analytes based on the recovery of spiked isotope surrogates, which easily controlled the variability in absolute recoveries (Wu et al., 2012). Due to the low, environmentally-relevant concentrations studied in complex matrices, variability is introduced through several avenues, including matrix effects on ionization, analyte loss during sample preparation, and variations in the instrumental response. After correction, recoveries of targeted PPCPs and hormones spiked plant samples were 94 to 107% (Table 3), indicating that the isotopic dilution method was able to provide good recovery during the simultaneous analysis of a broad range of compounds in a complex matrix.

Limits of detection (LODs) for this method were calculated according to an established method (Vanderford and Snyder, 2006). Limits of quantification (LOQs) were used as reporting limits in this study and are calculated as three times the corresponding LODs. In this method, LODs were in the range of 0.04 to 2.60 μg kg^{-1} dry-weight plant tissue (dw) (Table 3), which were similar to a previous study (Wu et al., 2012). These relatively low LODs make this method appropriate for the detection of trace residues of targeted PPCPs and hormones, especially in studies of vegetables impacted by reclaimed or otherwise contaminated waters.

3.3. PPCP and Hormone Accumulation in Lettuce Plants

Greenhouse studies were performed to quantify the uptake of PPCPs and steroid hormones from nutrient solutions into edible plants. Experiments addressed four research objectives: (1) effect of initial PPCP and hormone concentrations in hydroponic solutions on their accumulation in lettuce plants; (2) effect of exposure duration; (3) effect of depuration period; and (4) effect of lettuce plant cultivars. Due to the short cultivation period before lettuce reaches commercial size (~42 d), lettuce makes an ideal model for PPCP and hormone accumulation into leaf vegetables that are typically eaten raw. Two cultivars of lettuce, ‘Green Rex Butterhead’ (GRB) and ‘Red Lollo’ (RL), were utilized to assess Objective 1, 3, and 4 (Figure 5), while experiments for Objective 2 only used GRB.

Table 3. Recoveries, Limits of Detection (LOD), and Limits of Quantification (LOQ) of the targeted PPCPs and hormones in spiked tomato fruit tissue (reported by dry-weight).

Compound	Absolute Recovery (%)	Corrected Recovery (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
Caffeine	79 ± 6	102 ± 6	1.4	4.2
Carbamazepine	103 ± 7	106 ± 4	0.4	1.2
Naproxen	121 ± 18	102 ± 6	1.0	3.0
Ibuprofen	61 ± 2	99 ± 8	0.6	1.8
Gemfibrozil	88 ±17	99 ± 9	0.04	0.12
Triclosan	52 ± 8	93 ± 8	0.8	2.4
Sulfamethoxazole	70 ± 21	102 ± 3	0.08	0.24
Estrone	84 ± 9	98 ± 3	1.5	4.5
17β-Estradiol	91 ± 11	94 ± 7	1.9	5.7
17α-Ethinylestradiol	70 ± 9	101 ± 10	2.6	7.8

Figure 5. Lettuce cultivars used in greenhouse experiments. Left: Green Rex Butterhead. Right: Red Lollo.

3.3.1. Study 1: Effect of Initial Concentrations of PPCPs and Hormones on their Plant Uptake

3.3.1.1. Root Uptake

Lettuce cultivars were exposed for 3 weeks to nutrient solution containing various concentrations of PPCPs and hormones. After exposure to 0.5 μg L^{-1} of each compound, accumulated targeted contaminants in lettuce roots ranged from 8 μg kg^{-1} (dw) (for βE2 in GRB) to 960 μg kg^{-1} (dw) (for SMO in RL) (Figure 6). CAF, NAP, TCS, and SMO were all detected at concentrations > 100 μg kg^{-1} (dw), suggesting these compounds have high potential to

accumulate in plant roots exposed to low levels of PPCPs and hormones. In contrast, hormones were detected at the lowest concentrations in lettuce roots, at levels < 30 μg kg^{-1} (dw).

In experiments with nutrient solution amended with 50 μg L^{-1} of each targeted compound, root concentrations were between 0.16 to 29 mg kg^{-1} (dw) (Figure 7). SMO was most highly accumulated, with a concentration > 15 mg kg^{-1} (dw) in both cultivars, while IBU and CAF were least accumulated, with concentrations < 1 mg kg^{-1} (dw).

For experiments performed at the highest initial spiking concentration (500 μg^{L-1}), the concentrations of PPCPs and hormones in roots ranged between 0.7 to140 mg kg^{-1} (Figure 8). SMO again was the most highly detected compound (> 130 mg kg^{-1} (dw) in each cultivar), which supports previous studies showing SMO has high potential for accumulation in plants (Herklotz et al., 2010). NAP was the second most accumulated contaminant, with concentrations > 100 mg kg^{-1}, while CAF was the least accumulated, with concentrations of only 0.78 to 0.97 mg kg^{-1} in the roots of both lettuce cultivars.

Across the three concentrations of PPCPs and hormones in nutrient solutions, root uptake of each compound increased as spiking concentrations increased. However, these relationships varied among the targeted compounds. For CBZ and SMO, the plant uptakes increased by the same magnitude as the solution concentrations. For example, the concentrations of CBZ in GRB roots were 0.0244, 2.07, and 24.7 mg kg^{-1} (dw) after exposure to 0.5, 50, and 500 μg L^{-1} solutions, respectively (Figure 6-8). Other compounds had also strong relationships between root accumulation and exposure concentrations, though they were not a proportional increase. For instance, NAP and GEM concentrations in plant roots increased only by one order of magnitude between the 0.5 and 50 μg L^{-1} exposure concentrations, but by two orders of magnitude between 50 and 500 μg L^{-1} exposure concentrations. The most complex behavior was observed for CAF, which had relatively high root uptake (0.173 to 0.331 mg kg^{-1}) after exposure to 0.5 μg L^{-1}, but only marginally accumulated greater amounts at 50 and 500 μgL^{-1} (0.643 to 0.732 mg kg^{-1} and 0.775 to 0.972 mg kg^{-1}, respectively), suggesting a rapid root uptake but also readily translocation from roots into leaves (discussed in Sections 3.3.1.2 and 3.3.3.).

After exposure at each of the solution concentrations (0.5, 50, and 500 μg L^{-1}), all targeted PPCPs and hormones were detected in lettuce roots of both cultivars. Experimental controls showed no detectable PPCP and hormone contaminants in lettuce roots or leaves grown in nutrient solutions without PPCP and hormone additions. Small differences were identified in the root accumulation between different lettuce cultivars, but it is unknown whether other leafy vegetables would accumulate PPCPs and hormones into roots to the same extent. Overall, these results support that plants roots can adsorb or take up PPCP and hormone contaminants from irrigation water with environmentally relevant levels of these compounds. Accumulation varies among compounds and some compounds, especially SMO, may pose a high risk of accumulating into plant roots from contaminated irrigation water.

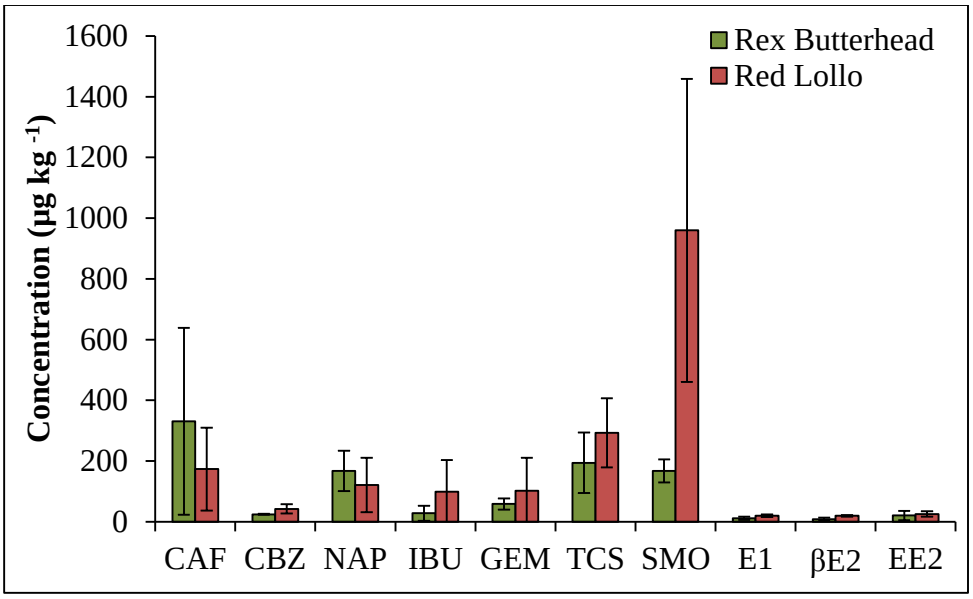


Figure 6. Concentrations of PPCP and hormone contaminants in lettuce roots (dw) after three weeks of exposure to nutrient solution containing each compound at 0.5 µg L^{-1}.

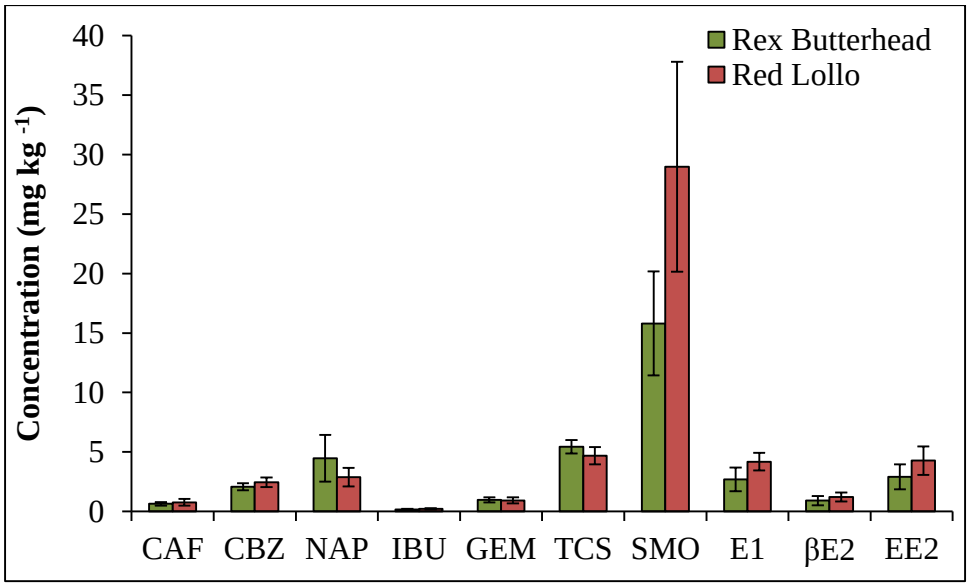


Figure 7. Concentrations of PPCP and hormone contaminants in lettuce roots (dw) after three weeks of exposure to nutrient solution containing each compound at 50 µg L^{-1}.

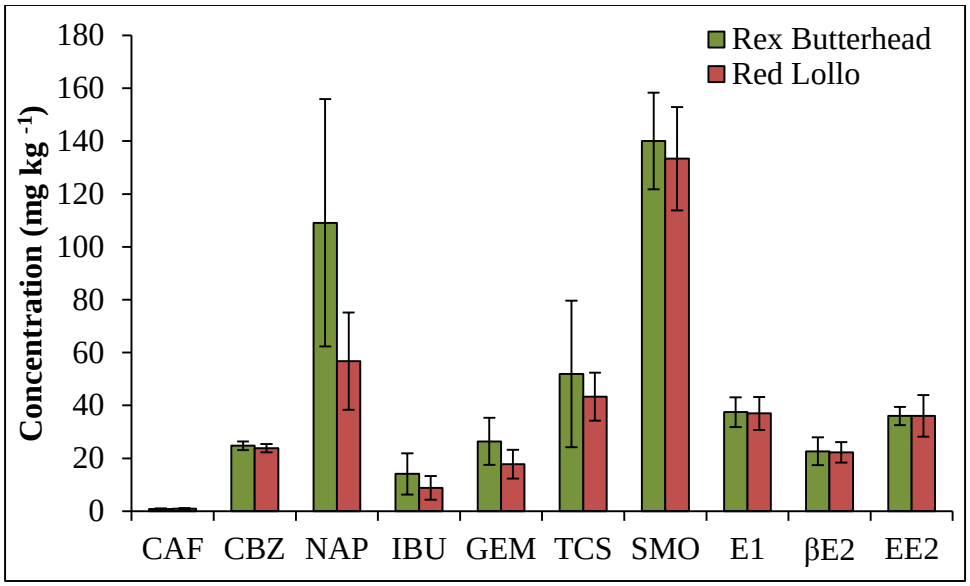


Figure 8. Concentrations of PPCP and hormone contaminants in lettuce roots (dw) after three weeks of exposure to nutrient solution containing each compound at 500 μg L^{-1}.

3.3.1.2. Leaf Accumulation

To examine concentration effects, lettuce plants were cultivated for a total of three weeks in nutrient solutions spiked with targeted PPCPs and hormones at 0.5, 50, or 500 μg L^{-1}. Most PPCPs were detected in lettuce leaves of GRB and RL after exposure at 0.5 μg L^{-1} (Figure 9), with concentrations ranging from 3.04 μg kg^{-1} (dw) for NAP in GRB to 132.6 μg kg^{-1} (dw) for SMO in RL. SMO, CAF, and CBZ were the most accumulated compounds. In contrast, TCS, E1, βE2, and EE2 were not detected in lettuce leaves for this treatment concentration (Figure 9).

After exposure to 50 μg L^{-1} of each targeted compound (Figure 10), lettuce leaves had accumulated between 9.0 μg kg^{-1} (dw) for EE2 in GRB up to 7.1 mg kg^{-1} (dw) for CBZ in RL. At this exposure level, CBZ was accumulated the most and had concentrations > 5 mg kg^{-1} (dw) in lettuce leaves of both cultivars. Many compounds showed only slightly raised levels compared to their accumulation from solution with 0.5 μg L^{-1} amendment, while TCS and βE2 were still not detectable in lettuce leaves (Figure 10).

After lettuce exposure to solution with 500 μg L^{-1} of each compound, CBZ had the highest concentration in leaves of both cultivars, with levels 51.8 to 77.9 mg kg^{-1} (dw) (Figure 11). Even at this elevated solution concentration, accumulation of many PPCPs and hormones did not significantly increase, suggesting these compounds are poorly translocated to leaves or readily metabolized within leaves. Comparing GRB and RL cultivars, RL plants consistently had higher leaf accumulation of PPCP and hormone contaminants than GRB across the range of exposure concentrations. This occurrence deserves further investigation to determine the cause of this trend, which may be related to growth behavior, transpiration needs, or translocation mechanisms.

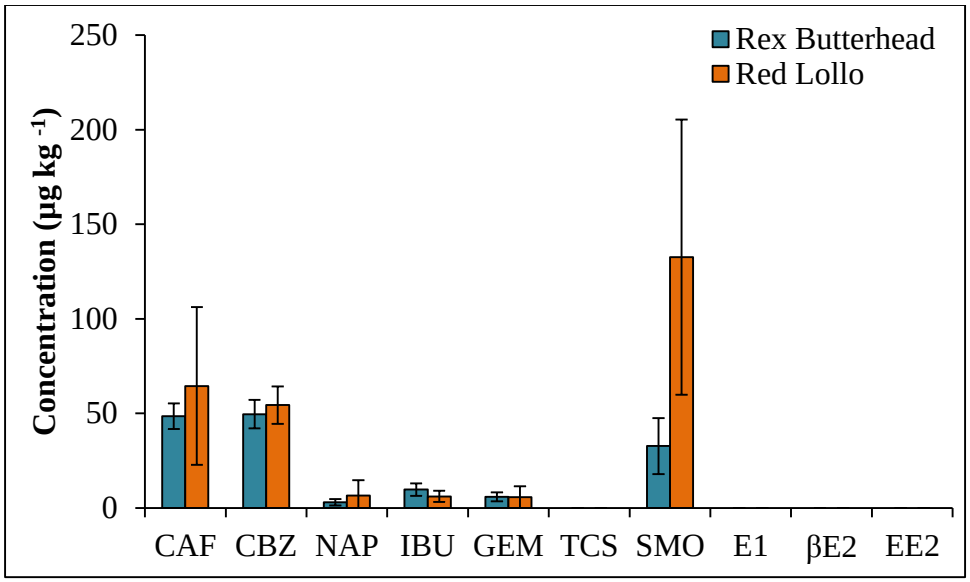


Figure 9. Concentrations of PPCP and hormone contaminants in lettuce leaves (dw) after three weeks of exposure to nutrient solution containing each compound at 0.5 µg L^{-1}.

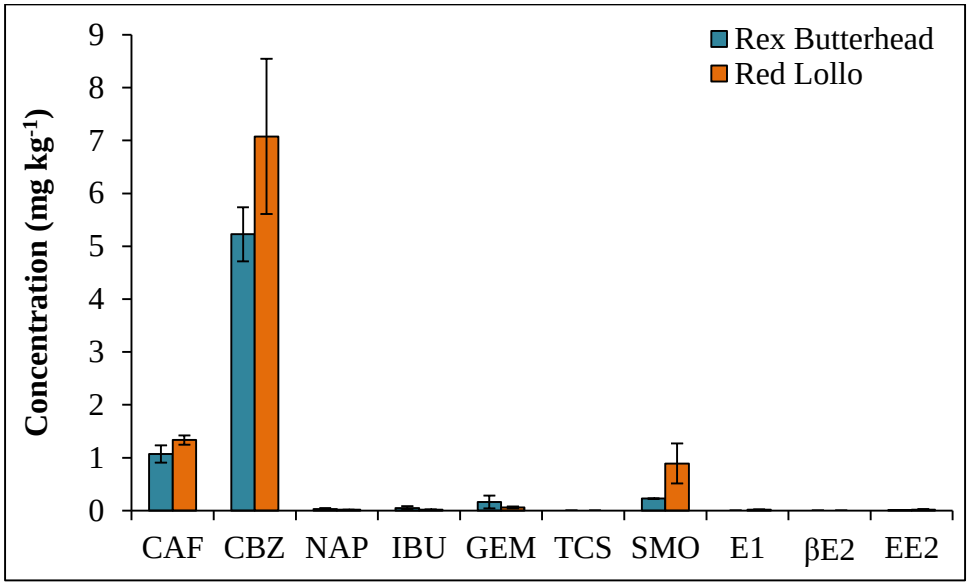


Figure 10. Concentrations of PPCP and hormone contaminants in lettuce leaves (dw) after three weeks of exposure to nutrient solution containing each compound at 50 µg L^{-1}.

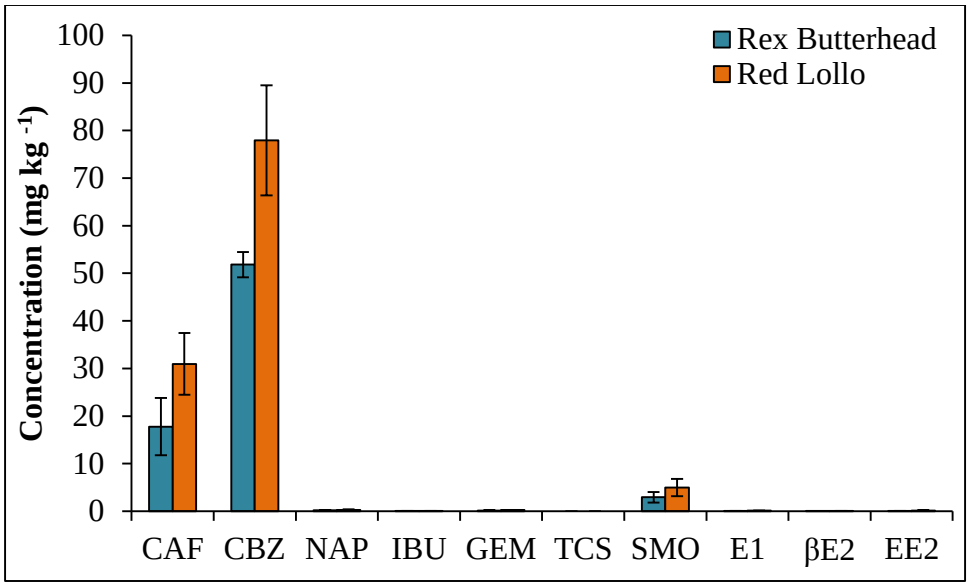


Figure 11. Concentrations of PPCP and hormone contaminants in lettuce leaves (dw) after three weeks of exposure to nutrient solution containing each compound at 500 µg L^{-1}.

Relationships between exposure concentration and accumulation in leaves were also assessed. For leaf tissue, only CBZ concentrations increased at the same proportion as solution concentrations increased. For example, in GRB CBZ leaf concentrations were 0.0496 mg kg^{-1}, 5.23 mg kg^{-1}, and 51.8 mg kg^{-1} when exposed to 0.5 µg kg^{-1}, 50 µg kg^{-1}, and 500 µg kg^{-1}, respectively. In contrast, SMO, CAF, and GEM exhibited increased accumulation with increased solution concentration, but it was not proportional. All other compounds showed little relationship between solution concentrations and leaf accumulation, suggesting that translocation of PPCPs and hormones from root tissue to leaf is likely controlled by plant tissue and metabolism processes.

Unlike lettuce roots, where all targeted PPCPs and hormones were detected and sometimes at high levels, only some PPCPs and hormones were detected in lettuce leaves. Previous studies have shown that many factors impact the uptake and translocation of organic compounds within plants, including chemical hydrophobicity and ionization (Dodgen et al., 2015; Herklotz et al., 2010; Trapp, 2004). For example, significantly greater accumulation in leaves was observed for neutral compounds than anionic compounds in collard plants (Dodgen et al., 2013), because molecular ionization of organic compounds may reduce their ability to permeate cell membranes and thereby result in a reduced internal transfer potential (Trapp, 2004). Because CAF and CBZ are not ionized at neutral pH, while the other studied PPCPs are partly ionized (Table 1), it is reasonable to expect these compounds would have the highest leaf accumulation (Figure 9-11).

Accumulation of PPCP and hormone contaminants into lettuce leaves is an issue of food security as humans may be exposed to these compounds through dietary consumption of these edible parts. These results suggest that only some compounds are likely to accumulate into lettuce leaves due to their chemical properties, and that risk assessments of dietary exposure should include CAF and CBZ for leaves and SMO for roots. Considering the relatively high

accumulation of SMO in both root and leaf tissues, it might be investigated as a potential marker for emerging contaminant accumulation into leafy food plants.

3.3.1.3. Bioaccumulation Factors

Bioaccumulation Factors (BAFs) are defined as the ratio of detected concentrations (dw) of targeted compounds in the plant tissues to their initial concentrations in the nutrient solutions. In this study, BAF values represent accumulation capacities of PPCPs and hormones in lettuce roots or leaves after three weeks of exposure to contaminating nutrient solutions.

$$\text{BAF} = \frac{\text{Concentration of PPCP or hormone in plant part } (\mu g\ kg^{-1}, \text{dw})}{\text{Concentration of PPCP or hormone in initial solution } (\mu g\ L^{-1}, \text{dw})} \quad (1)$$

BAFs after exposure to 0.5 μg L^{-1} of each targeted compound in solutions are shown in Figure 12. At this exposure level, root BAFs ranged from 16.3-1,920, but most were under 400. The extreme high BAF value was for SMO in RL, while the lowest values were contributed by hormones. For leaf tissue, BAFs ranged from 0 to 265, with hormones and TCS showing no leaf accumulation and SMO again being most accumulated. SMO had high BAFs in both leaf and root tissues because it is predominantly neutral at neutral pH and has low hydrophobicity (Table 1), which facilitates its transfer through plant cell membranes (Dodgen et al., 2015; Trapp and Legind, 2011). These high BAFs suggest that SMO should be prioritized for further research as a marker for emerging contaminant accumulation into food crops and evaluation of human risks.

After exposure to 50 μg L^{-1}, BAF values ranged from 3.2 to 580 in roots and 0 to 105 in leaves (Figure 13). Compared to BAFs for 0.5 μg L^{-1} exposure, these BAFs were similar (for CBZ, SMO, E1, βE2, and EE2) or much lower (for other PPCPs). For instance, the BAF value of CAF in GRB was 662 in the 0.5 μg L^{-1} exposure and decreased to 12.9 in the 50 μg L^{-1} exposure.

BAF values after exposure to 500 μg L^{-1} of PPCPs and hormones ranged from 1.6 to 280 in roots and 0 to 156 in leaves (Figure 14), and overall were similar to BAFs for 50 μg L^{-1} exposures (Figure 13). Except for CAF and CBZ, the BAF values in lettuce roots of all targeted compounds for two of the exposure concentrations (50 and 500 μg kg^{-1}) were much higher than those in leaves (Figure 13 and 14).

All BAF values of PPCPs and hormones in lettuce roots were more than 1 (Figure 12-14), suggesting that while many of these compounds are present at low levels in reuse water, they have potential to accumulate in plant roots to much higher levels. Potentially high root accumulation should be taken into account for any safety thresholds developed for emerging contaminants in plants. Also, further study of edible root plants, such as carrots or sweet potatoes, is necessary to assess whether these plants accumulate PPCPs and hormones to the same extent.

In lettuce leaves, BAF values were generally low for all targeted compounds except three PPCPs: CBZ, SMO, and CAF. This indicates that these three PPCPs are readily accumulated in lettuce leaves and may pose a potential risk to public health through consumption of this leafy vegetable. Therefore, the potential occurrence of these PPCPs in leafy vegetables including lettuces, cabbages, and spinaches irrigated with reclaimed water needs to be preferentially investigated.

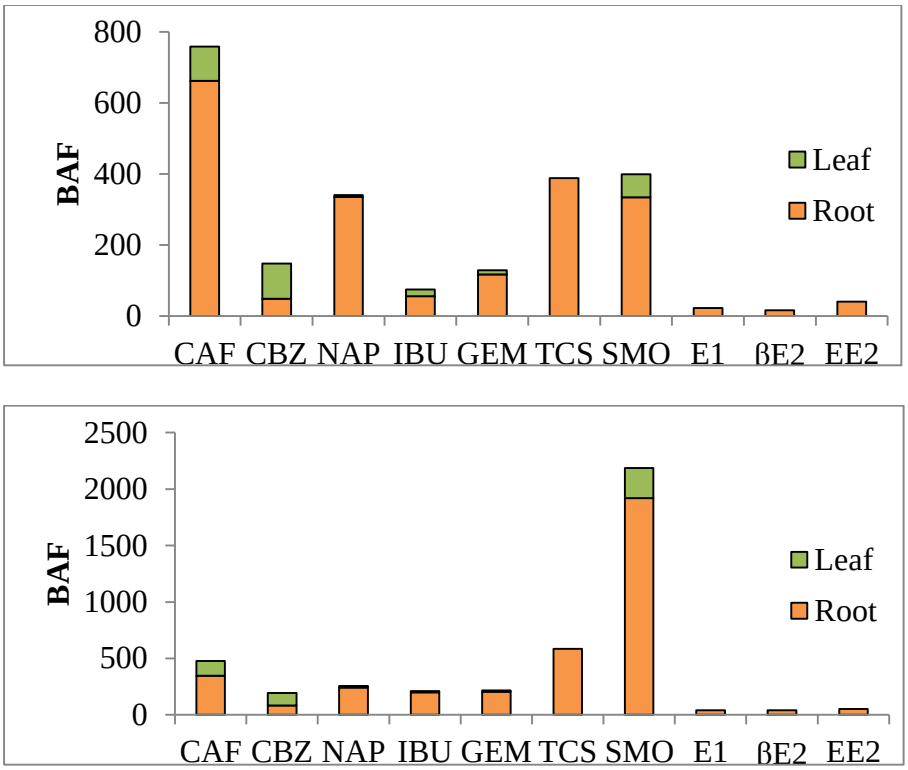


Figure 12. Bioaccumulation factors for PPCPs and hormones into lettuce tissues after exposure to 0.5 μg L^{-1} concentration in nutrient solutions. Top: GRB lettuce, Bottom: RL lettuce.

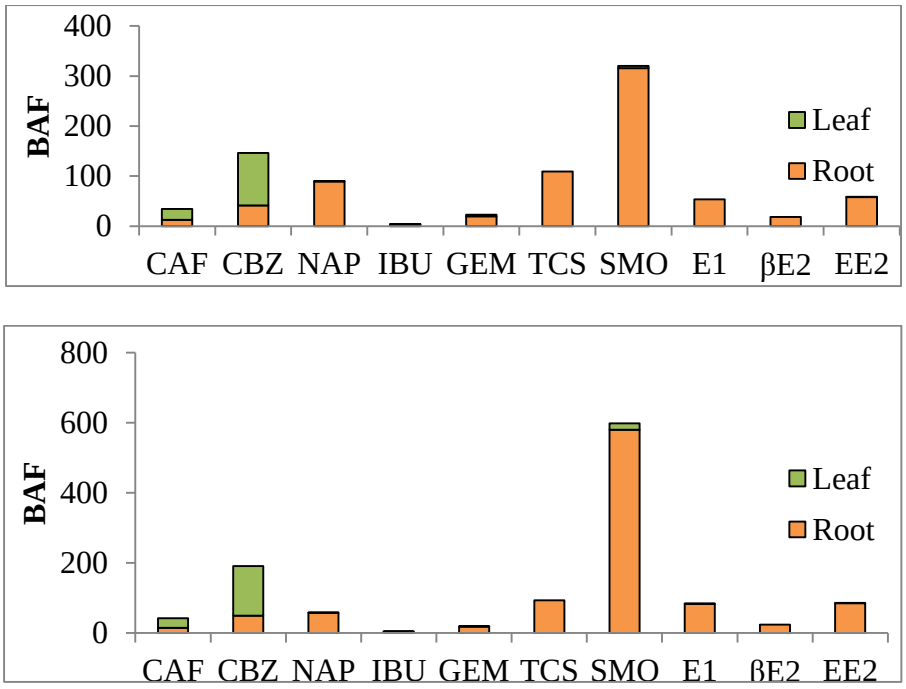


Figure 13. Bioaccumulation factors for PPCPs and hormones into lettuce tissues after exposure to 50 μg L^{-1} concentration in nutrient solutions. Top: GRB lettuce, Bottom: RL lettuce.

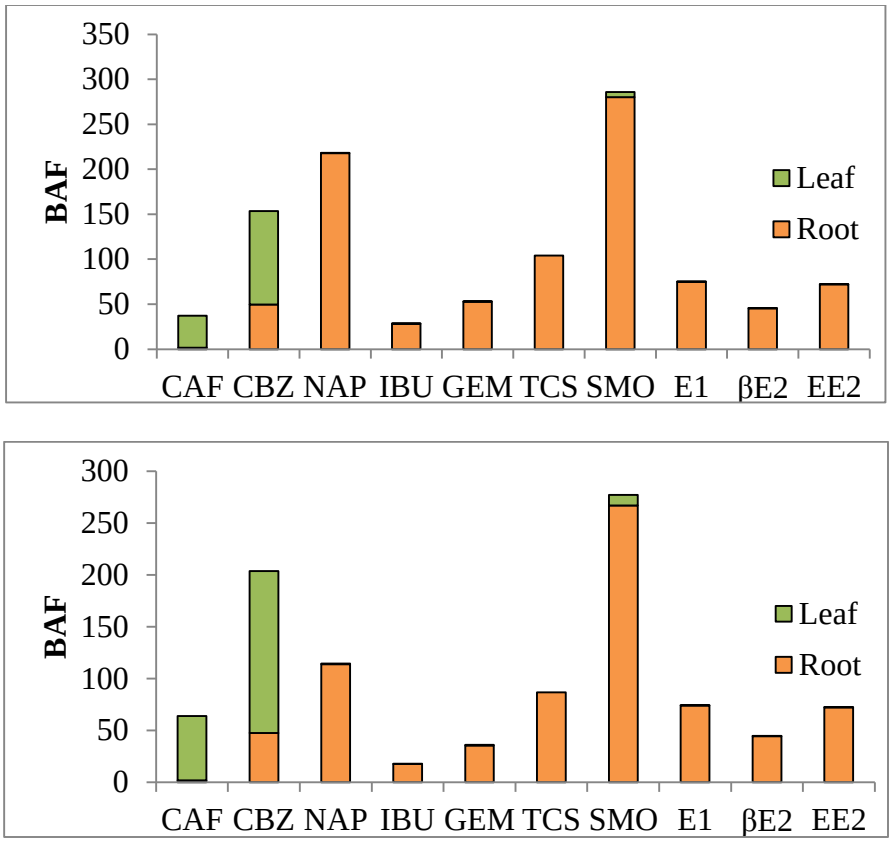


Figure 14. Bioaccumulation factors for PPCPs and hormones into lettuce tissues after exposure to 500 μg L^{-1} concentration in nutrient solutions. Top: GRB lettuce, Bottom: RL lettuce.

3.3.1.4. Translocation Factors

In order to uniformly evaluate translocation of PPCPs and hormones from roots to leaves in both lettuce cultivars, a translocation factor (TF) was calculated for each compound based on its amounts in leaves relative to those in roots.

$$TF = \frac{\text{Concentration of PPCP or hormone in leaf (μg kg}^{-1}\text{, dw)}}{\text{Concentration of PPCP or hormone in root (μg kg}^{-1}\text{, dw)}} \quad (2)$$

TF values for each compound in lettuce plants are shown in Figure 15. Except for CAF and CBZ, the calculated TF values of the other PPCPs and hormones were very small (<<1) in both lettuce cultivars, suggesting poor translocation of these chemicals from roots to leaves after uptake. Considering that these chemicals preferentially accumulate in plant roots as compared to above-ground parts, the potential risk for human consumption from those contaminants may be significantly greater for root vegetables such as radishes and carrots.

On the other hand, most TF values of CAF and CBZ for both lettuce cultivars were greater than 1 (Figure 15), suggesting that these two PPCPs can easily translocate from plant roots to leaves via water transpiration. A previous study illustrated that the translocation of non-ionized chemicals from plant roots into shoots is a passive process that occurs in proportion to

the amount of water transpired (Briggs et al., 1982). The TF values of CAF into lettuce increased by an order of magnitude with each increase in solution concentration, such as in GRB where TF values were 0.14, 1.67, and 22.94 at 0.5, 50, and 500 μg L^{-1}, respectively. In contrast, the TF values of CBZ generally remained the same across solution concentrations (1.29 to 3.27 in both cultivars), which suggests that leaf and root concentrations would increase at the same rate with increasing exposure. This difference between TFs in CAF and CBZ may be due to lower hydrophobicity of CAF, which may lower its ability to transfer into phloem and leaf aerial tissue and redistribute to root tissue, whereas CBZ has moderate hydrophobicity that would facilitate its transfer across all plant tissues (Briggs et al., 1982; Trapp and Legind, 2011).

Other PPCP compounds, including NAP, IBU, GEM, and SMO were found at TF values < 1, indicating that their contamination was mainly relegated to the roots of the lettuce plants. This is likely due to the fact that these compounds are partly ionized at a neutral pH, which would limit their ability to transfer through cell walls in the plant (Dodgen et al., 2015; Trapp, 2000). This interpretation is consistent with previous research which indicated that molecular ionization of organic compounds may reduce their ability to permeate cell membranes and thereby result in a reduced internal transfer potential (Trapp, 2004). In addition, the results from this study also showed that, as solution concentration increased, TF values for all of these compounds decreased, suggesting that translocation is rate-limited inside the plant. For instance, SMO in RL had a TF value of 0.14 after exposure to 0.5 μg L^{-1} solutions but a TF value of only 0.04 after exposure to 500 μg L^{-1} solutions.

The behavior of TCS was unique among the targeted PPCPs and hormones in that no accumulation was detected in lettuce leaves at even the highest exposure rate, leading to TF values of 0 for all treatments. Considering the high hydrophobicity of TCS and its undetectable translocation, it is likely that TCS was only poorly taken up by plant roots and that the majority of the root concentration was caused by TCS adsorbed onto the root exterior. If other hydrophobic emerging contaminants act in this manner, it is possible that mechanical methods of cleaning root vegetables, such as peeling carrots, may be useful in minimizing human exposure to these particular compounds.

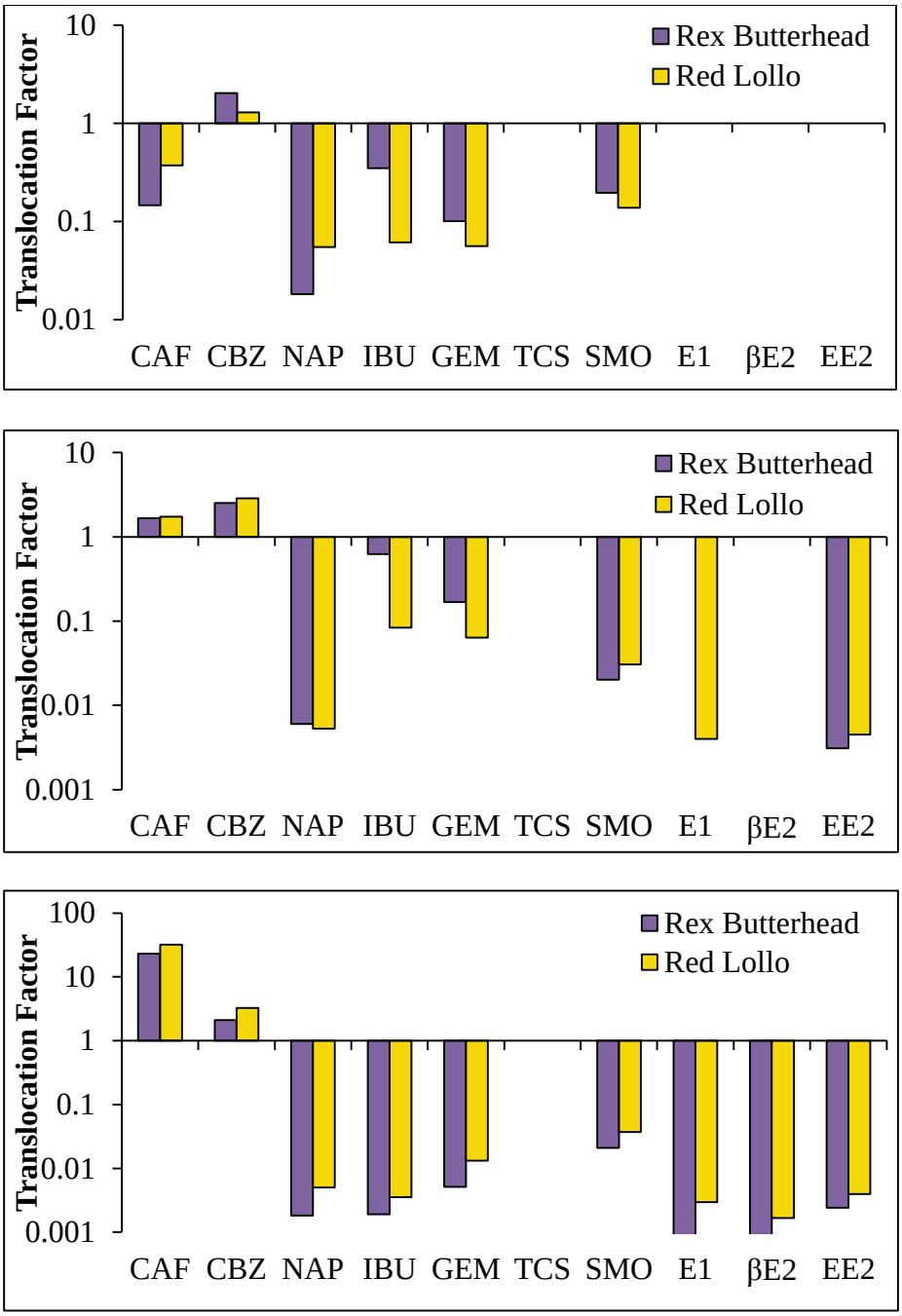


Figure 15. Translocation factors of PPCPs and hormones from roots to leaves of lettuce grown in solutions containing emerging contaminants at: Top: 0.5 µg L^{-1}; Middle: 50 µg L^{-1}; and Bottom: 500 µg L^{-1}.

3.3.2. Study 2: Effect of Exposure Duration

In this study, 20 GRB lettuce plants were grown for three weeks in nutrient solutions spiked with 50 μg L^{-1} of each targeted compound. After each week of growth in the spiked solutions, a third of the plants were harvested for analysis of the accumulation of PPCPs and hormones in their leaves and roots (Figure 16). Results showed that most compounds had similar concentrations in the leaves or roots, regardless of exposure length. For example, CAF was detected at 1,084, 1,148, and 1,152 μg kg^{-1} in lettuce roots after one, two, and three weeks, respectively. This result indicates that in less than one week, accumulation of PPCPs and hormones may reach a steady level in lettuce plants with exposure through contaminated water. The weight of leaf biomass was also measured for each treatment group, and total dry weight was 29.1, 70.8, and 237.0 g for plants harvested at one, two, and three weeks, respectively. By comparing the concentrations of PPCPs and hormones in leaf biomass for each treatment, it was observed that targeted compounds accumulated in leaf tissue at a similar rate as overall plant growth, effectively preserving overall contaminant concentration.

The stable compound concentrations during the growth period show that the plants were continually taking up contaminants for the duration of exposure. It is likely that ongoing accumulation in leaves occurred in proportion to the increased transpiration needs of a larger plant, and not to a coincidental similarity between contaminant uptake rate and plant growth rate. Indeed, it has been suggested previously that transpiration is the primary cause of contaminant translocation to aerial plant tissues (Dodgen et al., 2015; Trapp and Legind, 2011). As plant transpiration increases with growth, it is probable that contaminant accumulation could continue throughout the growth period for a variety of plants. Accordingly, this could potentially result in concentrations of biological relevance to human health. This relationship would simplify some aspects of modeling human exposure, as plant age could be omitted. However, it is unclear from this study what effect timing of exposure has and whether plants may depurate accumulated PPCPs and hormones if exposure ceased previous to harvest.

TFs in this study were calculated according to Equation (*2*) and were similar among treatment groups (Figure 17). However, NAP, IBU, and GEM exhibited decreasing TFs across exposure length, suggesting that they may be taken into roots faster than they translocate to leaves, in agreement with observations in Section 3.1.1.

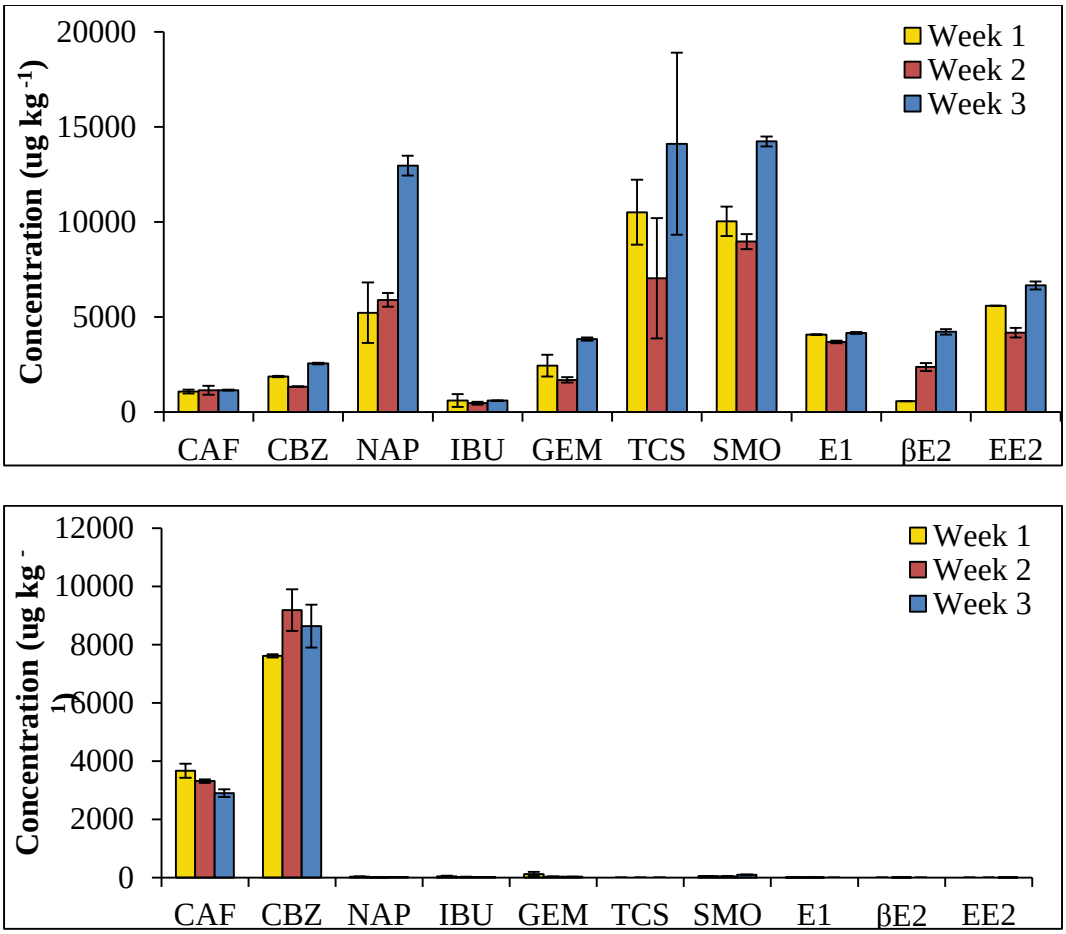


Figure 16. Accumulation of PPCPs and hormones into GRB lettuce tissue after one, two, or three weeks of growth in nutrient solutions amended with 50 µg L^{-1} of each compound. Top: roots. Bottom: leaves.

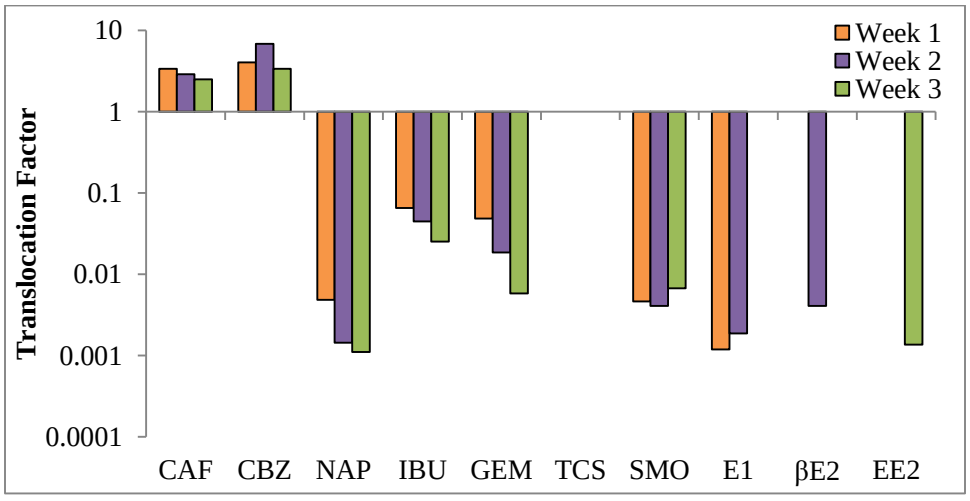


Figure 17. Translocation factors for PPCPs and hormones in GRB lettuce after exposure to 50 µg L^{-1} of each compound for one, two, or three weeks.

3.3.3. Study 3: Effect of Exposure Timing and Depuration

This study investigated the impact of exposure timing on accumulation of PPCPs and hormones in lettuce. Two groups of 20 lettuce plants each were grown in nutrient solutions. One treatment group was grown in nutrient solutions spiked with each targeted compound at 50 µg L^{-1} for 1.5 weeks, and then grown in unspiked nutrient solutions for a further 1.5 week period (hereafter referred to as the "Beginning" treatment). The second treatment group was grown in unspiked nutrient solutions for 1.5 weeks and then in spiked solutions for 1.5 weeks ("End" treatment). In these experiments, exposure to PPCPs and hormones was divided into two separate hydroponic systems to determine which types of compounds were the cause of a hormetic increase in microbial growth in the nutrient solutions that had been observed.

Lettuce plants were harvested after a total of three weeks of treatment. The concentrations of PPCPs and hormones were measured in roots and leaves. Plants in the End treatment (i.e., exposed close to harvest) had substantially greater root concentrations of almost all PPCPs than plants in the Beginning treatment (Figure 18). For example, IBU was detected in RL roots at 55 µg kg^{-1} in the Beginning plants, but at 737 µg kg^{-1} in End plants. In contrast, hormones (E1, βE2, and EE2) and TCS were detected at similarly high root concentrations in both treatments.

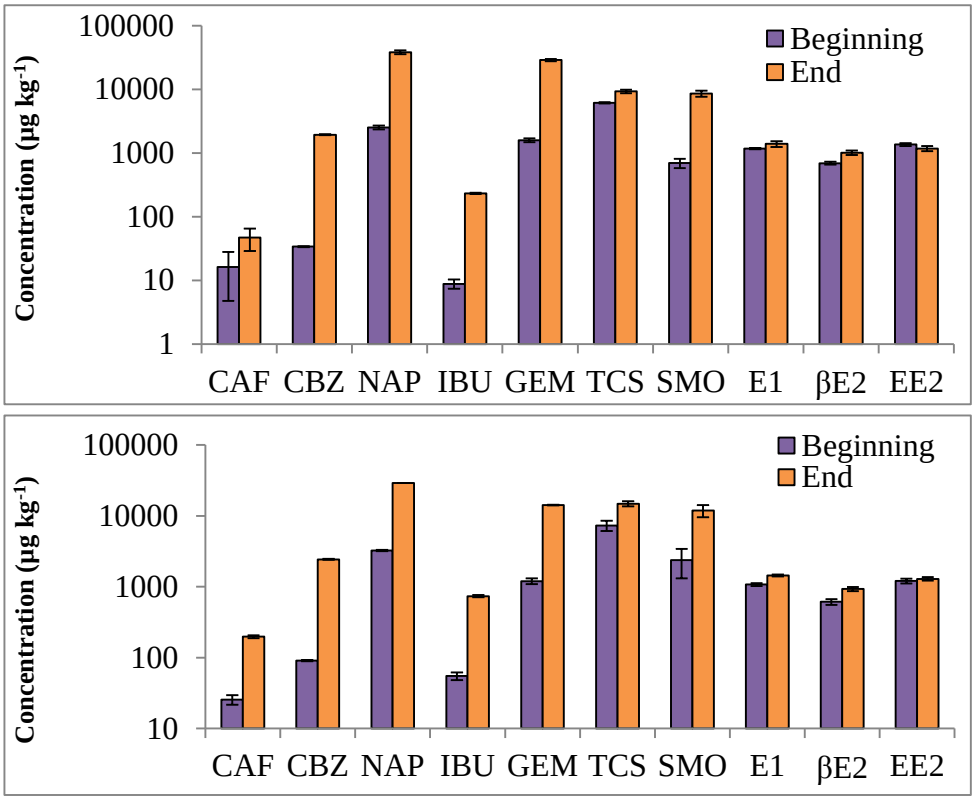


Figure 18. Accumulation of PPCPs and hormones into lettuce roots following exposure to 50 µg L^{-1} at the Beginning or End of the treatment period. Top: GRB lettuce. Bottom: RL lettuce.

PPCPs also accumulated in lettuce leaves in both treatments (Figure 19). A few compounds were generally not detectable in lettuce leaves, namely TCS, E1, βE2, and EE2. For the remaining compounds, leaf concentrations were greater in End plants than Beginning plants, although this difference was not as large as for roots. For example, IBU was detected in GRB at 11.3 μg kg^{-1} in the Beginning plants and at 37.8 μg kg^{-1} in End plants. An exception to this was SMO, which was detected at similar leaf concentrations in both treatments.

The generally higher concentrations of PPCPs and hormones in plants that were recently exposed (the End treatment) may be attributed to the depuration of early contaminant residues through plant metabolism for the Beginning treatment. Plants are able to metabolize xenobiotics through processes of conjugation, catabolism, and binding to tissues (Collins et al., 2011; McCutcheon and Schnoor, 2004), and some work has found evidence of these processes for chemical contaminants (Bokern and Harms, 1997; Macherius et al., 2012). Also, it must be noted that PPCPs and hormones that have been taken up by the Beginning plants may be secreted by the plants into the unspiked nutrient solutions and thereby decrease their accumulation in the plants. However, no PPCP and hormone residues were detected in the unspiked nutrient solutions for the Beginning treatment. Therefore, further work is needed to assess the capacity of plants to metabolize and bind PPCPs or hormones in order to evaluate food safety. This depuration observed may be an approach to reduce emerging contaminant load in food crops by irrigating with high-grade water for a brief period of time before harvest. But the metabolic products of these compounds should also be studied to assess their toxicity relative to their parent compounds.

TFs were calculated for each treatment according to Equation (*2*); some compounds were not detectable in leaves and had TF values of zero. In this study, TFs were almost entirely greater for Beginning plants than End plants (Figure 20). For example, CBZ had TF values of 36.1 for RL Beginning plants, but only 8.9 for RL End plants. The only exception was for CAF, where TFs were similar across treatments (TF = 8.7 to 16.1).

The higher TF values in the Beginning plants for most PPCPs suggests that translocation may be rate-limited compared to root uptake from nutrient solutions. This relationship would require more time for PPCPs to move up to leaf tissues, which is supported by higher TF values in plants exposed earlier. Section 3.3.1 discussed the likelihood that CAF experiences rapid translocation from roots to leaves after root uptake, as evidenced by increasing TF values with increasing solution concentrations. This rapid translocation would explain the similar TF values of CAF for plants in this study and support the hypothesis that other compounds may experience slower translocation. This area deserves further study due to its implications for managing irrigation with reuse water. Current information on PPCP and hormone accumulation by food crops is frequently based on laboratory-scale studies that grow plants for a limited time-frame, which may not encompass the behavior of field plants exposed to emerging contaminants for longer periods, such as slow translocation into leaf tissue.

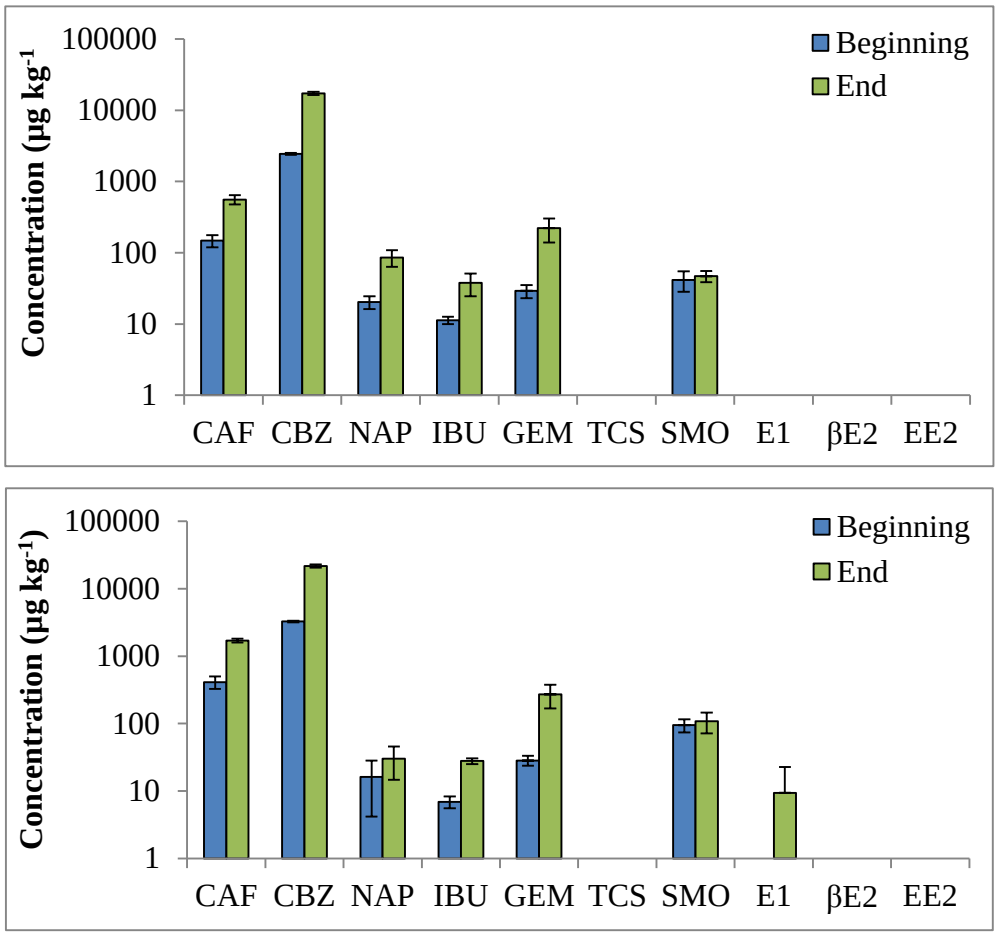


Figure 19. Accumulation of PPCPs and hormones into lettuce leaves following exposure to 50 µg L^{-1} at the Beginning or End of the treatment period. Top: GRB lettuce. Bottom: RL lettuce.

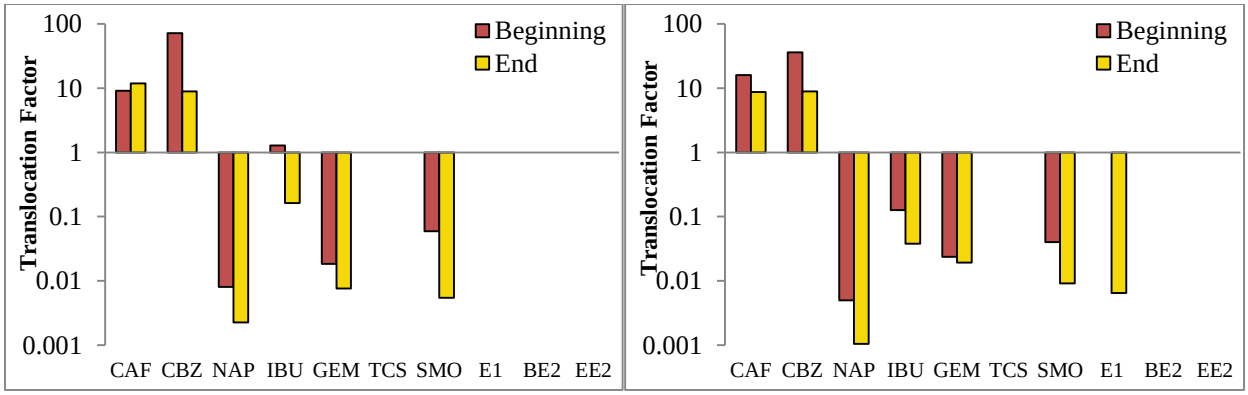


Figure 20. Translocation factors for PPCPs and hormones after exposure to 50 µg L^{-1} of each compound at either the Beginning or End of a three-week period. Left: GRB; Right: RL.

3.4. PPCP and Hormone Accumulation in Tomato Plants

3.4.1. Concentrations of PPCPs and Hormones in Plant Tissues

Fruiting tomato plants of two cultivars, 'Cherry Cascade' (CC) and 'Tiny Tim' (TT), were grown for five weeks in nutrient solution amended with 0.5, 50, or 500 μg L^{-1} of each compound. Roots, leaves, stems, and fruits were then harvested (Figure 3) to analyze for accumulated PPCPs and steroid hormones.

3.4.1.1. PPCP Accumulation

All PPCPs were detected in tomato roots after exposure at 0.5 μg L^{-1} (Figure 21), which shows that these compounds are likely to accumulate in plant roots when present at levels in reuse water. Concentrations for most PPCPs were below 200 μg kg^{-1} (dw), with the exception of TCS, which had very high root concentrations of 800.0 μg kg^{-1} (dw) in CC and 1732.3 μg kg^{-1} (dw) in TT.

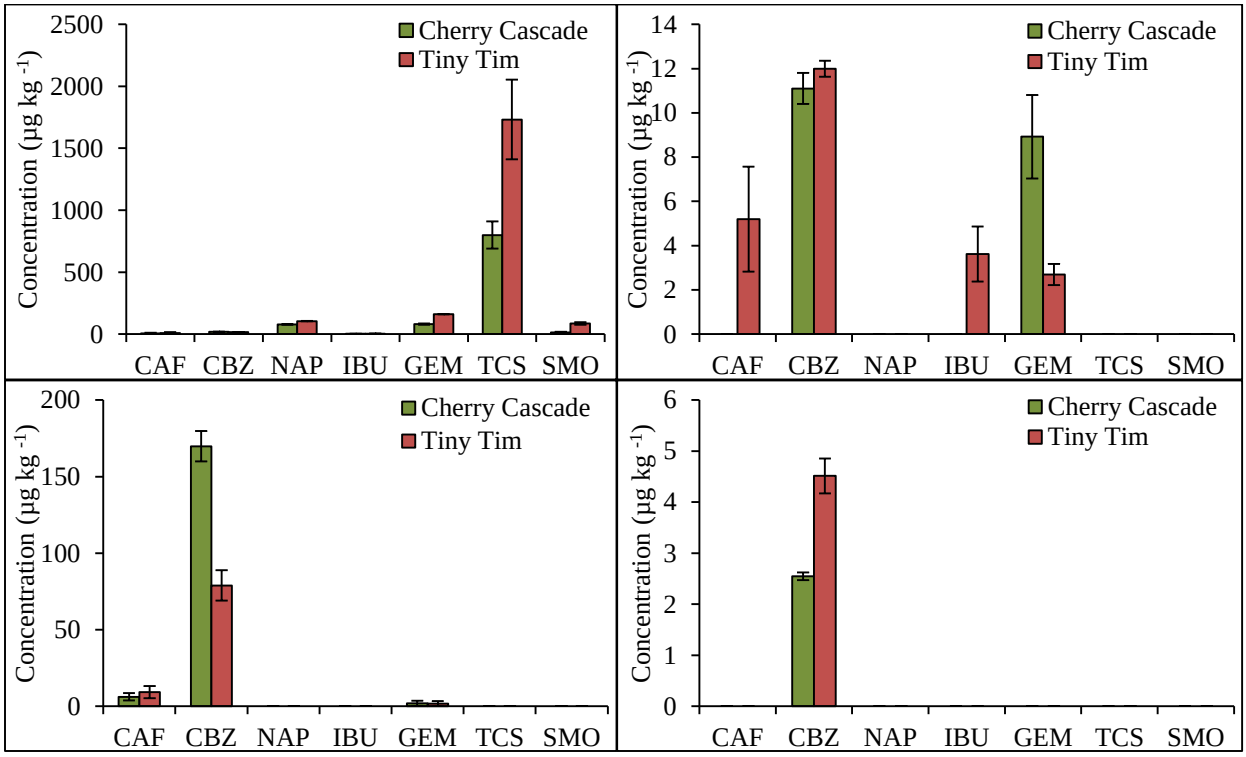


Figure 21. Accumulation of PPCPs into CC or TT tomato plants after exposure to 0.5 μg L^{-1} in nutrient solution. Top-left: root. Top-right: stem. Bottom-left: leaf. Bottom-right: fruit.

Accumulation of PPCPs into stems, leaves, and fruits was more selective than into roots for all exposure levels. Only four PPCPs were found both in roots and stems (CAF, CBZ, IBU, and GEM). CAF, CBZ, and IBU were found at similar levels in the stems and roots (Figure 21). For example, CAF was measured at 5.2 and 8.2 μg kg^{-1} (dw) in TT stems and roots, respectively. In contrast, GEM levels were much lower in stems (8.9 μg kg^{-1} (dw) in CC) compared to roots (80.7 μg kg^{-1} (dw) in CC).

In leaves, only CAF, CBZ, and GEM were found (Figure 21). Concentrations of GEM continued to decrease with decreasing proximity to roots, while CBZ was found at much higher levels in leaves than in roots, similar to its behavior in lettuce plants. In CC leaves, CBZ was measured at 169.8 μg kg^{-1} (dw) while it only accumulated to 18.8 μg kg^{-1} (dw) in roots. In contrast to its behavior in lettuce plants, CAF was found at similar levels in roots, stems, and leaves.

In the fruit, only CBZ was detectable, with concentrations ranging from 2 to 5 μg kg^{-1} after exposure to only 0.5 μg L^{-1}. Fruit accumulation is expected to be related to a compound's ability to partition into phloem (Trapp and Legind, 2011). As shown in Figure 21, CBZ seems to easily translocate in the tomato plants and has the greatest potential for fruit accumulation compared to other targeted PPCPs. Further research is warranted to determine if the accumulation of CBZ is principally caused by its ability to readily distribute and whether a recalcitrance to plant metabolism is also involved. Overall, at this environmentally-relevant exposure level, few PPCPs accumulated to high levels, with the exception of TCS in roots.

When exposed to nutrient solution with 50 μg L^{-1} of each targeted compound, concentrations of PPCPs in roots ranged from 10.8 μg kg^{-1} (dw) for CAF in TT to 20.9 mg kg^{-1} (dw) for SMO in CC (Figure 22). Overall, a wider selection of PPCPs accumulated to high levels compared to solutions amended with 0.5 μg L^{-1}, where only TCS exhibited high accumulation. In stems, leaves, and fruit, CBZ was the most accumulated PPCP (as it also was for the lower exposure level). For example, in stems CBZ was found at 1209.7 to 1504.5 μg kg^{-1} (dw), while concentrations of any other PPCPs were < 48 μg kg^{-1} (dw) (Figure 22).

After exposure to 500 μg L^{-1} of each compound, tomato plants accumulated very high levels of some compounds (Figure 23). All compounds were detected in roots, and GEM had the highest concentration at 105.1 to 156.3 mg kg^{-1} (dw). This result contrasts with other exposure levels where SMO or TCS were the compounds that were accumulated the most for the 50 or 0.5 μg L^{-1} solutions, respectively. This variation shows that PPCP concentration may be an important factor in root uptake and accumulation behavior, and it should be considered when developing risk assessment methods for PPCP uptake by plants.

To summarize PPCP detection in fruits, after exposure to environmentally-relevant levels (0.5 μg L^{-1}) only CBZ was detected in tomato fruits. However, after exposure to 50 μg L^{-1}, NAP, IBU, GEM, and SMO were also detected in tomato fruit, and exposure to 500 μg L^{-1} caused detectable accumulation of CAF as well, showing that sufficiently high exposure can cause accumulation of a variety of PPCPs into edible fruits.

Comparing various plant compartments, roots clearly accumulate greater levels of most PPCPs than stems, leaves, or fruits. The exception to this trend was the high accumulation of CAF and CBZ in leaves compared to their concentrations in tomato roots. In particular, CBZ was detected as the most highly accumulated compound in tomato stems, leaves, and fruits, suggesting that this contaminant has more potential for translocation in tomato plants compared to other targeted PPCPs.

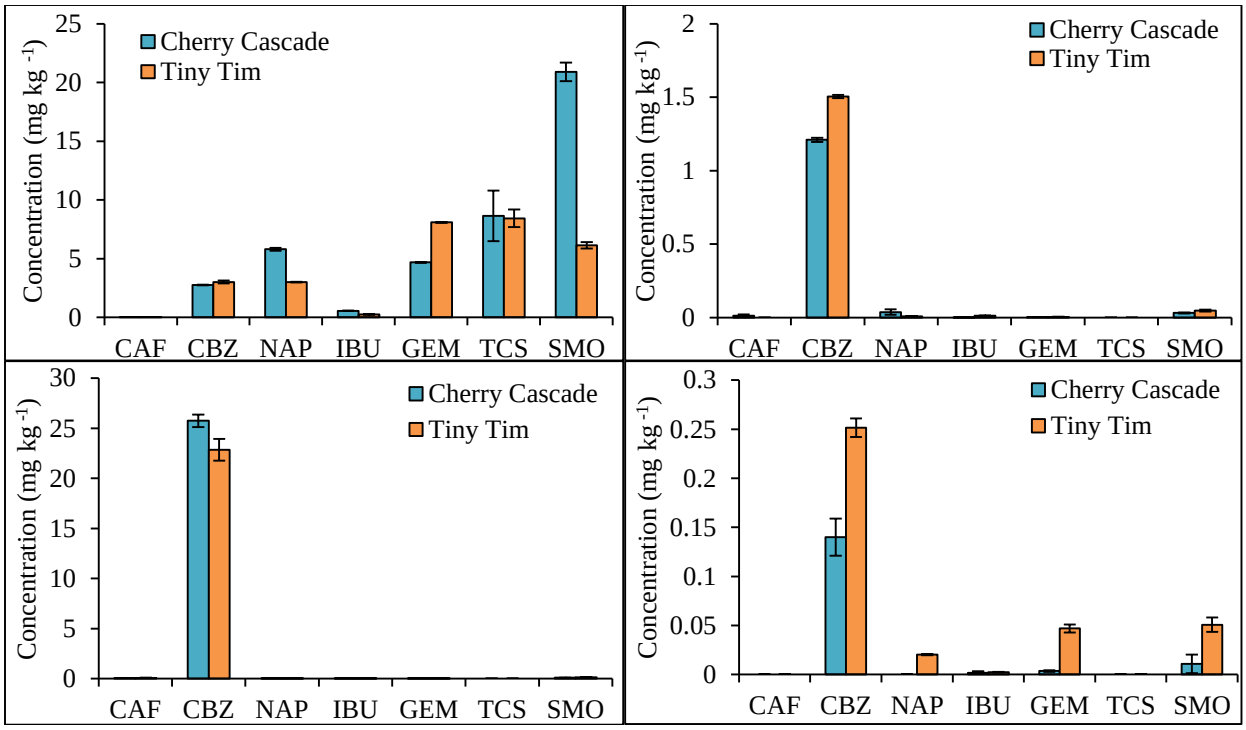


Figure 22. Accumulation of PPCPs into CC or TT tomato plants after exposure to 50 µg L^{-1} in nutrient solution. Top-left: root. Top-right: stem. Bottom-left: leaf. Bottom-right: fruit.

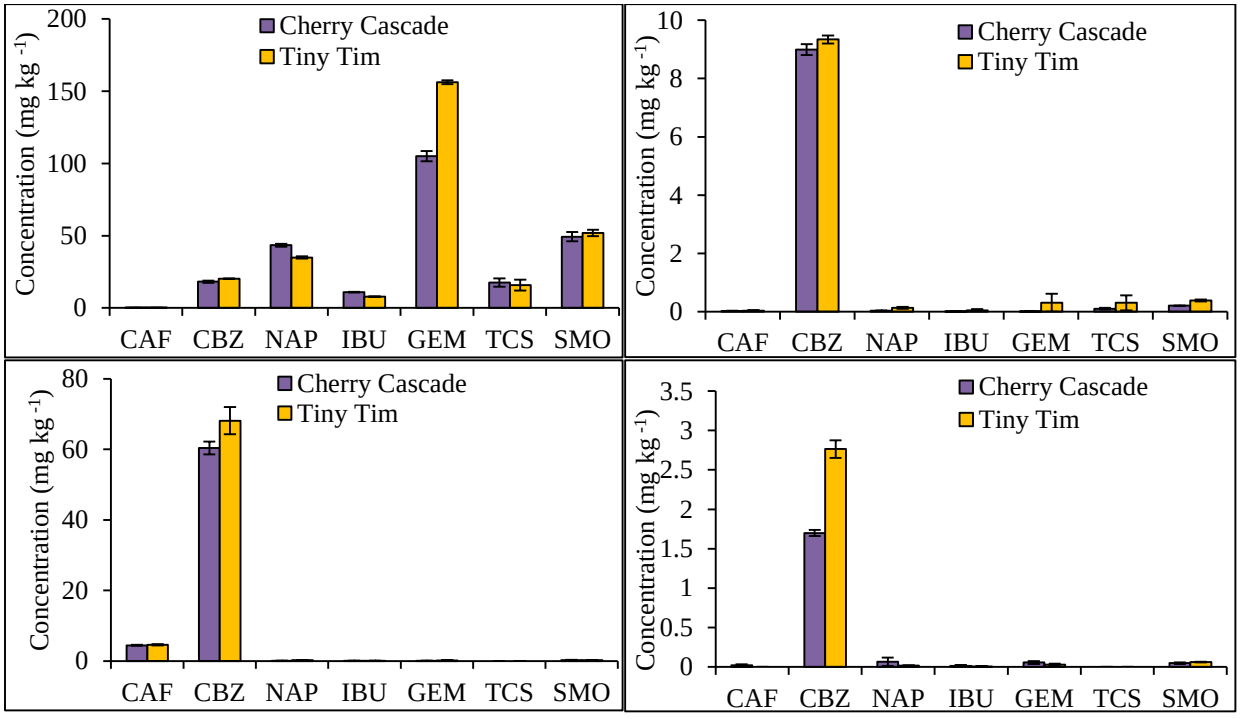


Figure 23. Accumulation of PPCPs into CC or TT tomato plants after exposure to 500 µg L^{-1} in nutrient solution. Top-left: root. Top-right: stem. Bottom-left: leaf. Bottom-right: fruit.

3.4.1.2. Hormone Accumulation

Hormones, specifically E1, βE2, and EE2, were spiked with the PPCPs into nutrient solutions for tomato plant cultivation. But even after exposure to 500 μg L^{-1} of hormones, tomato leaves and fruits had no detectable accumulation of the hormones. The concentrations of hormones in roots and stems are shown in Figure 24. Only one hormone, EE2, was detectable in tomatoes exposed at 0.5 μg L^{-1} and was measured at 9 μg kg^{-1} (dw) in roots. No compounds were detectable in stems at that exposure (Figure 24). In plants exposed to 50 μg L^{-1}, all three hormones were detected in roots at concentrations ranging from 51.5 μg kg^{-1} (dw) for βE2 in CC to 589 μg kg^{-1} (dw) for EE2 in CC, while these hormones were still undetectable in stems. After exposure to 500 μg L^{-1}, root concentrations had increased to 1.6 to 2.3 mg kg^{-1} (dw). At this exposure, E1 and βE2 were detected in stems at 10.1 to 22.3 μg kg^{-1} (dw) while EE2 was still not detectable.

Compared to the PPCPs tested, hormone accumulation was very limited. Levels of hormones in roots were similar to concentrations of CAF, the least accumulated PPCP. However, CAF generally had greater accumulation in leaves and stems, whereas hormones were typically undetectable in those plant parts. The highly limited accumulation of hormones in all plant parts may seem to suggest that these compounds are of limited concern for food safety. However, because hormones strongly interact with the human endocrine system and may elicit effects even at minute concentrations, this assumption needs to be verified in a systematic way through further research.

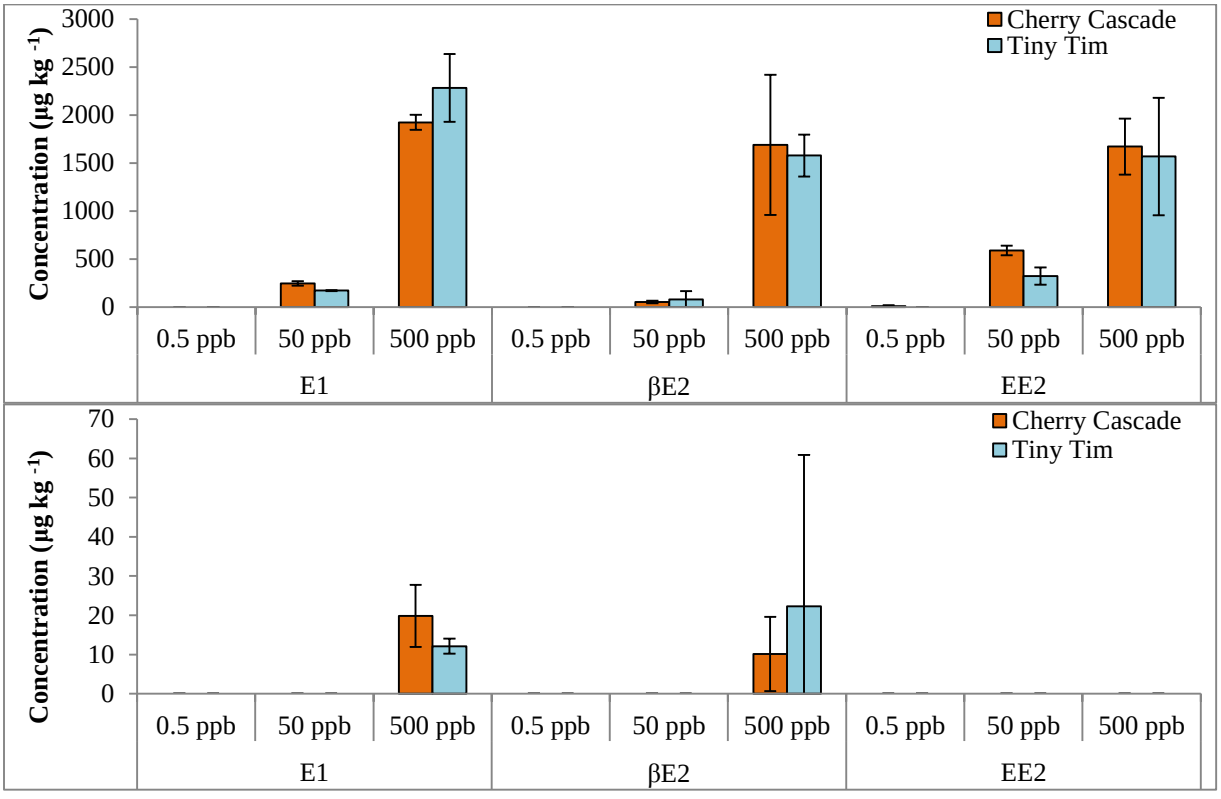


Figure 24. Accumulation of hormones into CC or TT tomato plants after exposure in nutrient solutions. Top: roots; Bottom: stems.

3.4.2. Bioaccumulation Factors

Bioaccumulation factors (BAFs) of PPCPs and hormones from solution into tomato plant parts were calculated using Equation (*1*), allowing comparisons among the exposure levels. TCS had the greatest total BAF among PPCPs for tomato plants exposed to solutions with 0.5 μg L^{-1}, largely due to also having the greatest root BAF (Figure 25). The second greatest total BAF was for CBZ, which had the largest leaf BAF.

Compared to 0.5 μg L^{-1} exposure, BAFs for most PPCPs in each plant part remained similar when exposed to solutions at 50 μg L^{-1} (Figure 26) or 500 μg L^{-1} (Figure 27). The exceptions were CAF and NAP, which had gradually decreasing BAFs with increasing solution concentration, and TCS, which had drastically decreasing BAFs. As an example, in CC roots, NAP BAFs were 157, 116, and 87 after exposure to 0.5, 50, and 500 μg L^{-1}, respectively, while TCS BAFs were 1600, 173, and 35 for the same exposures. The different accumulation of these compounds is likely related to their properties, such as hydrophobicity and ionization potential.

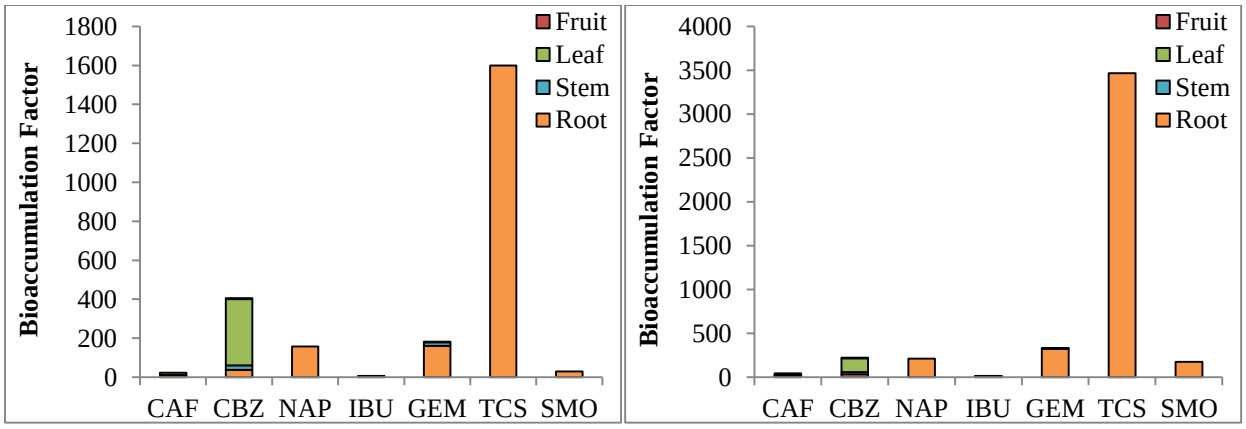


Figure 25. Bioaccumulation factors for PPCPs into tomato tissues after exposure to 0.5 μg L^{-1} concentrations in nutrient solution. Left: CC tomato plants. Right: TT tomato plants.

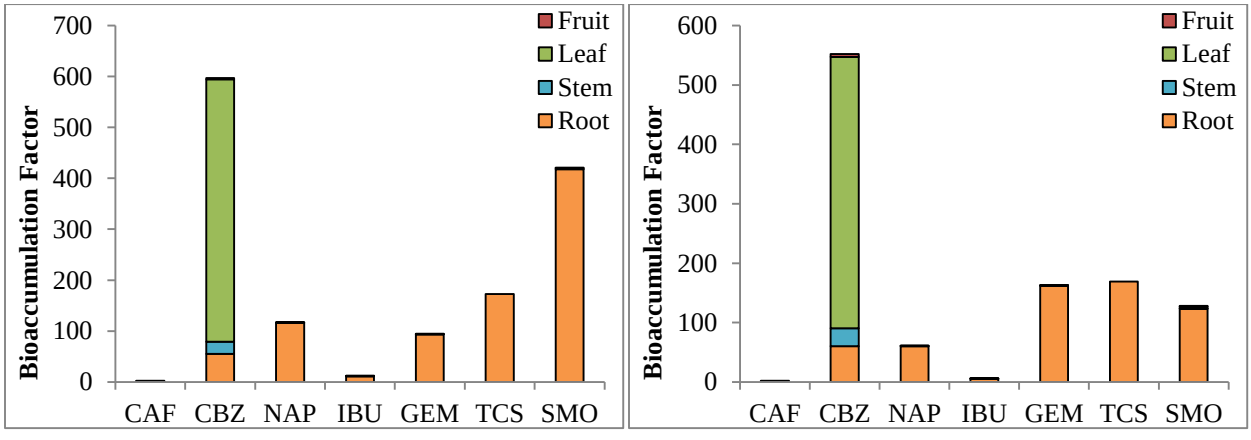


Figure 26. Bioaccumulation factors for PPCPs into tomato tissues after exposure to 50 μg L^{-1} concentrations in nutrient solutions. Left: CC tomato plants; Right: TT tomato plants.

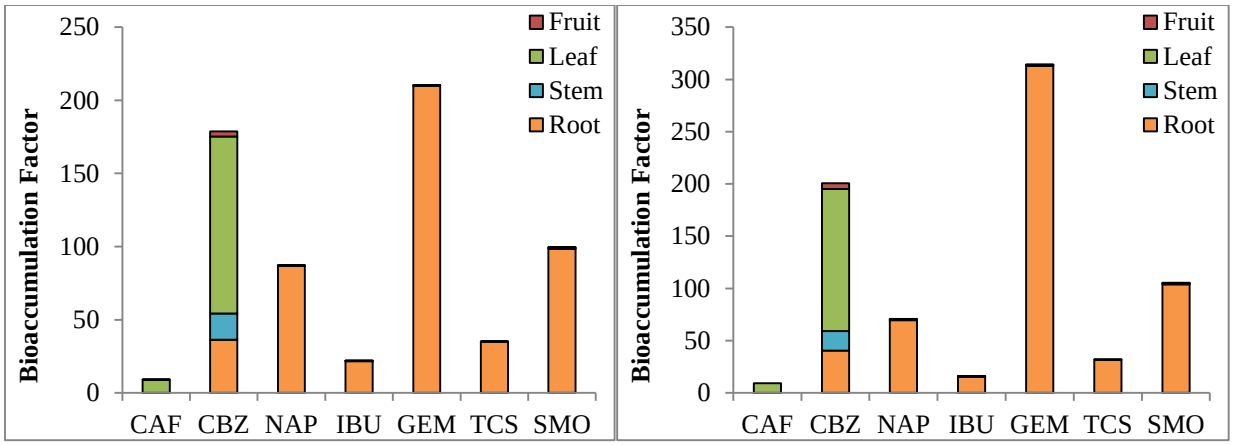


Figure 27. Bioaccumulation factors for PPCPs into tomato tissues after exposure to 500 μg L^{-1} concentrations in nutrient solutions. Left: CC tomato plants; Right: TT tomato plants.

As also noted above, the exposure concentration affected the accumulation of PPCPs into roots. For example, in CC, TCS was the most accumulated PPCP (BAF = 1,600) after exposure to 0.5 μg L^{-1} concentration solutions, SMO was the most accumulated (BAF = 418) after exposure to 50 μg L^{-1}, and GEM was the most accumulated (BAF = 210) after exposure to 500 μg L^{-1}. This complex interplay between exposure concentration and compound behavior deserves further investigation so that it can be successfully modeled for risk assessment.

In contrast to root accumulation, CBZ had the highest leaf BAF values for all tomato treatments. In TT, BAFs were 158, 457, and 136 after exposure to 0.5, 50, and 500 μg L^{-1}, respectively, suggesting that leaf accumulation is generally high and some exposure concentrations may experience enhanced accumulation. CBZ accumulation into fruit was found at all exposure levels as well, with BAFs of 5 to 9. Overall, CBZ had the largest leaf BAF values for both tomato and lettuce plants. Moreover, it was the only targeted contaminant to accumulate in tomato fruits, suggesting that CBZ has high potential for leaf and fruit accumulation and could be used as a marker when assessing human risk from consumption of leafy vegetables and fruits contaminated with PPCPs.

Hormones had minimal accumulation into tomato roots, little accumulation into stems, and no accumulation in leaves or fruits (Figure 28). Further study should investigate whether hormones in roots are simply adsorbed to the root surface, in which case directing the use of preparative measures (such as peeling carrots) can minimize human exposure, or whether accumulation is occurring in interior root tissues. Accumulation within roots has greater potential impact on human health, because even minute concentrations of hormones have great biological activity.

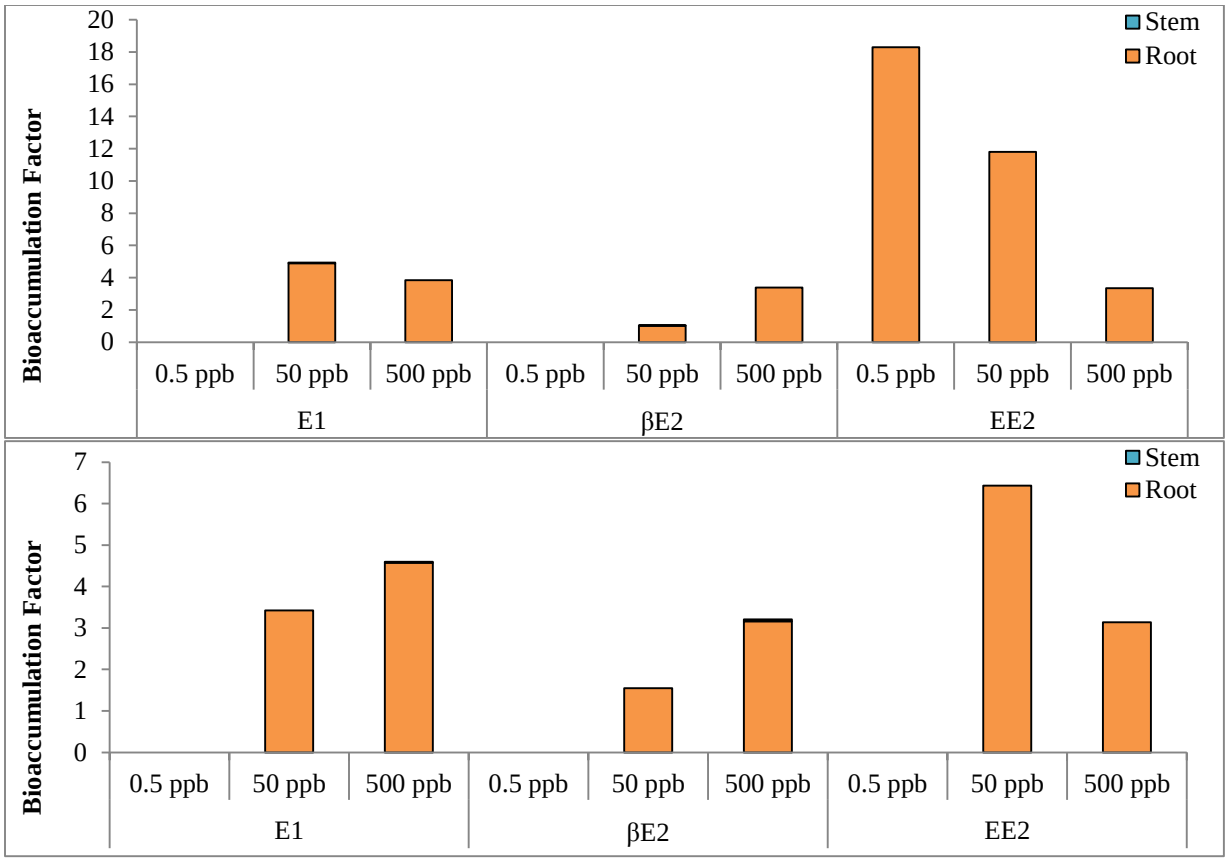


Figure 28. Bioaccumulation factors for hormones into tomato tissues after exposure in nutrient solutions. Top: CC tomato plants; Bottom: TT tomato plants.

3.4.3. Translocation Factors

To assess the translocation of PPCPs and hormones from root to aerial tomato tissues, the following equation was used.

$$TF = \frac{\text{Sum of PPCP or hormone in stem, leaf, and fruit } (\mu g\ kg^{-1}, dw)}{\text{Concentration of PPCP or hormone in root } (\mu g\ kg^{-1}, dw)} \quad (3)$$

All PPCPs and hormones, besides CAF and CBZ, were found predominantly in roots, with minimal or no translocation to aerial tissue (Figure 29 and 30), producing TFs of 0 to 0.13. The only exception was IBU in TT, which had a TF of 1.13 (Figure 29). For these PPCPs and hormones, TF values mostly decreased with increasing exposure concentration. Comparing BAF values (Figure 25-28), it is clear that this effect was mostly due to the very low detection of any of these emerging contaminants in aerial tissues while root concentrations remained mostly stable. The low translocation of these PPCPs and hormones suggest they bear little risk to contaminate leafy vegetables, and attention should instead be paid to their root accumulation.

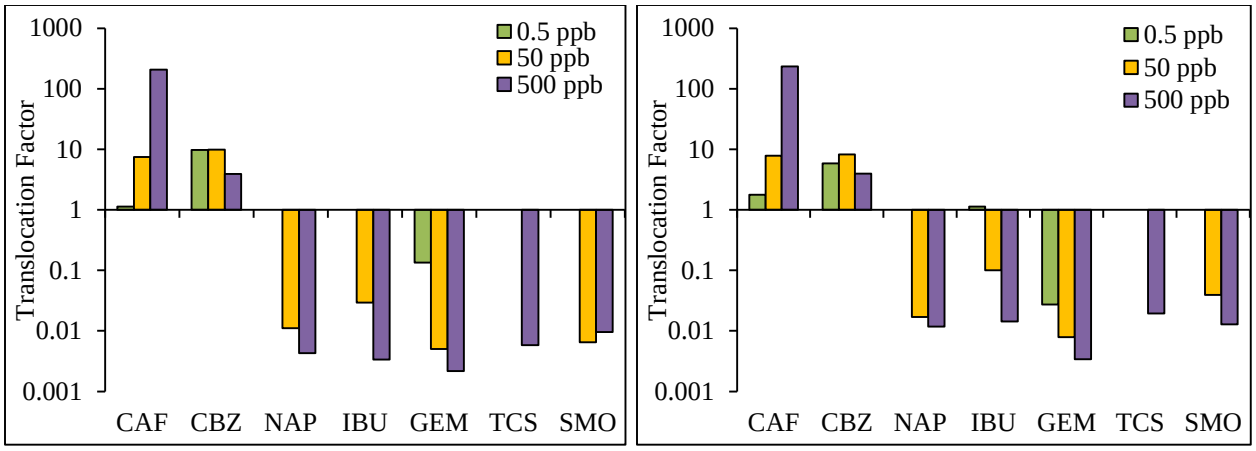


Figure 29. Translocation factors of PPCPs in tomatoes at three exposure concentrations. Left: CC tomato plants; Right: TT tomato plants.

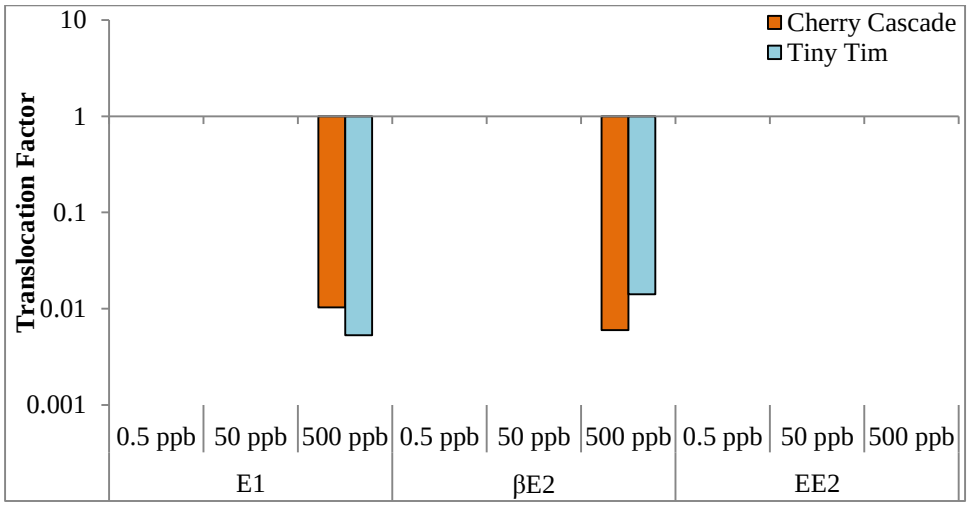


Figure 30. Translocation factors of hormones in tomato tissues at three exposure concentrations.

In marked contrast, CAF and CBZ had high TFs ranging from 1.12 to 233.62. CAF exhibited a direct relationship between exposure and translocation, with TF values increasing about an order of magnitude with each increase in exposure concentration. CBZ did not exhibit this behavior but remained relatively stable across exposures. These behavior trends are very similar to those observed in lettuce (Figure 15). In lettuce, CAF and CBZ also had high TF values, which in the case of CAF were related to exposure, but in the case of CBZ were not related. This similarity across plant species and cultivar suggests that aerial accumulation of targeted contaminants was strongly connected to their chemical properties and that neutral, hydrophilic compounds such as CAF and CBZ have great potential to accumulate in leafy vegetables (Dodgen et al., 2015; Zhang et al., 2013).

3.5. Evaluating Human Exposure and Risk

The accumulation of PPCPs and hormones in lettuce leaves and tomato fruits indicates a potential risk to human health through dietary uptake. In this study, potential human exposure from consumption of food crops grown in nutrient solutions containing PPCPs and hormones at 500 μg L^{-1} were evaluated. Although this concentration level is much higher than the typical levels of emerging contaminants in the environment, sometimes this concentration could be detected in reclaimed water (Anderson et al., 2010; Choi et al., 2008; Heberer, 2002a; Karnjanapiboonwong et al., 2011*b*; Li et al., 2013; Zheng et al, 2008) that may be used for agricultural irrigation. Also, the use of the highest accumulation value for the evaluation may represent a "worst case" exposure. After exposure to 500 μg L^{-1} of each targeted compound, six of the seven PPCPs were detected in lettuce leaves and tomato fruits. Three targeted hormones were found in lettuce leaves, but not in tomato fruits.

To place the detected concentrations in some context, Table 4 shows values of acceptable daily intake (ADI) for all targeted compounds for a 65 kg human. ADIs are developed by regulatory agencies to estimate an exposure level that will have no observable effect, even for susceptible populations. To estimate human exposure, values from the United States Environmental Protection Agency (U.S. EPA) were used. It reports an average per capita consumption of 0.23 g (ww) lettuce leaves and 0.72 g (ww) tomato fruit per kg (body weight) per day (U.S. Environmental Protection Agency, 2011). The highest detected concentrations of each contaminant in lettuce leaves and tomato fruits were used to calculate potential human exposure (Table 4). These calculations represent a "worst case" exposure estimation. Worst case estimations are useful because they err toward human safety and allow further scrutiny to be focused on compounds that exceed these protective limits.

Acceptable intake values range from 0.0065 to 37,050 μg d^{-1} for a 65 kg individual. The lowest ADIs belong to hormones, due to their potent biological activity, while the PPCPs have much higher values. Comparing ADIs with estimated intake (Table 4), it is evident that an average American may be exposed to far less than the ADI of any one contaminant through lettuce leaf and tomato fruit consumption. The only exceptions were CBZ and EE2, where a typical American might consume more than the ADIs from lettuce grown in high contaminated water (Table 4). These calculations are for average consumption, so an individual who consumed more than average amounts of the vegetables would have a greater exposure to these emerging compounds. Also, humans are likely exposed to PPCP and hormone contaminants from many sources, including food crops, drinking water, and home and work environments. When all inputs are summed, it is possible for humans to be exposed to greater amounts of emerging contaminants than those allowed by their ADIs.

Actual information for PPCPs and hormones in commercial food crops is largely unavailable. Only a few studies have suggested that emerging contaminants may be present in commercial crops if they were irrigated with reclaimed water (Calderón-Preciado et al., 2011; Wu et al., 2014). A concern is that ADIs are developed for a single compound in isolation, while humans are likely exposed to a simultaneous mixture of many emerging contaminants. Mixtures of PPCPs and/or hormones have been shown to have additive or synergistic toxic effects *in vivo* (Cleuvers, 2004), which has clear implications of enhanced potential human risk from exposure to hundreds of emerging contaminants in contaminated food, water, and other living environments.

Table 4. Acceptable Daily Intake (ADI) for a 65 kg human and estimated daily exposure by ingestion of lettuce leaves and tomato fruits for targeted PPCPs and hormones.

Compound	ADI (µg)	Exposure in Lettuce (µg d^{-1})	Exposure in Tomato (µg d^{-1})
Caffeine	1248[a]	23.13	0.063
Carbamazepine	22.1[b]	58.26	9.05
Naproxen	37050[b]	0.21	0.21
Ibuprofen	1625[c]	0.023	0.044
Gemfibrozil	26.65[b]	0.18	0.18
Triclosan	4875[b]	0	0
Sulfamethoxazole	33150[b]	3.72	0.20
Estrone	0.845[b]	0.027	0
Estradiol	3.25[b]	0.11	0
Ethinylestradiol	0.0065[b]	0.082	0

a – no ADI available; based on Fisher Scientific MSDS oral-rat LD50 of 192 mg kg^{-1} using an Uncertainty Factor of 10 000, b - Snyder et al., 2008, c - Ricardo-AEA, 2014.

Currently, hydroponics is a growing area of commercial food production. Compared to soil cultivation systems irrigated with reclaimed water, the use of hydroponics utilizing reclaimed water may result in greater accumulations of PPCPs and hormones in vegetables, because contaminant sorption on soils can reduce their availability for plant uptake (Shenker et al., 2011). However, the concentrations of emerging contaminants in bio-solids such as manure and STP sludge are usually much higher than those in reclaimed water. Land application of these bio-solids on food crops may result in an enhanced uptake and accumulation of PPCP and hormone contaminants in vegetables compared to irrigation with reclaimed water. The effects of different cultivation systems and management practices on the accumulation of emerging contaminants in food crops need to be further evaluated.

4. Conclusions

Lettuce plants were used to address several knowledge gaps, including (1) effect of initial PPCP and hormone concentrations on their accumulation, (2) effect of exposure duration, (3) effect of depuration period, and (4) effect of lettuce cultivars. In addition, two cultivars of tomato plants were also grown to assess the accumulation of PPCPs and hormones into edible fruits at various exposure concentrations.

4.1. Study 1: Evaluating Effects of PPCP and Hormone Concentrations

When lettuce cultivars were exposed to nutrient solutions with varying concentrations of PPCPs and hormones, all of the contaminant compounds were detected in roots even at a very low exposure concentration that was representative of recycled water. Greater exposure concentration generally led to greater PPCP and hormone accumulation. Among the test compounds, SMO accumulated the most in lettuce roots. Leaf tissue had detectable levels for most compounds except TCS. Levels of CAF and CBZ were higher in leaves than in roots, while other targeted emerging contaminants predominantly accumulated in roots. CBZ, CAF, and SMO showed very high BAF values in lettuce leaves, suggesting that these three compounds can easily translocate from lettuce roots to leaves and thereby accumulate in plant leaves.

4.2. Study 2: Evaluating Effects of Exposure Duration

When lettuce plants were grown for one, two, or three weeks in nutrient solutions spiked with PPCPs and hormones, very little difference in accumulated concentrations of most targeted compounds was observed among plants with different exposure duration. However, NAP, SMO, and βE2 showed some increase in their concentrations in roots after three weeks of exposure. Also, increased exposure duration showed a tendency for NAP, IBU, and GEM to gradually distribute to greater extents in roots, perhaps due to an "ion trap" of these anionic organic compounds (Trapp, 2009). The similar concentration in root tissues and leaf tissues across exposure times suggests that the plants took up greater amounts of PPCPs and hormones as their biomass increased, likely due to increased transpiration needs in larger plants (Dodgen et al., 2015). It also suggests that accumulation of PPCPs and hormones in plant tissues may rapidly reach a steady level (< 1 week), meaning field crops may quickly reflect changes in irrigation water quality.

4.3. Study 3: Evaluating Effects of Depuration

When plants were exposed to PPCPs and hormones and then grown for 1.5 weeks without exposure, the concentrations of chemicals in the leaves and roots were lower than in plants of similar age which were harvested immediately after exposure. Comparison between the results of this study and Study 2 (described in Section 4.2) suggests that the different accumulation levels of targeted PPCPs and hormones are not attributed to their difference in total uptake or plant biomass, but are likely due to metabolism of the accumulated compounds by the

plant (Bokern and Harms, 1997; Macherius et al., 2012). The only exceptions were for SMO which had similar levels in leaves and TCS and the hormones which had similar levels in roots after the 1.5 weeks. The persistence of these compounds, even after 1.5 weeks without new exposure, suggests that lettuce plants have limited ability to metabolize them and their residues may remain in lettuce plants.

4.4. Study 4: Accumulation of PPCPs and Hormones into Fruiting Plants

When fruiting tomato plants were exposed to various concentrations of PPCPs and hormones for five weeks, plants accumulated detectable levels of every PPCP in their root tissues, even at low exposure concentrations (0.5 $\mu g\ L^{-1}$). TCS was most highly accumulated in tomato roots after exposure to environmentally-relevant levels. In contrast, hormones were scarcely detectable, even after high exposure concentrations. This result implies that the accumulation of steroid hormones in tomato plants irrigated with hormone-containing water is unlikely. PPCPs, except for CAF and CBZ, showed little ability to translocate from tomato root tissues to other plant parts. Their accumulation in tomato plants generally decreased with decreasing proximity to roots, i.e., roots had greater levels of PPCPs than stems, which had greater levels than leaves or fruit. CBZ was the most accumulated PPCP in tomato fruits among all the targeted compounds.

4.5. Estimation of Human Risk from Consuming Contaminated Plants

An estimate of human exposure was calculated using the highest contaminant concentrations in lettuce leaves and tomato fruits and average per capita consumption of those foods. Values for acceptable daily intake (ADIs) of these PPCPs and hormones were developed from literature for comparisons. Overall, daily exposure through lettuce and tomato is much be less than the ADI values for most targeted compounds, even under the "worst case" scenario with highest concentrations of targeted compounds. However, estimated exposures for CBZ and EE2 in lettuce leaves were greater than their ADIs, suggesting that these two compounds require further study to determine the potential human risk from consumption of these contaminated food plants. In addition, this estimation method needs further expansion because it does not encompass individuals with higher than average consumption, does not include total exposure from the various food and environmental sources, and does not consider synergistic effects of simultaneous exposure to mixtures of PPCPs and hormones.

5. Recommendations

Some targeted PPCPs and hormones, namely CAF, CBZ, TCS, and SMO, were found to consistently accumulate in lettuce and tomato plants after exposure to levels that are relevant to reclaimed water. SMO was frequently detected in aerial and root tissues, while TCS predominantly partitioned to roots, and CAF and CBZ typically translocated to aerial tissues. CBZ was the only PPCP that accumulated in tomato fruits after exposure to environmentally-relevant water concentrations (0.5 μg L^{-1}). The wide variety of physico-chemical properties of these four compounds and their extensive uptake into plant tissues suggest that they may be useful as markers for plant uptake and for modeling human exposures from food crops.

Simple estimates of human exposure suggest that plant accumulation of most PPCPs and hormones from contaminated water will not be a significant source of risk, as dietary intake would be far below ADI values. However, CBZ and EE2 could accumulate in lettuce leaves to very high levels that surpass their ADI values when the vegetable is irrigated with highly contaminated water. This result suggests that total exposure to these compounds from all dietary and environmental sources may have a potential risk on human health, and thus should be further investigated.

Currently, approximately 8% of water used in agriculture and other applications in the U.S. is treated wastewater. Moreover, water reuse is growing by 15% each year (Miller, 2006). Current practices of irrigating with reuse water are not expected to cause human health risks due to the low PPCP and hormone levels in reuse water approved for food crops (Anderson et al., 2010). However, the emerging contaminants mentioned above should be given priority for future work. Root vegetables, such as sweet potatoes and carrots, should also be evaluated for uptake of these compounds, because most PPCPs and hormones have a high potential for root accumulation. A soil system should also be used to assess accumulation of a suite of PPCPs and hormones. Soil is a complex matrix that acts to sorb and degrade chemicals and may have a profound impact on their plant accumulation. Amendment of soil with biosolids or manures should be investigated because concentrations of some PPCPs and hormones may be substantially higher in these materials and their addition can change soil properties and affect the transport and persistence of these chemicals. Also, because the use of pharmaceuticals and other chemicals is increasing worldwide and water reuse is likewise increasing, the accumulation of PPCPs and hormones in food plants requires extensive evaluation to assure food safety.

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