Environ Sci Pollut Res (2014) 21:6025–6033
DOI 10.1007/s11356-013-2474-3

RESEARCH ARTICLE

Uptake and accumulation of antimicrobials, triclocarban and triclosan, by food crops in a hydroponic system

Shiny Mathews · Shannon Henderson · Dawn Reinhold

Received: 31 July 2013 / Accepted: 16 December 2013 / Published online: 25 January 2014

Abstract Commonly used in personal care products, triclocarban (TCC) and triclosan (TCS) are two chemicals with antimicrobial properties that have recently been recognized as environmental contaminants with the potential to adversely affect human health. The objective of the study described herein was to evaluate the potential of food crops to uptake TCC and TCS. Eleven food crops, grown in hydroponic nutrient media, were exposed to a mixture of 500 μg L^{-1} TCC and TCS. After 4 weeks of exposure, roots accumulated 86–1,350 mg kg^{-1} of antimicrobials and shoots had accumulated 0.33–5.35 mg kg^{-1} of antimicrobials. Translocation from roots to shoots was less than 1.9 % for TCC and 3.7 % for TCS, with the greatest translocation for TCC observed for pepper, celery, and asparagus and for TCS observed for cabbage, broccoli, and asparagus. For edible tuber- or bulb-producing crops, the concentrations of both TCC and TCS were lower in the tubers than in the roots. Exposure calculations using national consumption data indicated that the average exposure to TCC and TCS from eating contaminated crops was substantially less than the exposure expected to cause adverse effects, but exceeded the predicted exposure from drinking water. Exposure to antimicrobials through food crops would be substantially reduced through limiting consumption of beets and onions.

Keywords Antimicrobials · Triclocarban · Triclosan · Uptake · Translocation · Exposure

Introduction

An increasing awareness of pollution of water and land resources with trace concentrations of pharmaceuticals and personal care products has prompted concerns about unintentional human exposure (Barcelo and Petrovic 2007; Bruce et al. 2010). Triclocarban (TCC) and triclosan (TCS) are chlorinated aromatic chemicals commonly used as antimicrobial agents in consumer products such as soaps and toothpastes (Perencevich et al. 2001; USEPA 2002, 2010). Both TCC and TCS are hydrophobic organic chemicals with log octanol–water partitioning coefficients (log K_{OW}) of 4.8 and 4.9 (respectively) and with high affinities for organic matter (Chen et al. 2011; Kwon et al. 2010; Ying et al. 2007a). While TCC and TCS are not currently considered health hazards by the EPA or FDA, animal studies have demonstrated that antimicrobials can disrupt endocrine function and the central nervous system, prompting further investigations (Paul et al. 2010; USEPA 2002; USFDA 2010). The predicted No Observed Adverse Effect Level (NOAEL) for oral repeated-dose toxicity of TCC for humans is 25 mg per kg body weight per day (USEPA 2008b). The NOAEL values for TCS for acute and chronic dietary exposures are 30 mg kg^{-1} day^{-1} and 0.3 mg kg^{-1} day^{-1}, respectively (USEPA 2008a, b). Additionally, while information on bacterial resistance to TCC is limited, studies have documented increased microbial resistance to TCS in *Escherichia coli* and *Staphylococcus aureus* at concentrations of micrograms per kilogram in aqueous and

Responsible editor: Laura McConnell

Electronic supplementary material The online version of this article (doi:10.1007/s11356-013-2474-3) contains supplementary material, which is available to authorized users.

S. Mathews (✉) · S. Henderson · D. Reinhold
Department of Biosystems and Agricultural Engineering, Michigan State University, 524 S. Shaw Lane, East Lansing, MI 48824, USA
e-mail: shinymj@gmail.com

Present Address:
S. Mathews
College of Natural Resources, University of Wisconsin, 800 Reserve Street, Stevens Point, WI 54481, USA

soil environments (Escalada et al. 2005; McMurry et al. 1998; Sivaraman et al. 2004; Suller and Russell 2000).

Antimicrobials enter the environment primarily through consumer discharge to municipal wastewater treatment plants. The average concentrations of antimicrobials entering wastewater treatment plants are 6,100±2,000 ng L^{-1} TCC and 4,700±1,600 ng L^{-1} TCS and, during wastewater treatment, TCC and TCS are only minimally transformed (Heidler and Halden 2007; Heidler et al. 2006). Instead, these antimicrobials are removed predominantly through sorption to particulate matter (settled organic matter and sludge) which are subsequently land applied as biosolids (Chu and Metcalfe 2007; Heidler et al. 2006; Sapkota et al. 2007). For example, 78±11 % of TCC and 80±22 % of TCS were partitioned to sludge, resulting in accumulation of 51,000±15,000 μg TCC and 30,000±11,000 μg TCS per kilogram of dry biosolids, respectively (Heidler and Halden 2007; Heidler et al. 2006). While studying the fate of TCC and TCS in a wastewater treatment system, the combined TCC and TCS concentrations decreased in the water by 97 %. There was a higher removal of TCC (79 %) compared to TCS (64 %) into solids (Lozano et al. 2013). As approximately 50 % of biosolids are land applied (USEPA 2007), three quarters of TCC that is used by consumers is ultimately released into the environment through land application of biosolids. However, a relevant fraction of antimicrobials still remains in effluent from wastewater treatment plants. For example, of the TCC, TCS, and methyl TCS released from wastewater treatment plants, 4.15 kg day^{-1}, 5.37 kg day^{-1}, and 0.58 kg day^{-1}, respectively, are seen in sludge and 0.13 kg day^{-1}, 0.24 kg day^{-1}, and 0.021 kg day^{-1}, respectively, are seen in effluent (Lozano et al. 2013). Wastewater treatment plant effluents contain 110 to 170 ng L^{-1} TCC and 800 to 37,800 ng L^{-1} TCS (Halden and Paull 2005; Heidler et al. 2006). At least 5,800 kg of TCC and 2,600–10,400 kg of TCS are discharged into U.S. water resources from activated sludge treatment plants (Halden and Paull 2005).

The major mechanism dominating fate of antimicrobials that are applied to agricultural fields in the form of biosolids is sorption, with greater sorption of TCC than TCS (Cha and Cupples 2009; Wu et al. 2009). Microbial degradation of TCC and TCS occurs under aerobic conditions, but is limited under anaerobic conditions (Ying et al. 2007b). The half-life of TCC was higher than TCS (87 to 231 days and 20 to 58 days, respectively) in biologically active soils (Wu et al. 2009). The half-life of TCS when present in biosolid applied to agricultural soils was 107 days (Lozano et al. 2010). Both microbial and photodegradation of TCC and TCS can form products such as chloroanilines, methyl-triclosan, dichlorodioxins, and chlorophenols that are environmentally persistent and have worse health effects than the parent TCC and TCS (Lawrence et al. 2009; Ozaki et al. 2011). In a long-term field study, the half-lives of TCS and its degradation products methyl-triclosan was found to be 104 days and 443 days, respectively, indicating a higher persistence of the degradation product when compared to triclosan (Lozano et al. 2012).

Plants, including food crops, are capable of accumulating many organic contaminants into their biomass (Kim et al. 2004; Loffredo et al. 2010; Murano et al. 2009; Zhang et al. 2009). While few fate studies have examined the interactions between antimicrobials and plants, an extensive body of literature on phytoremediation has examined the interactions between plants and other organic contaminants. Organic contaminants internal to plant cells can be conjugated and sequestered (i.e., phytometabolism) or can accumulate in plant shoots (i.e., phytoaccumulation). Uptake and bioaccumulation of hydrophobic chlorinated aromatics (i.e., chlorinated pesticides and polychlorinated biphenyls) have been rigorously documented for *Cucurbita pepo* subspecies (Huelster et al. 1994; Lunney et al. 2004a; Wang et al. 2004; White et al. 2003). For example, pumpkin (*C. pepo*) extracted 0.301 % of weathered *p,p*-dichlorodiphenyldichloroethylene (DDE) from soils in 2 months, thereby accumulating 9,240 mg kg^{-1} of DDE in roots and 4,970 mg $kg^{-1}$of DDE in shoots (White et al. 2003). From an industrial site contaminated with 6.5 μg kg^{-1} polychlorinated biphenyls (PCBs), *C. pepo* ssp. *pepo* accumulated on average 21.5 μg $kg^{-1}$of PCBs in roots and 3.5 μg kg^{-1} of PCBs in shoots (Low et al. 2010). Studies examining hydroponic accumulation of polychlorinated dibenzodioxins and dibenzofurans by 12 agricultural crops demonstrated that while *C. pepo* subspecies (e.g., pumpkin and zucchini) accumulated the greatest concentrations of chlorinated organic contaminants, other species, including tomato and cabbage, can also accumulate chlorinated organic contaminants (Zhang et al. 2009).

Recent studies have also documented accumulation of TCC and TCS by plants. When soybean plants were treated with TCC and TCS, the antimicrobials accumulated in the roots and were translocated to the shoots and beans (Wu et al. 2010a). Similar results were observed by Pannu et al. (2012) where radish, lettuce, and bahia grass accumulated TCS in the roots. When grown in soils treated with biosolids, pumpkin and zucchini accumulated up to 55 mg kg^{-1} of combined antimicrobials in root tissues and up to 13 mg kg^{-1} combined antimicrobials in shoot tissues (Aryal and Reinhold 2011). When grown under hydroponic conditions with elevated concentrations of antimicrobials, the same varieties of pumpkin and zucchini accumulated up to 480 mg kg^{-1} combined antimicrobials in root tissues and up to 9.3 mg kg^{-1} combined antimicrobials in shoot tissues (Aryal and Reinhold 2013). Although hydroponic systems yielded higher root concentrations than those observed under more realistic, soil-based conditions, similar or decreased concentrations of antimicrobials were observed in shoot tissues, supporting the use of hydroponic systems to screen for the capabilities of plants to accumulate antimicrobials.

The primary aim of this study was to investigate the diversity of food crops capable of uptake and translocation of TCC and TCS. Specific objectives were to (1) compare uptake and translocation by fruit-producing, edible leaf-producing, and tuber- or bulb-producing vegetables, and (2) estimate potential human exposure to antimicrobials through consumption of vegetables.

Materials and methods

Experimental setup

Triclocarban [CAS 101-20-2], triclosan [CAS 3380-34-5], and C^{13}-triclocarban were purchased from Tokyo Chemical Industry, Calbiochem, and Cambridge Isotope Laboratory Inc., respectively. Stock solutions of both TCC and TCS mixtures were prepared in methanol. Ammonium acetate (>99.99 %), acetone (>99.7 %), and methanol (>99.99 % for LCMS) were purchased from VWR. Planting materials for the hydroponic study were procured from Garden Harvest Supply, Burpee, and Tasteful Garden. The plants used for the study were cucumber, tomato, cabbage, okra, pepper, potato, beet, onion, celery, and asparagus. Plants were raised from seeds [cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), cabbage (*Brassica oleracea*), okra (*Abelmoschus esculentus*), pepper (*Capsicum annuum*)], tubers [potato (*Solanum tuberosum*), beet (*Beta vulgaris*)], bulbs [onion (*Allium cepa*)], or whole plants [broccoli (*Brassica oleracea*), celery (*Apium graveolens*), and asparagus (*Asparagus officianalis*)]. Germinated seeds, tubers, bulbs, or whole plants were raised in a potting mix until the four to five leaf stage and then were transferred to hydroponic growth systems with a basal nutrient media (APHA A, WEF 1999) and constant aeration for 1 week of acclimatization.

After acclimatization, plants were transferred to 1-L amber glass jars with 900 mL test solution. Each plant was exposed to 500 μg L^{-1} TCC and 500 μg L^{-1} TCS mixture in nutrient media (APHA A, WEF 1999) for 1 month, with five replicates per plant. Two types of controls were used for the study: (a) controls with media containing antimicrobials, but no plants, to understand loss of TCC and TCS overtime in the absence of plants; and (b) controls with plants grown in the absence of antimicrobials for toxicity comparisons. The test solutions were continuously aerated using aquarium aeration pumps and stainless steel needles (1 mm diameter). One pump each of 2.4 W was used for a batch of four reactors yielding similar aeration conditions per reactor. The temperature of the hydroponic system was maintained at 23±2 °C and light supply was provided for 12 h each day. Media lost through evapotranspiration and evaporation was replaced with nutrient solution once a week. The transpiration loss was calculated by subtracting the no-plant control media loss (evaporation) from the total amount of water lost from each planted bottle. The initial pH of the nutrient media ranged from 7.5±0.1 and the final media pH ranged from 7.5±0.2 and 7.7±0.4 in the unplanted and planted media, respectively. Additional studies were performed in triplicate to understand the behavior of 500 μg L^{-1} of TCC and TCS in (a) e-pure water, and (b) nutrient solutions and (c) different components (A, B, and C) of the nutrient solution in the absence of plants (discussed in supporting information).

Sample collection and analysis

Liquid samples (2 mL) were collected weekly and analyzed immediately. Collected media samples from all the experiments were diluted in 2 mL methanol and the resulting 4-mL sample was passed through a 0.2-μm PTFE filter for analysis. During experimentation, cloudiness was observed in the all experimental reactors, including the controls. At the conclusion of the experiment, the cloudy residue was allowed to settle to the bottom of the amber bottles. The gelatinous residue was then filtered out using Whatman 4 filter, dried, and weighed. The residue was then mixed with methanol for 5 min and analyzed for TCC and TCS.

After the test period of 4 weeks, the plants were removed from the bottles, washed, and rinsed in distilled water. The plant growth was compared to control plants to evaluate any phytotoxic effects of TCC and TCS. The plants were then separated into the shoots, roots, and, when available, flower, tubers, or bulbs, and the fresh masses of the plant parts were measured. The plant material was then dried for 2 days at 55 °C. The dried plant samples were ground, weighed, and extracted using a Dionex Accelerated Solvent Extractor 200. Cellulose thimbles were first placed in the extractor cells and were half filled with sand, followed by the sample and then filled again with sand. One of the samples in each set of the plants was spiked with 1.2 ppm of ^{13}C-labeled TCC to measure the extraction and analysis efficiency. The extractor specifications were temperature of 100 °C, pressure of 1,500 psi, static time of 5 min, and flush volume of 100 %. The solvent used for the extraction was 1:1 methanol/acetone. The collected extracts in amber vials were then dried in nitrogen gas and reconstituted in 3 mL of 1:1 methanol/acetone mixture.

The extracted and aqueous samples were analyzed for TCC and TCS using a Shimadzu LC-MS 2010 EV with an Allure biphenyl column (5 μm, 150×2.1 mm) from Restek. Qualitative analysis was done in negative electrospray ionization with scan mode and quantification by selected ion monitoring mode. TCC and TCS were identified by retention time (t_R± 0.1 min), specific molecular ions (*m/z* 313 of TCC and 287 for TCS), and reference ions (*m/z* 315 and 317 for TCC and *m/z* 289 and 291 for TCS) (Halden and Paull 2005). Mobile phases were 5 mM ammonium acetate and methanol; for a binary gradient from 75 % to 100 %, methanol was used to

enhance separation of TCC and TCS peaks. Standard curves were prepared with a minimum of six concentrations. Detection limits were determined using the USEPA method of determining the variability of TCC and TCS concentration in seven water samples (spiked at 10 ng/g) and plant samples (spiked at 20 ng/g) (Berthouex and Brown 2002). The detection limits of TCC and TCS were 0.01 μg L^{-1} for media and 0.1 μg kg^{-1} for plants. Concentrations were calculated relative the measured concentrations of ^{13}C-TCC to account for extraction efficiency. The ^{13}C-TCC concentrations from the spiked plant samples indicated a recovery of 97 %. The recovery of both TCC and TCS were compared to the ^{13}C-TCC concentrations to obtain the final recovery values of 93.5 ±6.5 % for TCC and 74.1±8.9 % for TCS.

Statistical analysis

Statistical analysis was performed using Sigma Stat (version 11.0). One-way ANOVA and Tukey's tests were used for all comparisons, with a criterion of $p < 0.05$ to determine statistical significance. Reported values are presented as mean± standard error of the mean.

Results and discussion

Media concentrations of triclocarban and triclosan

The concentration of TCC and TCS in the growth media decreased with time for all experimental reactors, including no-plant controls, over the 4-week experiment. Since the observed decreases were statistically similar for all treatments, one plant was selected from each category to depict the media data (Fig. 1). Media concentrations of TCC decreased by 50 % during the first week followed by an additional 25 % over the next 3 weeks; concentrations of TCS decreased from 25 to 50 %. Since antimicrobial concentrations in reactors with no plants also decreased with a similar trend, the majority of loss from the experimental systems was attributed to physicochemical or microbial processes.

Comparisons between the aqueous depletion of TCC and TCS in e-pure water and the aqueous nutrient media indicated that the presence of basal salts substantially increased the depletion of TCC and TCS over 4 weeks. Additionally, photodegradation contributed to the loss of TCS. While light exposure to the media was minimized through use of amber bottles, some light penetration was unavoidable in planted reactors through the bottle openings where the stems protruded from the media. Due to the absence of a carbon source in the nutrient media, a lack of observed microbial growth, and the recalcitrance of TCC and TCS, microbial degradation was not likely a major process contributing to the aqueous depletion of TCC and TCS in the reactors. A detailed discussion of these media studies, including results from further investigations on which basal salts may increase physicochemical loss, are discussed in the supporting information. Throughout the experiments, cloudiness was observed within the aerated nutrient media. When the nutrient media was left unaerated at the conclusion of the experiment, the cloudiness settled, forming a gelatinous residue. Concentrations of TCC and TCS in the gelatinous residue were 17.7±6.2 mg kg^{-1} TCC and 2.6± 0.2 mg kg^{-1}TCS, substantially higher than the concentrations in the filtered media. Consequently, a dominant portion of the observed decrease in aqueous concentrations of TCC and TCS in the control and experimental systems was attributed to precipitation or sorption of TCC and TCS to salt precipitates.

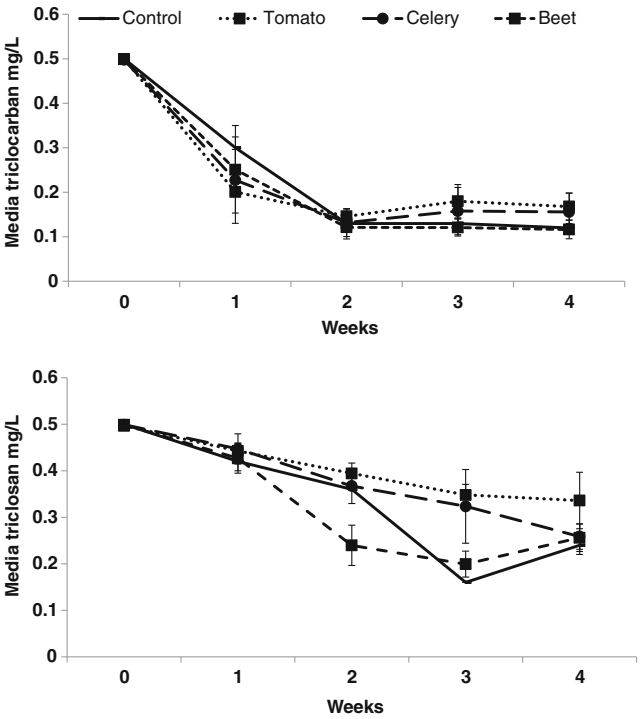

Fig. 1 Concentration of TCC and TCS in the growth media of **a** control, **b** tomato, **c** celery, and **d** beetroot when treated with 500 μg L^{-1} of TCC and TCS for 4 weeks

Plant TCC and TCS concentrations

TCC and TCS were detected in milligrams per kilogram concentrations in the roots and shoots of all 11 plant species, with a broader range of concentrations observed for root concentrations than for shoot concentrations. Generally, concentrations of antimicrobials were substantially higher in the roots than the shoots (Fig. 2). The translocation factors (TF), or ratios of the average concentration of antimicrobial in the shoot to the corresponding concentration in the root, and the root concentration factors (RCF), or ratios of the concentration of antimicrobial in the root to the final concentration in the hydroponic media, are provided in Table 1.

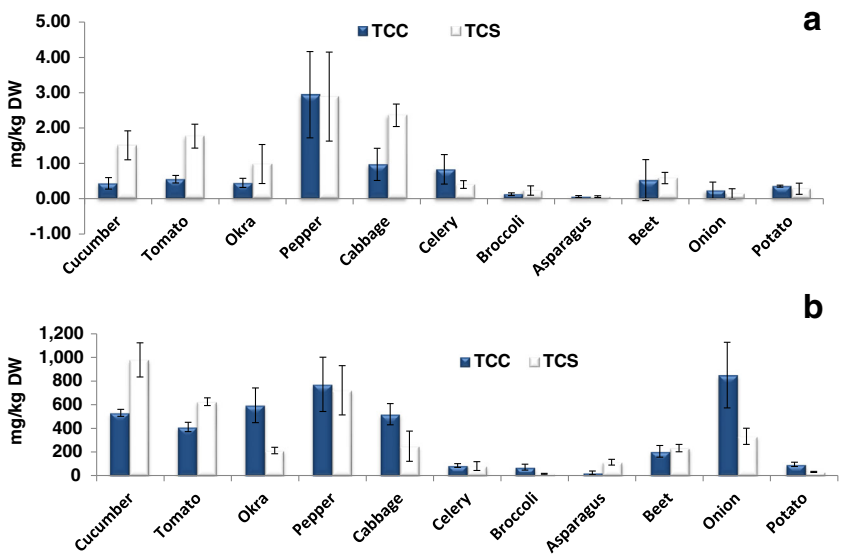

Fig. 2 Concentration of TCC and TCS in the **a** shoot and **b** root of different crops when treated with 500 μg L^{-1} of TCC and TCS for 4 weeks

For fruit-producing plants, accumulation of TCC in the shoot was greater in pepper (2.94 mg kg^{-1}) than in cucumber (0.44 mg kg^{-1}), tomato (0.53 mg kg^{-1}), and okra (0.45 mg kg^{-1}) (Fig. 2). Accumulation of TCC in the roots was independent of plant species for the experimental fruit-producing plants. These results are consistent with the study conducted by Wu et al. (2012). When exposed to TCC in soil systems, pepper and tomato accumulated similar root concentrations, but the shoot concentration factor was greater for pepper than for tomato (Wu et al. 2012). Likewise, in the present study, pepper had the highest TCC shoot concentrations among the fruit-producing crops, but exhibited similar root concentrations as other fruit-producing crops. The accumulation of both TCC and TCS by okra shoot was comparable to that of tomato and cucumber. However, the root TCS concentration was significantly lower (177 mg kg^{-1}) than tomato (520 mg kg^{-1}) and cucumber (815 mg kg^{-1}) ($p<0.05$). In other studies, the uptake of PAH from soil was higher for pepper roots than tomato and okra roots (Al Nasir and Batarseh 2008). Likewise, higher concentrations of PCB accumulated in pepper roots than in okra roots and tomato roots (Al Nasir and Batarseh 2008). A similar trend was observed in the current study with higher accumulation of both TCC and TCS in pepper root than in okra and tomato roots (Fig. 2). Pepper also accumulated higher concentrations of both PAH and PCB in its leaves than did okra and tomato (Al Nasir and Batarseh 2008). Likewise, the translocation factors for both TCC and TCS were highest for pepper than for okra and tomato (Table 1). Consequently, the observed trends for accumulation of organic contaminants by fruit-producing vegetables were similar for PAHs, PCBs, and antimicrobials.

Substantial research has been conducted on organic contaminant accumulation by cucurbits (e.g., cucumber, pumpkin, and zucchini). Translocation of TCC and TCS by cucumber (this study) was 0.082±0. 03 % and 0.16± 0.04 %, respectively, which is less than the translocation observed previously for hydroponically grown pumpkin and zucchini (0.13 % for TCC and 8.2 % for TCS) (Aryal and Reinhold 2013). A similar enhanced uptake of organic contaminants by pumpkin and zucchini, as compared to cucumber or other vegetables, has also been observed for DDT (Lunney et al. 2004b) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Huelster et al. 1994). While pumpkin and zucchini are from the same genus *Cucurbita*, cucumber is from the genus *Cucumis*, which may result in a different uptake pathway for contaminants (Huelster et al. 1994).

Table 1 Translocation factors (TF) and root concentration factors (RCF) for TCC and TCS

	Cucumber	Tomato	Okra	Pepper	Cabbage	Celery	Broccoli	Asparagus	Potato	Beet	Onion
TCC											
TF (%)	0.082±0.03	0.14±0.03	0.12±0.048	0.54±0.28	0.22±0.092	1.5±0.75	0.35±0.21	1.5±0.64	0.14±0.014	0.24±0.12	0.049±0.046
RCF	3,288±407	2,403±203	6,158±1,281	7,573±2,395	3,233±551	576±178	674±218	38.73±5.54	1,275±258	1,786±431	8,610±2,566
TCS											
TF (%)	0.16±0.04	0.29±0.06	0.42±0.19	0.98±0.79	3.2±1.3	0.53±0.28	3.2±2.5	3.7±1.7	0.99±0.55	0.29±0.1	0.12±0.12
RCF	2,576±536	1,538±122	1,353±303	4,896±927	732±354	327±101	96±28	15.49±3.05	304±45	783±126	1,892±267

Of the experimental leaf-producing crops (i.e., cabbage, celery, broccoli, and asparagus), cabbage accumulated the highest concentrations of both TCC and TCS in the shoot and root (Fig. 3) with translocation factors of 0.0022±0.0009 for TCC and 0.032±0.013 for TCS. However, after the second week of exposure, toxicity symptoms were observed with wilting of cabbage leaves when compared to the control. When cabbage was raised in soil with 433 μg kg^{-1} TCS, the observed translocation factor was 0.02 (Holling et al. 2012); consequently, the observed toxicity may have contributed to lower translocation of antimicrobials by cabbage in this study. Cabbage still accumulated 5.8 times more TCS in its leaves than did celery; however, shoot accumulation of TCC was similar for both cabbage and celery (Fig. 3). Root accumulation of both TCC and TCS was greater for cabbage than for celery by 6.1 and 2.1 times, respectively. In contrast, cabbage and celery accumulated similar concentrations of DDT in both roots and shoots (Tao et al. 2005). Consequently, the observed dependency of accumulation on species was not consistent for multiple organic contaminants for leaf-producing crops.

For root-producing crops, TCC and TCS concentrations in beet shoots (0.53 and 0.48 mg kg^{-1}, respectively) were greater than those in onion shoots (0.24 and 0.12 mg kg^{-1}, respectively), whereas the opposite trend was observed for onion roots (851 and 277 mg kg^{-1}, respectively) and beet roots (205 and 193 mg kg^{-1}, respectively). The edible portions of the roots accumulated substantially lower concentrations of antimicrobials. Concentrations of antimicrobials in beet tubers were 5.3 mg kg^{-1} TCC and 4.8 mg kg^{-1} TCS, and concentrations of antimicrobials in onion bulbs were 25.6 mg kg^{-1} TCC and 16.4 mg kg^{-1} TCS.

Potato accumulated substantially lower concentrations of antimicrobials in the roots than did beet and onion. The concentrations of TCC in the peel, middle section, and core of the potato tuber were 0.10, 0.10, and 0.24 mg kg^{-1}, respectively, while the concentrations of TCS were 0.10, 0.10, and 0.32 mg kg^{-1}, respectively. There was no significant difference the concentration of TCC or TCS in the three sections of the tuber, likely due to the limited numbers of samples that were analyzed. However, the observed increase in antimicrobial concentrations in the core of the potato tuber differs from that of accumulation of the antibiotic sulfamethazine, where concentrations were higher in the outer skin of the potato tuber (1.5 mg kg^{-1}) than the core (<0.5 mg kg^{-1}) (Bruce et al. 2010). When four varieties of potatoes were raised in organic farms in the presence of PAHs, PCBs, and organo-chlorine pesticides, all the varieties accumulated the chemicals in the peel and core. The concentrations of PAH and organo-chlorine pesticide in the peel was higher than the core, but were similar in the peel and core for PCBs—similar to the observations in the current study (Zohair et al. 2006). The partitioning of the chemicals in the peel and the core hence depends on the contaminant.

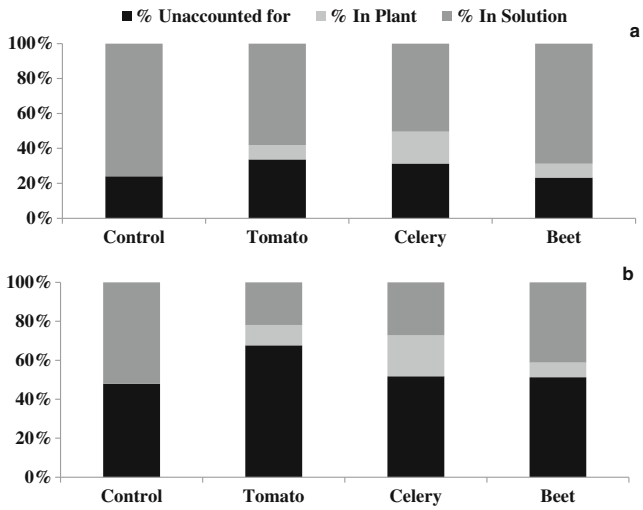

Fig. 3 Mass balance of **a** TCC and **b** TCS for control, tomato, celery, and beet

Transpiration and translocation

Once taken up by the roots, contaminants are translocated through the transpiration stream. Transpiration rate can be a good indicator of translocation of contaminants (Miguel et al. 2012; Zhao et al. 2012). The hydrophobicity of a contaminant, as quantified by log K_{OW}, is a factor that has been related to the passive translocation of a contaminant via the transpiration stream (Karnjanapiboonwong et al. 2011). The passive translocation of a pollutant in plants, on a mass basis, is directly proportional to the transpiration stream, provided factors such as the chemical characteristics of the compound, environmental conditions, and plant species are constant (Burken and Schnoor 1996). Contaminants with log K_{OW} values of approximately 2 are easily translocated via the transpiration stream, whereas contaminants with log K_{OW} values greater than 4 are generally not. Both TCC and TCS have log K_{OW} values greater than 4 at pH values expected in the transpiration stream, and hence reduced or no translocation via the transpiration stream is expected. However, translocation of TCS ranged from 0.12 to 3.7 % and was independent of volume of media transpired (r^2=0.0082). The lack of correlation between translocation factors and transpiration of antimicrobials, combined with the high log K_{OW} of antimicrobials, indicates that a mechanism beyond passive translocation (such as enzyme transport) may be responsible for shoot accumulation of antimicrobials.

The TF for TCC was less than that of TCS for every crop except celery and beet, implying that the translocation from the root to the shoot is generally more limited for TCC. TFs observed for celery and beet were similar for TCS and TCC. The observed translocation factors were within the same order of magnitude as those observed for pumpkin and zucchini in hydroponic studies (Aryal and Reinhold 2013), but were

considerably lower than those observed for pumpkin and zucchini in soil systems (Aryal and Reinhold 2011).

Mass balance

The mass balance of TCC and TCS was calculated for each crop. Measured biomass (dry weight) for the roots and shoots was highest for asparagus and celery. The lowest shoot biomass was observed for tomato, and the lowest root biomass was observed for onion (not including the weight of the bulb). Plant moisture contents ranged from 35.2 to 96.3 %. In general, more TCC was unaccounted for than TCS (Fig. 3). This is most likely attributed to the greater concentration of the TCC (17 mg kg^{-1}) than TCS (2 mg kg^{-1}) in the gelatinous precipitate that was lost during the filtration of the media before analysis. The mass balance distribution of selected representative plants is depicted in Fig. 3. The complete mass balance including the residue was studied only for beetroots. The mass balance of the beetroot plant indicated that 15.1 % and 1.7 % of TCC and TCS, respectively, was seen in the residue which accounts for 21 % and 4 % of the unaccounted fraction, respectively. The greatest mass of TCC in the plant was observed in okra, while the greatest mass of TCS was observed in celery. Plant accumulation accounted for 5–45 % of TCC fate and 2–22 % of TCS fate in the hydroponics—substantial contributions given the initial concentration of 500 μg L^{-1}. The total mass balance equation can be depicted as

$$M_{\text{total}} = M_{\text{residue}} + M_{\text{plant}} + M_{\text{unaccounted}} + M_{\text{solution}}$$

Potential exposure

Whether application of antimicrobial-laden biosolids to agricultural fields poses a threat to human health depends on the uptake of these contaminants in the edible portion of the plant. Previous studies on accumulation of antimicrobials by pumpkin and zucchini indicated that hydroponic studies yielded similar or slightly lower shoot concentrations of TCS and TCC (respectively) as studies using soil systems with environmentally relevant concentrations of antimicrobials (Aryal and Reinhold 2013). Consequently, hydroponic studies are valuable for assessing accumulation of antimicrobials by food crops. However, it is important to note that the exposure calculation herein conservatively assumes that shoot concentrations are equivalent to fruit concentrations, which may overestimate potential exposure as previous studies with accumulation of antimicrobials by pumpkin, zucchini, and soybeans indicate that leaf and fruit concentrations were less than stem concentrations (Aryal and Reinhold 2011; Wu et al. 2010b).

Table 2 Exposure assessments for TCC and TCS

Exposure	1–2 years Mean	Middle age Mean	Whole population Mean
ng TCC kg^{-1} day^{-1}			
Cucumber	8.4±3.4	2.8±1.1	3.3±1.2
Tomato	56.0±11.4	24.7±4.9	27.7±5.5
Cabbage	63.8±37.7	35.8±17.1	37.6±17.8
Pepper	NC	NC	42.9±17.8
Celery	22.1±11.7	8.4±4.2	9.1±4.6
Beet	NC	7,641.8±2,941.5	7,387.1±2,052.4
Onion	752.7±754.8	501.8±259.5	501.8±258.0
ng TCS kg^{-1} day^{-1}			
Cucumber	24.2±7.6	8.2±2.3	9.5±2.6
Tomato	150.3±29.7	66.2±12.7	74.3±14.3
Cabbage	129.2±49.2	72.6±11.1	76.1±10.9
Pepper	NC	NC	35.2±15.3
Celery	8.2±4.5	3.1±1.6	3.4±1.8
Beet	NC	7,225.2±2378.3	6,984.4±1,350.3
Onion	481.3±288.9	320.9±191.9	320.9±191.1

Predicted exposure to antimicrobials from consumption of food crops are summarized in Table 2. The exposure was calculated by using the daily consumption rates of the vegetables by different age groups and the mean analytical residue concentration of TCC and TCS accumulated in the plant in the present study (Rasmussen et al. 2002). The moisture content was also considered in estimating the exposure. The highest predicted exposure to TCC and TCS through vegetable consumption resulted from consumption of onions, which accounted for greater than 60 % for TCC and 45 % for TCS of the predicted exposure from the experimental crops. Consumption of root crops accounted for 72–86 % of predicted exposure to antimicrobials through consumption of food crops.

Overall, estimated exposure to TCC was greater than exposure to TCS; however, substantially higher exposure to

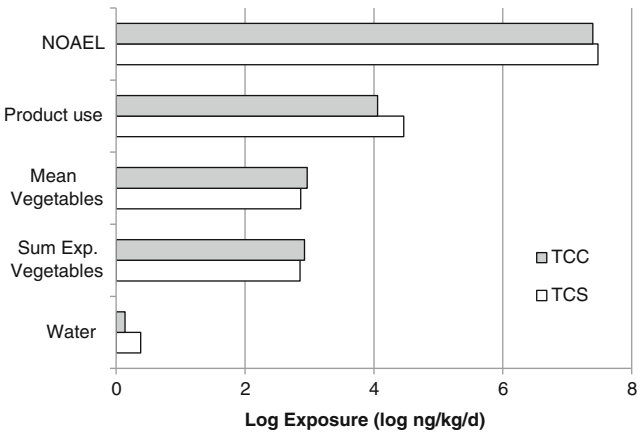

Fig. 4 Potential exposure to antimicrobials from food crops consumption and exposure from crops compared to other routes

TCC than to TCS from onion accounted for much of the TCC exposure. When root crops were excluded, exposure to TCC was less than exposure to TCS (120.6 ng kg^{-1} day^{-1} vs. 196.4 ng kg^{-1} day^{-1}, respectively). Consequently, TCS is of higher concern in terms of human health exposure than is TCC in the case of fruit- and edible leaf-producing crops. The exposure due to the consumption of pumpkin was 8 and 993 ng^{-1} kg^{-1} day TCC and TCS, respectively, and for zucchini 8.8 and 334 ng^{-1} kg^{-1} day (Aryal and Reinhold 2011). However, this difference may be due to difference in plant species and the longer experimental time in this study.

The estimated exposure from the mean of the crops compared to other routes of exposure is shown in Fig. 4. The estimated exposure from vegetables is 10^3 times greater than exposure from drinking water and $10^{0.5}$ times less than exposure from product use. The total estimated exposure is $10^{2.9}$ to $10^{3.3}$ times less than the acute NOAEL, indicating that current exposure does not present a regulated human health risk, even when unintended routes of exposure are considered.

Conclusions

All 11 experimental food crops were capable of uptaking and accumulating milligram-per-kilogram concentrations of TCC and TCS after 1 month of exposure. Concentrations of antimicrobials in the roots were two or three orders of magnitude greater than the concentrations of antimicrobials in the shoots. Translocation factors were higher for TCS than for TCC in the majority of the plants. Pepper had highest translocation of both TCC and TCS to the shoots. Pepper accumulated the highest concentrations of antimicrobials, supporting the general observation that pepper plants tend to accumulate greater concentrations of organic contaminants than most food crops. This observation is supported by literature documenting increased accumulation of PCBs and PAHs by pepper plants. Cabbage and onion accumulated the highest concentrations of antimicrobials when compared to other leaf- and tuber-producing plants (respectively).

Based on exposure assessments, the highest exposure from the edible fruit, leaf, and root categories were for pepper, cabbage, and onion, respectively, for both TCC and TCS. Eliminating consumption of onion was estimated to reduce exposure to antimicrobials from consumption of food crops by at least 50 %. Further research on this area is required to understand the harmful effects of TCC and TCS that can help in future recommendations in use of biosolids and wastewater effluents for fertilization and irrigation.

Acknowledgments The authors would like to thank Dr. Kurt Thelen and Dr. Yunjie Ding for the accelerated solvent extractor; Niroj Aryal and Kris Van Winkle for their assistance on the LC–MS. Funding for this project was made possible by a grant from the National Institute of Food and Agriculture (NIFA) within the United States Department of Agriculture (USDA) (Grant No. 2011-67019-20026).

References

Al Nasir F, Batarseh MI (2008) Agricultural reuse of reclaimed water and uptake of organic compounds: pilot study at Mutah University wastewater treatment plant, Jordan. Chemosphere 72:1203–1214

APHA A, WEF (1999) Standard methods for examination of water & wastewater. American Public Health Association, American Water Works Association. Water Environment Federation, Washington

Aryal N, Reinhold D (2013) Phytoaccumulation of antimicrobials by hydroponic *Cucurbita pepo*. Int J Phytorem 15:330–342

Aryal N, Reinhold DM (2011) Phytoaccumulation of antimicrobials from biosolids: impacts on environmental fate and relevance to human exposure. Water Res 45:5545–5552

Barcelo D, Petrovic M (2007) Pharmaceuticals and personal care products (PPCPs) in the environment. Anal Bioanal Chem 387:1141–1142

Berthouex PM, Brown LC (2002) Statistics for environmental engineers, 2nd edn. Lewis, Boca Raton

Bruce GM, Pleus RC, Snyder SA (2010) Toxicological relevance of pharmaceuticals in drinking water. Environ Sci Technol 44:5619–5626

Burken J, Schnoor J (1996) Phytoremediation: plant uptake of atrazine and role of root exudates. J Environ Eng 122:958–963

Cha J, Cupples AM (2009) Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. Water Res 43:2522–2530

Chen X, Nielsen JL, Furgal K, Liu Y, Lolas IB, Bester K (2011) Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions. Chemosphere 84:452–456

Chu SG, Metcalfe CD (2007) Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. J Chromatogr, A 1164:212–218

Escalada MG, Harwood JL, Maillard JY, Ochs D (2005) Triclosan inhibition of fatty acid synthesis and its effect on growth of *Escherichia coli* and *Pseudomonas aeruginosa*. J Antimicrob Chemother 55:879–882

Halden RU, Paull DH (2005) Co-occurrence of triclocarban and triclosan in U.S. water resources. Environ Sci Technol 39:1420–1426

Heidler J, Halden RU (2007) Mass balance assessment of triclosan removal during conventional sewage treatment. Chemosphere 66:362–369

Heidler J, Sapkota A, Halden RU (2006) Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. Environ Sci Technol 40:3634–3639

Holling C, Bailey J, Vanden Heuvel B, Kinney C (2012) Uptake of human pharmaceuticals and personal care products by cabbage (*Brassica campestris*) from fortified and biosolids-amended soils. J Environ Monit 11:3029–3036

Huelster A, Mueller JF, Marschner H (1994) Soil-plant transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans to vegetables of the cucumber family (Cucurbitaceae). Environ Sci Technol 28:1110–1115

Karnjanapiboonwong A, Chase DA, Canas JE, Jackson WA, Maul JD, Morse AN, Anderson TA (2011) Uptake of 17Î±-ethynylestradiol and triclosan in pinto bean, *Phaseolus vulgaris*. Ecotoxicol Environ Saf 74:1336–1342

Kim J, Drew MC, Corapcioglu MY (2004) Uptake and phytotoxicity of TNT in onion plant. J Environ Sci Health, Part A 39:803–819

Kwon J-W, Armbrust KL, Xia K (2010) Transformation of triclosan and triclocarban in soils and biosolids-applied soils. J Environ Qual 39: 1139–1144

Lawrence JR, Zhu B, Swerhone GDW, Roy J, Wassenaar LI, Topp E, Korber DR (2009) Comparative microscale analysis of the effects of triclosan and triclocarban on the structure and function of river biofilm communities. Sci Total Environ 407:3307–3316

Loffredo E, Eliana Gattullo C, Traversa A, Senesi N (2010) Potential of various herbaceous species to remove the endocrine disruptor bisphenol A from aqueous media. Chemosphere 80:1274–1280

Low JE, Aslund MLW, Rutter A, Zeeb BA (2010) Effect of plant age on PCB accumulation by *Cucurbita pepo* ssp *pepo*. J Environ Qual 39: 245–250

Lozano N, Rice CP, Ramirez M, Torrents A (2010) Fate of triclosan in agricultural soils after biosolid applications. Chemosphere 78:760–766

Lozano N, Rice CP, Ramirez M, Torrents A (2012) Fate of triclosan and methyltriclosan in soil from biosolids application. Environ Pollut 160:103–108

Lozano N, Rice CP, Ramirez M, Torrents A (2013) Fate of triclocarban, triclosan and methyltriclosan during wastewater and biosolids treatment processes. Water Res 47:4519–4527

Lunney AI, Zeeb BA, Reimer KJ (2004) Uptake of weathered DDT in vascular plants: potential for phytoremediation. Environ Sci Technol 38:6147–6154

McMurry LM, Oethinger M, Levy SB (1998) Triclosan targets lipid synthesis. Nature 394:531–532

Miguel S, Ravanel P, Raveton M (2012) A comparative study on the uptake and translocation of organochlorines by *Phragmites australis*. J Hazard Mater 244–245:60–69

Murano H, Otani T, Seike N, Sakai M (2009) Dieldrin uptake and translocation in plants growing in hydroponic medium. Environ Toxicol Chem 29:142–148

Ozaki N, Bester K, Moldrup P, Henriksen K, Komatsu T (2011) Photodegradation of the synthetic fragrance OTNE and the bactericide triclosan adsorbed on dried loamy sand—results from models and experiments. Chemosphere 83:1475–1479

Pannu MW, Toor GS, O'Connor GA, Wilson PC (2012) Toxicity and bioaccumulation of biosolids-borne triclosan in food crops. Environ Toxicol Chem 31:2130–2137

Paul KB, Hedge JM, DeVito MJ, Crofton KM (2010) Developmental triclosan exposure decreases maternal and neonatal thyroxine in rats. Environ Toxicol Chem 29:2840–2844

Perencevich EN, Wong MT, Harris AD (2001) National and regional assessment of the antibacterial soap market: a step toward determining the impact of prevalent antibacterial soaps. Am J Infect Control 29:281–283

Rasmussen L, Andersen KJ, Ando M, Bolger M, Fawell J, Fuge R, Kosmus W, Lamont WH, Magara Y, Moy G (2002) Environmental health and human exposure assessment, United Nations Synthesis Report on Arsenic in Drinking Water. WHO, Washington

Sapkota A, Heldler J, Halden RU (2007) Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry. Environ Res 103:21–29

Sivaraman S, Sullivan TJ, Johnson F, Novichenok P, Cui GL, Simmerling C, Tonge PJ (2004) Inhibition of the bacterial enoyl reductase FabI by triclosan: a structure–reactivity analysis of FabI inhibition by triclosan analogues. J Med Chem 47:509–518

Suller MTE, Russell AD (2000) Triclosan and antibiotic resistance in *Staphylococcus aureus*. J Antimicrob Chemother 46:11–18

Tao S, Xu FL, Wang XJ, Liu WX, Gong ZM, Fang JY, Zhu LZ, Luo YM (2005) Organochlorine pesticides in agricultural soil and vegetables from Tianjin, China. Environ Sci Technol 39:2494–2499

U. S. Environmental Protection Agency (U.S. EPA) (2002) High Production Volume (HPV) Chemical Challenge Program Data Availability and Screening Level Assessment for Triclocarban: The TCC Consortium. Report 201-14186A. Accessed 23 Apr 2013

USEPA (2007) Water: Sewage sludge (Biosolid). Office of Pollution Prevention and Toxics, Environmental Protection Agency, U. S., (Ed).

USEPA (2008a) Reregistration Eligibility Decision for Triclosan. Office of Prevention, Pesticides and Toxic Substances. EPA 739-RO-8009. Accessed 22 Apr 2013

USEPA (2008b) Screening-level hazard characterization of high production volume chemicals. In: RAD High Production Volume Chemicals Branch, Risk Assessment Division, Office of Pollution Prevention and Toxics. Accessed 23 Apr 2013

USEPA (2010) Triclosan Facts. In: USEP Agency (ed). http://www.epa.gov/oppsrrd1/REDs/factsheets/triclosan_fs.htm. Accessed 1 Mar 2013

USFDA (2010) Triclosan: What Consumers Should Know. In: USFaD Administration (ed). http://www.fda.gov/forconsumers/consumerupdates/ucm205999.htm. Accessed 23 Apr 2013

Wang XP, White JC, Gent MPN, Iannucci-Berger W, Eitzer BD, Mattina MJI (2004) Phytoextraction of weathered p(,)p'-DDE by zucchini (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) under different cultivation conditions. Int J Phytorem 6:363–385

White JC, Wang X, Gent MPN, Iannucci-Berger W, Eitzer BD, Schultes NP, Arienzo M, Mattina MI (2003) Subspecies-level variation in the phytoextraction of weathered p, p'-DDE by *Cucurbita pepo*. Environ Sci Technol 37:4368–4373

Wu C, Spongberg AL, Witter JD (2009) Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils. J Agric Food Chem 57:4900–4905

Wu C, Spongberg AL, Witter JD, Fang M, Czajkowski KP (2010) Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. Environ Sci Technol 44:6157–6161

Wu C, Spongberg AL, Witter JD, Sridhar BBM (2012) Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil. Ecotoxicol Environ Saf 85:104–109

Ying G-G, Yu X-Y, Kookana RS (2007) Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environ Pollut 150:300–305

Zhang H, Chen J, Ni Y, Zhang Q, Zhao L (2009) Uptake by roots and translocation to shoots of polychlorinated dibenzo-p-dioxins and dibenzofurans in typical crop plants. Chemosphere 76:740–746

Zhao M, Zhang S, Wang S, Huang H (2012) Uptake, translocation, and debromination of polybrominated diphenyl ethers in maize. J Environ Sci 24:402–409

Zohair A, Salim A-B, Soyibo AA, Beck AJ (2006) Residues of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides in organically-farmed vegetables. Chemosphere 63:541–553