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ACCUMULATION OF ANTIMICROBIALS BY PLANTS:
IMPACTS ON ANTIMICROBIAL FATE AND RISK

presented by

NIROJ ARYAL

has been accepted towards fulfillment
of the requirements for the

Master of Science degree in Biosystems Engineering

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Major Professor's Signature

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Date

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ACCUMULATION OF ANTIMICROBIALS BY PLANTS: IMPACTS ON ANTIMICROBIAL FATE AND RISK

BY

NIROJ ARYAL

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ABSTRACT

ACCUMULATION OF ANTIMICROBIALS BY PLANTS: IMPACTS ON ANTIMICROBIAL FATE AND RISK

BY

NIROJ ARYAL

Triclocarban and triclosan, two widely used antimicrobials in consumer use products, adversely affect ecosystems and potentially human health. The application of biosolids to agricultural fields introduces triclocarban and triclosan to soil and water resources through runoff. This research examined the effects of plant growth on the fate and migration of antimicrobials to water resources, focusing on plant accumulation of antimicrobials and a risk characterization following plant accumulation of antimicrobials.

Pumpkin, zucchini, and switch grass were grown in soil columns to which biosolids were applied. Leachate from soil columns was assessed every other week for concentration of triclocarban and triclosan. At the end of trial, concentration of triclocarban and triclosan was determined for soils, roots, stems, and leaves using liquid chromatography mass spectrometry. In addition, pumpkin, and zucchini were grown hydroponically in medium spiked with triclocarban and triclosan to study the phytoaccumulation potential of plants for antimicrobials.

Results indicated that plant growth reduced the leaching of antimicrobials from columns. Plants accumulated triclocarban and triclosan, but not at high enough concentrations to be considered hyperaccumulators. There was negligible risk of antimicrobials from eating pumpkin and zucchini fruits produced from fields to which biosolids have been applied.

Dedication

To my Late Grandmother

Mishri Maya Aryal

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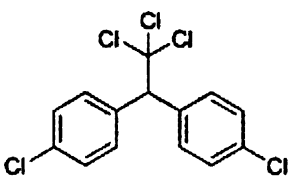
Chapter 1: Introduction

1.1 Rationale

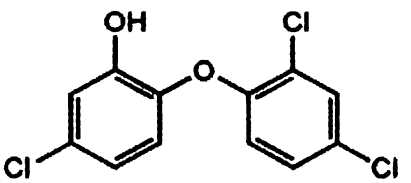
Triclocarban and triclosan are two very widely used antimicrobials worldwide. Added to daily use products like hand soaps, lotions, toothpastes, cosmetics and deodorants, annual per capita usage in the U.S. is 1,130 mg triclocarban and 1,030 mg triclosan. Consequently, the combined input of antimicrobials into the U.S. environment is 0.6 - 1 million kg (Halden and Paull 2005, EPA 2003). Triclocarban and triclosan are not readily biodegradable, persistent and hydrophobic (EPA 2002b, EPA 2008b). These "down the drain" contaminants are not readily degraded and strongly sorb to solids in wastewater treatment plants (Heidler, Sapkota and Halden 2006, Heidler and Halden 2007). However, a fraction of antimicrobial does migrate to water resources. The widespread practice of applying digested sludge from wastewater treatment plant to agricultural fields introduces triclocarban and triclosan into agricultural soils (Kinney et al. 2008, Cha and Cupples 2009). Once in agricultural soils, triclocarban and triclosan can contaminate runoff water and then water resources (Topp et al. 2008, Sabourin et al. 2009). Triclocarban is ranked top 10 in occurrence and top 20 in concentration among 96 organic pollutants in the United States water resources (Halden and Paull 2005).

Moreover, triclocarban and triclosan are toxic to animals, plants and potentially humans. For example, algae growth is affected at current environmental concentrations (Johnson et al. 2009). Antimicrobials also affect soil and aqueous ecological health. More details about the fate and effects of antimicrobials are discussed in Chapter 2: Literature Review.

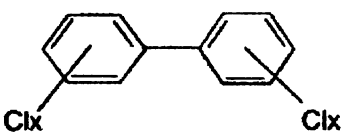
Continuous release of antimicrobials to water resources and soils necessitates exploration of sustainable management strategies. Phytoremediation could be a sustainable approach to managing these types of continuously released organic pollutants. Phytoremediation is inexpensive, environmentally friendly, and requires low maintenance. Polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and their metabolites, which have two chlorinated benzene rings similar to triclocarban and triclosan (Figure 1.1), have been shown to be phytoremediated (Aslund et al. 2007, Lunney, Zeeb and Reimer 2004). Specifically, pumpkin and zucchini can phyto or hyperaccumulate compounds like PCBs, and DDT and its metabolites (Dzantor, Chekol and Vough 2000, Lunney et al. 2004, Wang et al. 2004, Aslund et al. 2008, White et al. 2003).



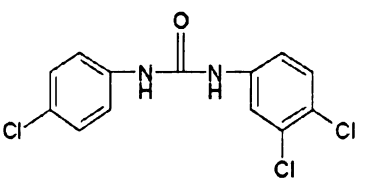
Dichlorodiphenyltrichloroethane (DDT)



Triclosan (TCS)



Polychlorinated biphenyls (PCBs)



Triclocarban (TCC)

Figure 1.1 Comparison of Chemical Structure of DDT, PCBs, TCS, and TCC

Given the widespread use, high production volume and established risks to humans and the environment, there is a pressing need to evaluate antimicrobials with

regards to occurrence, fate and effects in broad system-based scenario. Most research on triclocarban and triclosan has focused on occurrence and fate in water resources and wastewater treatment plants (Coogan et al. 2007, Halden and Paull 2004, Halden and Paull 2005, Heidler et al. 2006, Heidler and Halden 2007, Sapkota, Heldler and Halden 2007, Young et al. 2008, Chu and Metcalfe 2007, Yu and Chu 2009, Miller et al. 2008b, Hua, Bennett and Letcher 2005, Bester 2003, Vikesland, Rule and Greyshock 2004, Wu et al. 2007, Poiger et al. 2003, Nishi, Kawakami and Onodera 2008, Bester 2005, Thompson et al. 2005, Morrall et al. 2004, Sabaliunas et al. 2003, Montes et al. 2009) and very few on agricultural soils (Cha and Cupples 2009, Kinney et al. 2008, Chu and Metcalfe 2007). The fate of antimicrobials in agricultural fields with plants is unknown, especially with regards to the role of plant growth in migration of antimicrobials from fields to water resources after land application of biosolids from wastewater treatment plants. Understanding the interactions between plants and antimicrobials is crucial to both (i) quantify the phytoremediation potential of plants for these compounds and (ii) to assess accumulation of antimicrobials in vegetables grown in biosolid-applied field.

1.2 Objectives

The thesis presented herein evaluates the hypothesis that triclocarban and triclosan, being similarly structured to other chlorinated aromatic organic contaminants like DDT, DDE and PCB (Figure 1.1), can be phytoremediated to reduce antimicrobial concentrations in soil and the migration of antimicrobials to water resources. Pumpkin and zucchini were selected due to their reported potential of PCBs and DDT phytoremediation. This study also aims to quantify the human health effects of

consuming food grown on land to which biosolids are applied. Switch grass was selected as a non-vegetable plant that is a potential bioenergy crop and has potential to phytoremediate hydrophobic aromatic compound like PCBs (Dzantor et al. 2000).

The specific aims of this study were to:

(i) Assess the effects of growth of pumpkin, zucchini and switch grass on leaching and soil accumulation of triclocarban and triclosan from land applied biosolids,

(ii) Evaluate phytoaccumulation of triclocarban and triclosan in pumpkin, zucchini and switch grass,

(iii) Evaluate phytoaccumulation of triclocarban and triclosan in pumpkin and zucchini grown hydroponically, and

(iv) Characterize health risk associated with observed accumulation of triclocarban and triclosan by pumpkin and zucchini to humans.

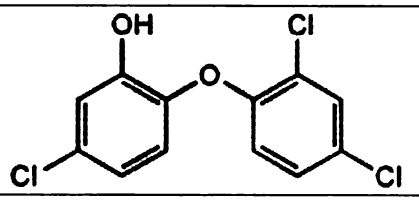
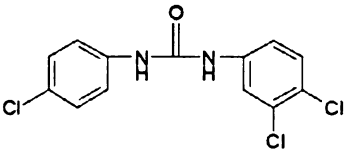
Chapter 2: Literature Review

2.1 Uses of Triclocarban and Triclosan

Antimicrobials such as triclocarban (3,4,4'-trichlorocarbanilide) and triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) are widely used in personal care products like toothpaste, soaps, deodorants, cosmetics and lotions (Ying, Yu and Kookana 2007). A retail survey of national brand liquid and bar soaps in 1999 to 2000 reported that antibacterial agents TCC and TCS were present in 76% of liquid soaps and 29% of bar soaps available nationally (Perencevich, Wong and Harris 2001). Added to soaps at levels of 0.5 to 5% (w/w), production of TCC exceeds one million pound per year. Triclosan is used in consumer products at concentrations of 0.69 – > 99 % (Heidler et al. 2006, EPA 2002b). Some personal consumer applications of triclosan include hand soaps, toothpaste, deodorants, laundry detergent, fabric softeners, facial tissues, antiseptics for wound care, and medical devices. Triclosan is used for commercial, residential and industrial applications to minimize bacterial growth on equipment and instruments. Industrial uses include conveyer belts, fire hoses, dye bath vats, ice making instruments, and HVAC coils. Triclosan is also used as a material preservative in toys, paints, mattresses, clothing, brooms, mulch, floors, shower curtains, awnings, tents, toilet bowls, urinals, garbage cans, refuse container liners, insulation, concrete mixtures, grouts, and upholstery fabrics adhesives, fabrics, vinyl, plastics, polyethylene, polyurethane, polypropylene, floor wax emulsions, textiles (footwear, clothing), caulking compounds, sealants, rubber, and latex paints (EPA 2008b).

The structure and properties of triclocarban and triclosan are shown in Table 2.1. Both are chlorinated aromatic organic compounds with low solubility in water and high octanol - water partition coefficient. Both are similarly- structured to poly- chlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) (Figure 1.1).

Table 2.1 Structure and Properties of Triclocarban and Triclosan

	Triclosan	Triclocarban
Molecular Structure		
CAS registry no.	3380-34-5	00101-20-2
Molecular Weight	289.55	315.59
Dissociation Constant, pK_a	8.14 at 20°C	12.7
Water Solubility	10 mg/L at 20°C	0.02366 mg/L at 25°C
Octanol -Water Partition Constant (Log K_{OW})	4.76 at 25°C, pH 7	4.90 at 25°C, pH 7

Reference: (Halden and Paull 2005) and EPI SUITE 4.0

2.2 Occurrence and Fate of Triclocarban and Triclosan

Diverse uses of triclocarban and triclosan lead to high volume use with an annual per capita usage of 1,130 mg triclocarban and 1,030 mg triclosan nationwide, resulting in annual disposal of greater than 330,000 kg triclocarban and 300,000 kg triclosan in the U.S. (Halden and Paull 2005). The primary route through which triclocarban and triclosan enter the environment is domestic sewage discharge to wastewater treatment plants (WWTP) (Chu and Metcalfe 2007, Heidler et al. 2006, Sapkota et al. 2007).

Removal of antimicrobials in WWTP predominantly results from sorption to wastewater particulate matter (78 ± 11 % for triclocarban and 80 ± 22 % for triclosan) (Heidler et al. 2006, Heidler and Halden 2007). Consequently, dewatered municipal sludge accumulates 51 ± 15 mg triclocarban and 30 ± 11 mg triclosan per kg of sludge (Heidler and Halden 2007, Heidler et al. 2006). Approximately 50% of US biosolids are land applied (EPA 2007). Heidler et al. (2006) estimated that major part of approximately

three-quarters of triclocarban used by consumers is ultimately released into the environment through land application of municipal sludge as biosolids for agriculture (Heidler et al. 2006). Combined input of triclosan and triclocarban into U.S. environment exceeds 0.6 to 1 million kg/year (EPA 2003), with only 5,800 kg of triclocarban and 2,600-10,400 kg of triclosan resulting from discharge of effluent of activated sludge treatment plants into U.S. water resources nationwide (Halden and Paull 2005). Agricultural soils previously amended with biosolids contains triclocarban and triclosan in the range of 1.20 - 65.10 μg/kg triclocarban and 0.16 - 1.02 μg/kg triclosan (Cha and Cupples 2009). Marine sediment samples at the outflow of urban wastewater treatment plant contained triclosan from 0.27 - 130.7 μg/kg (Aguera et al. 2003).

Triclocarban ranks in the top 10 in occurrence and top 20 in maximum concentration among 96 organic pollutants in U.S. water resources (Halden and Paull 2005). A summary of the occurrence of antimicrobials in the environment is shown in Table 2.2. Triclocarban is present in the environment at broader range than triclosan. Triclocarban is detected in U.S. rivers and streams from 0.09 – 1.55 μg/L (Halden and Paull 2005, Sapkota et al. 2007), in sediment pore water from 1.77-10.78 μg/L (Chalew and Halden 2009, Miller et al. 2008a), in WWTP influents from 4.1-8.1 μg/L (Heidler et al. 2006, Halden and Paull 2005) and in WWTP effluents from 0.11-0.17 μg/L (Heidler et al. 2006, Halden and Paull 2005). Triclosan is detected in rivers from <0.003-0.075 μg/L (Bester 2003, Ying and Kookana 2007), in WWTP influents from 3.8 to 16.6 μg/L (McAvoy et al. 2002) and in WWTP effluents from 0.800 – 37.8 μg/L (Aguera et al. 2003). Annual loadings of antimicrobials to water resources are attributed to activated

sludge treatment plants (39-67%), trickling filter plants (31-34%) and combined and sanitary sewer overflows (2-7% and <0.2% respectively) (Halden and Paull 2005).

Once introduced into the environment, triclocarban and triclosan tend to sorb to soil or sediment (Heidler and Halden 2007). Miller et al. (2008) indicated the persistence of triclocarban in agricultural soils (Miller et al. 2008a). Additionally, triclocarban and triclosan computer fate modeling for biodegradability using Quantitative Structure-Activity Relationship (QSAR) indicate that these compounds do not degrade quickly with primary biodegradation half-lives of weeks and ultimate biodegradation half-lives of months (Halden and Paull 2005, Ying et al. 2007). Experimental half-lives of triclocarban and triclosan were 108 and 18 days respectively, in aerobic soil, with longer half lives in anaerobic soils (Ying et al. 2007). Triclosan is reported to form even more persistent and toxic degradation products like 2,8-dichlorodibenzo-*p*-dioxin (DCDD) (Gledhill 1975, Aranami and Readman 2007).

Accumulation of triclocarban and triclosan has been reported in aquatic organisms and humans. Computer modeling using EPIWIN PBT (persistence, bioaccumulation and toxicity) profiler, developed by USEPA, estimated that triclocarban and triclosan are potentially bio-accumulative (Ying et al. 2007). Triclocarban and triclosan tend to adsorb to particulate matter in waters, which are eaten by aquatic organism like fish, crustaceans, clams, oysters, snails leading to detectable concentration of antimicrobials in their tissues (Balmer et al. 2004, Ramirez et al. 2009). For example, maximums of 58.7 μg/kg triclosan and 299 μg/kg triclocarban was detected in snails near WWTP effluent (Coogan and La Point 2008). Triclocarban can also bioaccumulate rapidly in filamentous algae

(*Cladophora spp.)* and in snail at three orders of magnitude greater than ambient water concentrations (Coogan et al. 2007, Coogan and La Point 2008).

Bioaccumulation of antimicrobials has been also observed in humans. A survey of the U.S. general population in 2003 - 2004 detected concentrations of triclosan in 74.6% of urine samples at the concentration of 2.4 - 3,790 μg/L (Calafat et al. 2008). Also, triclosan was detected in plasma and breast milk of Swedish mothers who did not use any personal care products containing triclosan, indicating systemic exposure (Allmyr et al. 2006).

Table 2.2 Occurrence of Triclocarban and Triclosan in the Environment. Concentrations in mean ± standard deviation. WWTP = Wastewater treatment plant

	Medium	Triclocarban, (µg/L or µg/kg)	Triclosan, (µg/L or µg/kg)	Location	References	Remarks
WWTP influent	Aqueous	6.7 ± 0.1	6.1 ± 1.6	MD, USA	(Halden and Paull 2005)	
	Aqueous	6.1 ± 2.0		East Coast, USA	(Heidler et al. 2006)	
	Aqueous		0.4 - 9.4		(Farre et al. 2008)	
	Aqueous		1.1 - 1.3	Germany	(Bester 2003)	
	Aqueous		0.8 - 10.8	Mid-Atlantic, USA	(Heidler and Halden 2007)	
	Aqueous		3.8-16.6		(McAvoy et al. 2002)	
	Aqueous		0.01-4.0	ON, Canada	(Lishman et al. 2006)	
WWTP	Aqueous	0.19	0.12	TX, USA	(Coogan et al. 2007)	At WWTP
	Algae	109.0	150 .0	TX, USA	(Coogan et al. 2007)	
WWTP effluent	Aqueous	0.08	0.06	TX, USA	(Coogan et al. 2007)	
	Algae	401.0	146.0	TX, USA	(Coogan et al. 2007)	
	Snail	9.8 - 299.0	5.9 - 58.7 wet wt	TX, USA	(Coogan and La Point 2008)	
	Aqueous	0.11 ± 0.0 1	0.03 ± 0.02	MD, USA	(Halden and Paull 2005)	
	Aqueous	0.17 ± 0.03		East Coast, USA	(Heidler et al. 2006)	
	Aqueous		0.8 -37.8	Spain	(Aguera et al. 2003)	
	Aqueous		0.04-0.06	Germany	(Bester 2003)	
	Aqueous		0.023-0.434	Australia	(Ying and Kookana 2007)	19 effluents
	Aqueous		0.001-0.240	Mid-Atlantic, USA	(Heidler and Halden 2007)	Tertiary effluent
	Aqueous		0.2 - 2.7		(McAvoy et al. 2002)	
	Aqueous		0.010-0.324	ON, Canada	(Lishman et al. 2006)	
Biosolids	Sludge	51,000±15,000 (dry)		East Coast, USA	(Heidler et al. 2006)	
	Sludge	2,170-5,970 (dry)	620.0-11,550 dry wt	ON, Canada	(Chu and Metcalfe 2007)	
	Sludge	4,890-9,280	90.00-7060	MI, USA	(Cha and Cupples 2009)	
	Sludge		1,000-1,300	WWTP at Germany	(Bester 2003)	
	Biosolid		10,500	Midwest, USA	(Kinney et al. 2008)	
	Biosolid		90.00-16,790 (db)	Australia	(Ying and Kookana 2007)	19 biosolids
	Sludge		20,000-55,000	Mid-Atlantic, USA	(Heidler and Halden 2007)	Digested, dewatered

Table 2.2 Cont'd

	Medium	Triclocarban, (µg/L or µg/kg)	Triclosan, (µg/L or µg/kg)	Location	References	Remarks
Biosolids	Sludge	19,300 ± 7,100		USA	(Sapkota et al. 2007)	Dried, primary; co-contaminant DCC, tetraCC also found
	Sludge	620.0-11,550 (dry)	2,170-5,970 (dry)	ON, Canada	(Chu and Metcalfe 2007)	Samples of activated sludge and treated biosolids
	Sludge		500.0-15,600 (dry)		(McAvoy et al. 2002)	Digested
Soil (agricultural)	Soil	1.2 - 65.10	0.16 - 1.02	MI, USA	(Cha and Cupples 2009)	Previously biosolid amended
	Soil		833.0	Midwest, USA	(Kinney et al. 2008)	Previously biosolid amended
	Soil		160.0	Midwest, USA	(Kinney et al. 2008)	Minimally affected by manures
	Soil		69.0	Midwest, USA	(Kinney et al. 2008)	Previously Swine manure applied
Surface water	River	5.6 - 6.7			(Halden and Paull 2004)	Known input or wastewater
	River		<0.003-0.010	Germany	(Bester 2005)	
	Rivers		Up to 0.075	Australia	(Ying and Kookana 2007)	Outfall, upstream, downstream
	River	0.084±0.110		USA	(Sapkota et al. 2007)	Downstream of sewage treatment plants
	River	0.012±0.015		USA	(Sapkota et al. 2007)	Upstream of sewage treatment plants
	Runoff	0.003 ± 0.002	0.109 ± 0.081	Canada	(Sabourin et al. 2009)	Runoff from dewatered municipal sludge applied field
	Runoff		0.258 ±0.039	Canada	(Topp et al. 2008)	Runoff from biosolid applied field
Sediment pore water	Aqueous	1.770 ± 0.670 (avg); 3.96 (max)		USA	(Miller et al. 2008a)	Mono -, di -, and nonchlorinated carbanilide found
	Aqueous	4.05 ±3.38; 10.78 (max)		USA	(Miller et al. 2008a)	DCC=15.5±1.8 mg/kg
		0.24-32.90	0.26-382.8		(Chalew and Halden 2009)	Estimated
Sediment		700.0-1,600 dry wt	80.0 ± 10.0	USA	(Miller et al. 2008a)	MCC=4.1±2.4 mg/kg; NCC=0.5±0.1 mg/kg
		<20.00 – 8,200		USA	(EPA 2002a)	
			0.270-130.7	Spain	(Aguera et al. 2003)	Sediments at outflow of WWTP
			0.006	Spain	(Morales-Munoz et al. 2005)	Sediment at WWTP outflow

2.3 Impacts of Triclocarban and Triclosan

Release of antimicrobials into the environment and subsequent accumulation in organisms raises a number of concerns about human health and ecosystem health. Figure 2.1 shows threshold toxicity values and reported occurrences of TCS and TCC in different environmental matrix (Appendix A.1 and A.2) published in different literatures.

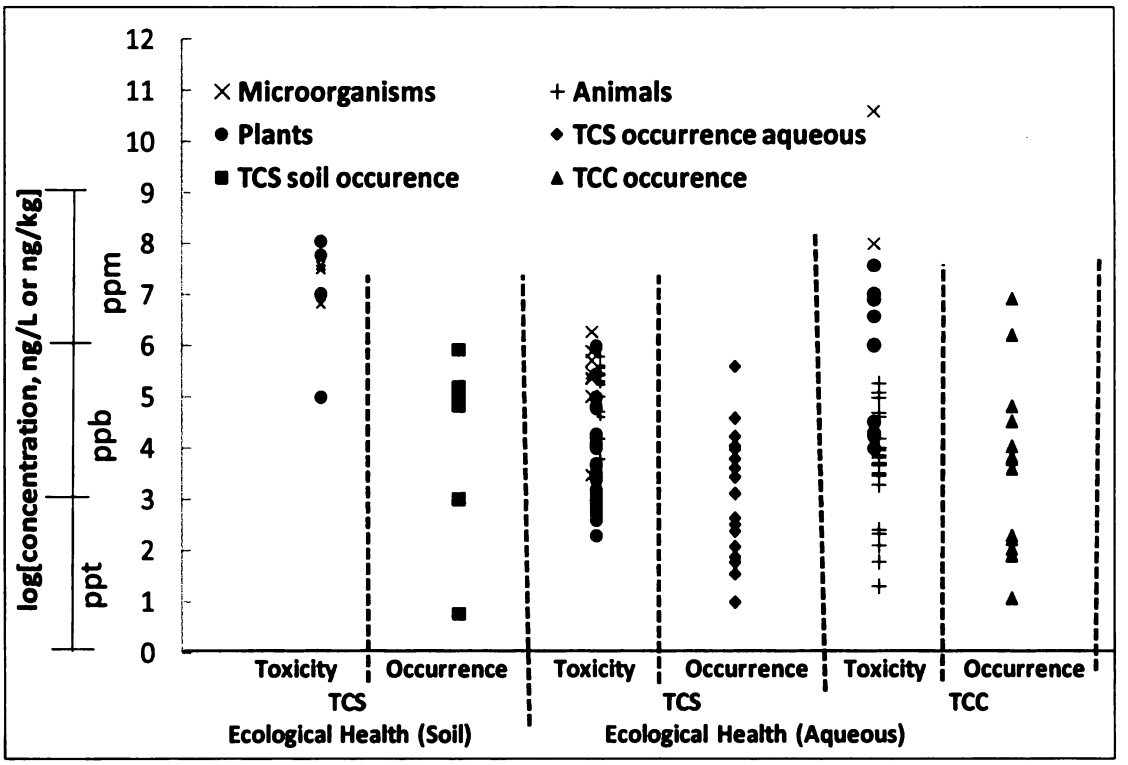


Figure 2.1 Comparison of Occurences and Threshold Toxicity Values of Triclosan and Triclocarban to Microorganisms, Animals and Plants in Both Soil and Aqueous Ecosystem. References are given at Appendices A.1 and A.2

Human Health. Multiple studies have documented adverse human health effects from antimicrobials. Triclocarban significantly reduced mammalian reproduction in rats at 0.25 % of diet and caused methemboglobeminema in humans (1971, Ponte et al. 1974, Johnson, Navone and Larson 1963). Methemboglobeminema was caused by the use of enema solutions made from soaps containing triclocarban. Margin of exposure (MOE),

which is ratio of no-observable adverse effect level (NOAEL) to the estimated exposure dose, is used to quantify risk. Exposure of triclosan or its residues can be from dietary (i.e., food and drinking water), residential, and other non-occupational sources like hand soaps and toothpaste, and from all known or plausible exposure routes (oral, dermal, and inhalation). MOE for triclosan for children from 6 years to adults ranged from 4,700-19,000 and was 290 for infants (EPA 2008b). Nolen and Dierckman (1979) found significant decrease in rat conception rate, in the number of pups, and in the number of pups that survived until weaning at 0.25% of diet (Nolen and Dierckman 1979). Maternal toxicity, including weight losses, abortions, and deaths were observed in rats at 50 mg/kg/day (Nolen and Dierckman 1979). Additionally, triclocarban and triclosan disrupt endocrine activity at concentrations of 29-3,150 μg/L potentially affecting human and animal health (Ahn et al. 2008). Endocrine disruption resulted from bioactivity amplification of endogenous hormones (Chen et al. 2008).

Ecosystem Health. Triclocarban and triclosan adversely impact both aqueous and soil ecosystems. Triclocarban and triclosan are toxic to microorganisms and hence can disrupt critical ecological processes like nitrification in WWTP and waste recycling (Neumegen, Fernandez-Alba and Chisti 2005, Dokianakis, Kornaros and Lyberatos 2004). In aqueous ecosystems, antimicrobials can disrupt river biofilm community structure and function, microbial community composition, algal biomass, algal architecture and algal activity (Lawrence et al. 2009). River biofilms are thin layer of microorganisms on solid surfaces like rocks, sediments, and plants which are different from their suspended or free floating counterparts (Beer 2006). River biofilms are responsible for majority of

microbial processes in aquatic ecosystem. Freshwater microbial communities are sensitive to 2.895 μg/L triclosan, which is within the range of observed freshwater triclosan concentrations (Johnson et al. 2009). Additionally, triclocarban at concentration of 17.04 μg/l and triclosan at 3.400 - 5.210 μg/l inhibit algal growth, adversely affecting primary productivity (Yang et al. 2008, Tatarazako et al. 2004). Concentrations of triclosan as low as 0.150 μg/L can cause both behavioral and physiological effects on thyroid hormone, body weight, and hind limb development in frogs (Fraker and Smith 2004, Veldhoen et al. 2007). Genotoxic and cytotoxic effects in Zebra mussel (*Dreissena polymorpha)* hematocytes by triclosan may be potentially dangerous to entire aquatic biocenosis (Binelli et al. 2009). In terrestrial ecosystems, triclocarban and triclosan inhibited soil respiration and nutrient recycling and plant growth (Liu et al. 2009). Development of microbial and drug resistance has also been reported (Heath et al. 1999, Heath et al. 2000, Heath et al. 1998, Hoang and Schweizer 1999, McMurry, Oethinger and Levy 1998, Walsh et al. 2003).

2.4 Plant Interaction with Chemicals and Phytoremediation of Chlorinated Organics

Phytoremediation is the use of green plants to remove, contain or detoxify pollutants in the environment. Phytoremediation processes include:

- rhizodegradation or phytostimulation (degradation of organic contaminants in the rhizosphere);
- phytostabilization (immobilization of contaminants in the rhizosphere);

- phytoaccumulation or hyperaccumulation (translocating and concentrating contaminants into plant tissues);
- phytodegradation or phytotransformation (uptake of organic contaminants with subsequent transformation, conjugation and sequestration); and
- phytovolatilization (uptake and release of same contaminant during transpiration (ITRC 2001).

Characteristics that influence the uptake of organic pollutants by plants are hydrophobicity, polarity, sorption properties, and solubility (ITRC 2001) . Hydrophobicity, measured by a chemical's octanol - water partitioning coefficient (K_{OW}), was initially used to characterize uptake and subsequent translocation of an organic molecule with an optimum range of $1 \leq \log K_{OW} \leq 3$ (Dzantor 2007). At log K_{OW} values ≥ 3, the pollutant is tightly sorbed onto roots, limiting translocation to shoots. At log K_{OW} values less than 1, the specific affinity of the pollutant to be sorbed onto plant roots is limited (Dzantor 2007).

The previous notion of plant uptake of chemicals only occurring in intermediate hydrophobicity range is not universally applicable (Dettenmaier, Doucette and Bugbee 2009). Lipid content of plant species and K_{OW} were related to partitioning and uptake of lipopholic organic chemicals with log K_{OW} values > 3 in root and water systems (Gao et al. 2008). Sites contaminated with organic hydrophobic pollutants with log K_{OW} >3 have been remediated by direct plant uptake (ITRC 2001). Additionally, the uptake of halogenated phenols by *Lemna minor* was affected primarily by subsequent positioning, but not by hydrophobicity as number of halogen substituents did not affect uptake rate (Tront et al. 2007, Reinhold and Saunders 2006). Pollutant uptake rate was controlled by

rate of enzymatic transformation internal to plants, not by rate of partitioning into plants, and was not related to log K_{OW} or dissociation constant (pK_a) (Tront et al. 2007, Reinhold and Saunders 2006).

After a pollutant is uptaken and translocated by plants, it can be metabolized to reduce phytotoxicity associated with the pollutant. Plant metabolism of organic pollutants, vital for phytoremediation, appears to follow detoxification or elimination metabolic processes collectively known as 'green liver model' described in separate section below (Burken 2003). The extensive number of substrates with which plant enzymes react allows plants to transform many xenbiotic organic pollutants (Burken 2003).

Studies have demonstrated that plants such as pumpkin, zucchini and switch grass can be used as phytoaccumulators, hyperaccumulators, and phytostimulators for hydrophobic chlorinated organic pollutants. Hyperaccumulators are distinguished from phytoaccumulators by the ability to accumulate at least 10^6 µg/kg (dry weight) of a chemical (McCutcheon and Schnoor 2003, ITRC 2001). In situ field experiments on the phytoextraction of polychlorinated biphenyls (Aroclor 1254/1260), *Cucurbita pepo spp* pepo cv. Howden (pumpkin) plants took up, translocated, and hyperaccumulated 7,600 µg/kg PCBs in plant shoots, indicating that pumpkin is a potential PCB phytoextractor (Aslund et al. 2007). An exponential decrease was observed in PCB concentration in the stem of pumpkin as the distance from root increased (Aslund et al. 2007). Shoot bioaccumulation factor (BAF_{shoot}), defined as ratio of concentration in shoot to that in soil, was 0.15 in pumpkin plants (Aslund et al. 2008). *Cucurbita pepo* species extracted and translocated dichlorodiphenyltrichloroethane (DDT) and its metabolites, dichlorodiphenyldichloroethhane (DDD) and dichlorodiphenyldichloroethylene (DDE),

with translocation factors (ratio of shoot concentration to root concentration) of 1.8 and 1.2 and bioaccumulation factors (ratio of plant concentration to soil concentration) of 3.3 and 2.0 for zucchini and pumpkin, respectively (Lunney et al. 2004). The authors concluded that extraction was related to high transpiration rates, large above-ground biomass, and composition of root exudates. Zucchini (*Cucurbita pepo* spp prop cv Black Beauty) phytoextracted 1.3% of weathered p, p'-DDE with 98% in aerial tissues under field conditions (Wang et al. 2004). Subspecies variation in phytoextraction of p, p'-DDE was observed in 21 cultivars of *C. pepo* spp *taxana* and *C. pepo* spp *pepo* (White et al. 2003). Average bioaccumulation factor of 0.283 was observed in zucchini fruit in phytoextraction study of p, p'- DDE compared to 0.87 for leaves, 5.40 for stems and 7.22 for roots, indicating decreasing concentration along the stem from root (White et al. 2003). Hulster et al. (1994) found that polychlorinated dibenzo-p-dioxins and dibenzofurans were uptaken through roots and translocated to shoots and fruits by pumpkin and zucchini (Hulster, Muller and Marschner 1994). Green house scale experiment on the evaluation of ability of switch grass (*Panicum variegatum L.)* to stimulate dissipation of PCB in soil in the laboratory confirmed the potential of switch grass to do so (Dzantor et al. 2000).

2.4.1 Green liver-model.

The "green liver" model first appeared in 1977 based on metabolic processes observed in enzyme studies from plant cell cultures and the metabolism of nonpolar compounds like DDT and benzo(a)pyrene (Sandermann 1994). Burken (2003) has described green-liver model in three common phases: transformation, conjugation, and

elimination or storage. After pollutants enter the plant tissue, the first phase is the transformation of the initial substrate, including oxidation, reduction, or hydroxylation (Burken 2003). Plant enzymes like Cytochrome P- 450 and peroxidases are common to phase I metabolism (Burken 2003).

Conjugation (phase II) follows transformation to generally produce more water-soluble and less-toxic compounds that can be deposited in vacuoles or incorporated into bound residues through sequestration in phase III (Burken 2003). Exceptions include organic pollutants with easily conjugated functional groups such as phenols, which are directly conjugated through phase II metabolism without undergoing phase I transformation. Phase II metabolism includes conjugation with malonic acid, D-glucose, glutathione, cysteine, and other amino acids and carbohydrates utilizing the functional groups produced from phase I transformations (Burken 2003).

Phase III, sequestration, follows conjugation and includes isolation of phase II products to three terminal fates, specifically the vacuole, the apoplast, or cell walls of the plant (Burken 2003). In phase II, active transportation of conjugates is facilitated and controlled by a glutathione pump (Burken 2003). Some residues are bound to stem or leaves or lignified, for example, residues of DDT in experiments with Canadian waterweed *(Elodea canadensis)* (Garrison et al. 2000).

2.5 Phytoremediation and Hydroponics

Hydroponics is a technology for growing plants in nutrients solution or water without use of soil. Practiced for centuries to produce food and vegetables, hydroponics research evolved to space applications, and waste recycling, and phytoremediation (Jones

1997). Screening of plants suitable for removing particular type of pollutant and identifying exact removal mechanisms of pollutants are eased by hydroponic systems (Nzengung 2007). Additionally, better control of the environment like light, temperature, humidity, nutrients input, root zone, air, bioavailability of targeted contaminant and less complexity of soil-related processes due to absence of soil make hydroponics system ideal for phytoremediation studies (Nzengung 2007). Identifying fate of any pollutants in vegetated systems using hydroponic systems gives freedom of fewer variables due to controlled environment.

However, hydroponics research might exaggerate the results as doses are higher than environmental concentrations. Interactions between pollutants and environmental matrices, like soil, can affect plants response (Zabudowska et al. 2009). Root biology may differ in hydroponics than in soil systems and selection of species for field site based on hydroponic experimentation might mislead (Zabudowska et al. 2009). Hydroponic research is difficult for water insoluble contaminants and for larger trees and shrubs.

2.6 Extraction Procedures for Antimicrobial Analysis

The goal of solid liquid extraction is to remove the analyte from a solid matrix (e.g., soil, biosolid, plant) to a liquid solvent with minimal loss, degradation, or contamination. While slight variations in protocol are numerous, there are only a few basic techniques to extract organic chemicals from plant tissues (Anderson et al. 1997).

Among several clean-up extracts including solid phase extraction (SPE), liquid-liquid extraction (LLE), gel permeation chromatography (GPC) and semi-preparative HPLC, SPE has been preferred in most instances because it is fast with low organic

solvent use, presents low contamination risk and can be used in-line (Diaz-Cruz, de Alda and Barcelo 2003).

Pretreatment techniques used to solubilize and remove organic chemicals from plant tissues include digestion (treatment of sample with strong alkaline or acidic solutions before extracting with an organic solvent), sonication (using high-intensity ultrasonic vibrations to lyse cells rapidly and increase solvent contact with the sample), homogenization (disrupting plant cells before solvent extraction), and solvent extraction (using affinity of a compound for a particular solvent) (Anderson et al. 1997). Personal care products from solid matrices have been extracted using ultrasonication and by simple stirring of the sample with polar organic solvents or mixtures of solvents, or with aqueous solutions containing additives or buffers (Diaz-Cruz et al. 2003). Homogenization or high intensity mechanical extraction methods are simple, quick, and efficient in removing chemicals from solid matrices, including plant tissues and employ tissue grinders or homogenizers to disrupt the sample and increase contact with the solvent (Anderson et al. 1997). A disadvantage of sonication and homogenization, however, is the increase in temperature from the sonication probe that can lead to chemical losses from volatilization and sample degradation (Anderson et al. 1997). Thus, it is extremely important to establish the percent recovery by using spiked controls or internal standards. Solvent extraction of plant tissues with an organic solvent has also been carried out in a separatory funnel of a soxhlet apparatus (Anderson et al. 1997).

With advancements in extraction techniques, the use of more advanced extraction techniques, such as pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE) are increasingly being utilized in extraction of triclocarban and

triclosan. A review of methods used to extract and clean-up triclocarban and/or triclosan from solid matrix is given in the appendix A.1 (Table A.1).

Chapter 3: Materials and Methods

Research consisted of two sections: soil column experiments and hydroponic experiments. In soil column experiment, four pumpkin, four zucchini, three switch grass and three controls (no plant) were grown in biosolids applied soil in columns to meet objective I and II. To meet objective III, five pumpkin, five zucchini and five controls were grown hydroponically in nutrient solution spiked with triclocarban and triclosan. The results from soil column experiment were used to characterize risk mentioned in objective IV.

3.1 Soil Column Experiment

In soil column experiments, plants were grown in soil columns inside laboratory. Leachate samples were analyzed for 22 weeks for concentration of triclocarban and triclosan and soil and plant samples were analyzed at the end of the experiment.

3.1.1 Soil, Seeds and Biosolids

The crops used in this study were pumpkin (*Cucurbita pepo* Howden cultivar), zucchini (*Cucurbita pepo* cultivar Gold Rush) and switch grass (*Panicum variegatum).* Pumpkin and zucchini seeds were obtained from Johny Seeds, Winslow, Maine, and switch grass seedlings were obtained from the Department of Crop and Soil Sciences, Michigan State University. Soil used in this study was collected from Michigan State University to represent locally available soil. The field had not been previously amended with biosolids. The texture of soil was sandy clay loam (sand 50.8 %, silt 28.4 % and clay 20.8 %). The collected soil was screened through a 2 mm sieve prior to use.

Biosolids were collected from Delhi Charter Township wastewater treatment plant in Michigan in February 2009. The biosolids was analyzed for triclocarban and triclosan prior to use by previously developed methods using liquid chromatography tandem mass spectrometer (LC-MS-MS) (Cha and Cupples 2009). Triclocarban and triclosan were present at concentrations of 8.18 ± 0.56 mg/kg and 0.18 ± 0.01 mg/kg dry mass of biosolids, respectively.

3.1.2 Chemicals

A list of chemicals used in the experiment, along with the manufacturer and the purity, are given in Table 3.1.

Table 3.1 Chemicals Used in the Experiment

Chemicals	Company	Purity
Triclocarban [CAS 101-20-2]	Tokyo Chemical Industry Co. Ltd.	>98 %
Triclosan [CAS 3380-34-5]	Calbiochem	>98%
Ammonium acetate	Sigma Aldrich	>99.7 %
Acetic acid	Columbus Chemical Industry Inc.	>99.7%
Acetone	J.T. Baker	>99.7%
Methanol	J.T.Baker	>99.8%
Methanol	EMD Chemical Inc.	>99.99%
Water (MS solvents, for nutrient preparation, standards preparation)	Millipore system	18.2 MΩ
Hoagland basal salt	Sigma Aldrich	

Combined stock solution for both chemicals was prepared using methanol and stored at 4^0C. Working standard solutions were prepared for every batch analysis by diluting stock solution.

3.1.3 Experimental Columns.

Plants were grown in experimental columns with diameter of 14.7 cm and length of 30 cm. The column size was selected to give enough depth and width to the pumpkin roots while being not too large. Plants were grown in the selected columns to test growth before deciding on the size of the columns. The experimental columns featured leachate collection at the bottom through a tightly held funnel (Figure 3.1). The bottom 7.6 cm of the soil column was filled with soil without mixing biosolids. To replicate actual field practice, the next 15.2 cm of soil was thoroughly mixed with biosolids at the application rate of 0.73 dry Mg per 1000 m^2 (3.25 dry tons per acre) (Cha and Cupples 2009). A depth of 15.2 cm is equivalent to plough depth. Additional triclocarban and triclosan were not added. Solid content of biosolids was 4.8 % (dry basis) and 200 g of biosolids were applied before seed sowing, with an additional 60 g of biosolids applied after 8 weeks of seed sowing to simulate a second field application. The second application of biosolids was done to study migration behavior of triclocarban and triclosan and once plants were established to provide nutrients to plants.

Figure 3.1 Experimental Setup Showing Six Columns and Amber Bottles to Collect Leachate Water

Experimental design included quadruple columns for pumpkin, zucchini, and switch grass and triplicate for no plant controls. The quadruple columns were selected to guarantee three replicates if any of the four replicates were to die. One switch grass plant died during the experimental period. Seeds were sown except for switch grass, for which plants were transplanted directly from the greenhouse, Department of Soil and Crop Sciences, MSU. Soil was kept moist during plant establishment. Plants were maintained at a constant temperature (23 ± 2^{0} C) under plant growth lights using a light regime of 16 h light: 8 h dark (Figure 3.2). The temperature and light duration were selected based on the average temperature and light duration during the months in which pumpkin are grown around East Lansing.

Figure 3.2 Plants Growing in Columns under Constant Regime Lights

3.1.4 Sampling

For leachate sampling, columns were flooded with equal volumes of water so that a minimum of 100 mL of water leached from each column every two weeks. Water samples were collected in amber bottles under each soil column. Samples were stored immediately at 4^0C until sample preparation.

Plants were harvested at the end of 22 weeks when plants started to senescence. Plant tissues were separated into roots, leaves, and stems for pumpkin and zucchini and into shoots and roots for switch grass. Plant tissues were rinsed carefully with deionized water to remove soil and dust particles, air-dried, weighed and stored in amber bottles in refrigerator at 4^0C until sample extraction. Leaves and stems samples of each plant were stored in an amber bottle without sub-sampling. Before preparation of samples for extraction, leaves and stems were sampled to triplicate sub-samples each.

Soil samples representative of different depths (0-15.2 cm) of soil around the root zone were collected from each column. Plant residues were removed and soil was homogenized by mixing and stored at 4^0C until sample preparation. Soil from each column was stored in an amber bottle. Before extraction, soil from each column was sampled to triplicate sub-samples.

3.1.5 Sample Preparation

Aqueous samples were prepared as published previously (Halden and Paull 2005, Halden and Paull 2004). The procedure began with filtering the samples through Whatman qualitative glass filter paper (7.0 cm) without applying vacuum to remove soil particles. Exactly 100 ml of filtrate was passed through a solid phase extraction (SPE) cartridge (Oasis HLB 3 cc, Waters Corporation) and eluted with 4 ml of 50 % methanol and 50 % acetone containing 10 mM acetic acid (Figure 3.3). Elutes were dried under a gentle stream of nitrogen, reconstituted in 1 ml of 50:50 methanol acetone, filtered through 13 mm syringe filter with 0.2 μm PTFE membrane to amber autosampler vials and analyzed by liquid chromatography mass spectrometer in negative electrospray ionization mode (LC/ESI(-)/MS). Stock solutions for analyzing water samples were prepared by spiking known concentrations following similar procedures to water sample preparation.

Figure 3.3 Solid Phase Extraction

Soil samples were prepared and extracted as previously published (Cha and Cupples 2009) by pressurized liquid extraction (PLE) using a Dionex ASE 200 accelerated solvent extractor (Figure 3.4). Triplicate subsamples of soil from each column sample were extracted and a fourth subsample was weighed and dried for at least 24 h at 105^0C and again weighed for moisture determination. A glass fiber filter was inserted at the outlet of stainless steel extraction cell (11 ml) body, a thin layer of Ottawa sand added from top followed by 5 g of thawed soil sample and a thin layer of Ottawa sand, and sealed on both sides using frits, rings and caps. Extraction on ASE (accelerated solvent extractor) was carried out in acetone with conditions: oven temperature of 100°C, extraction pressure of 1500 psi, static time of 5 min and flush volume of 100%. The extracts were evaporated to dryness under nitrogen gas, reconstituted in 940 µl of 50% methanol and 50% acetone mixture, spiked with 6 µg/ml triclosan and triclocarban in

50% methanol and 50% acetone, and filtered to auto-sampler vials through 0.2 μm filter before analysis by LC-MS. For quality assurance, all analytical runs included a blank, a control and three sub-samples from a column. The recovery from the methods was 94.03 ± 9.76 % for triclocarban and 83.39 ± 19.48 % for triclosan.

Figure 3.4 Accelerated Solvent Extractor Used in the Experiment

For plant samples, frozen plant tissues were oven dried at 105°C, grounded in mortar and pestle and extracted using the same method as for soil described above. For quality assurance, cutting of plant samples was done with metal blades rinsed with methanol between uses to minimize cross contamination. All analytical runs included a blank, a control and three sub-samples except for roots, where sufficient biomass for replicate analysis was not available. A blank run, control run and a sample re-run was performed once for each 15 samples to check the accuracy and precision of LC-MS.

3.1.6 LC-MS Analysis

A Shimadzu LC-MS 2010 EV was used to analyze samples for triclocarban and triclosan (Figure 3.5). Samples were injected by auto-injector in the autosampler controlled by Shimadzu lab solutions software (v 3). Triclocarban and triclosan were separated using Allure biphenyl column (5 µm, 150 × 2.1 mm) from Restek Cor. MS (Mass Spectrometer) parameters were: curved desolvation line (CDL) of 1.5 V, Block and CDL temperature of 30^0C and nitrogen desolvation gas flow rate of 1.5 L/min. MS negative electrospray ionization with scan mode was used for method development and identification whereas selected ion monitoring (SIM) mode was used for quantification.

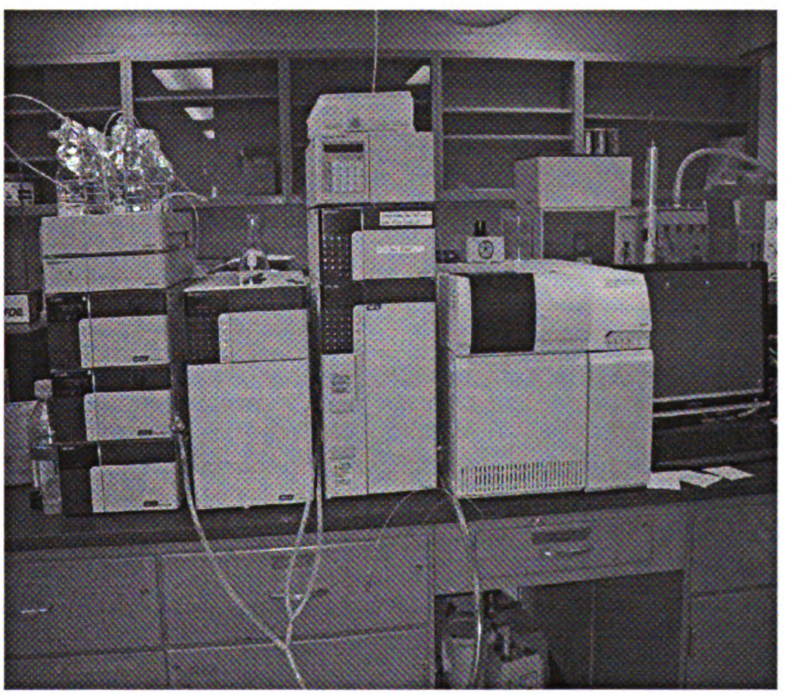


Figure 3.5 LC- MS Used in the Experiment

Retention time ($t_R \pm 0.1$ min), detection of characteristics molecular ions (de-protonated molecular mass [M-H$^-$]): 313 of triclocarban and 287 for triclosan, and detection of reference ions, m/z (molecular mass) 315 and 317 for triclocarban and m/z 289 and 291 for triclosan were used to identify the target molecules (Halden and Paull

2005). Reference ions were present due to naturally occurring ^{37}Cl atoms. Mobile phases were 5 mM ammonium acetate as phase A and methanol as phase B. The total flow rate was 0.2 mL min^{-1}. A binary gradient 20 min method with LC flow time program of 75% B for 0-2 minutes, increased linearly to 100% B in 13 min, held to 14 min, decreased linearly to 75% B in 15 min and held up to 20 min for re-equilibration, was used. Quantification was performed using external, linear calibration and a minimum of five calibration levels (Figure 3.6) (Halden and Paull 2005). The regression coefficient (R^2) of the curves was greater than 99.5 %. The detection limits were 10-100 ng/L for water, and 100-1000 ng/kg for plants and soils.

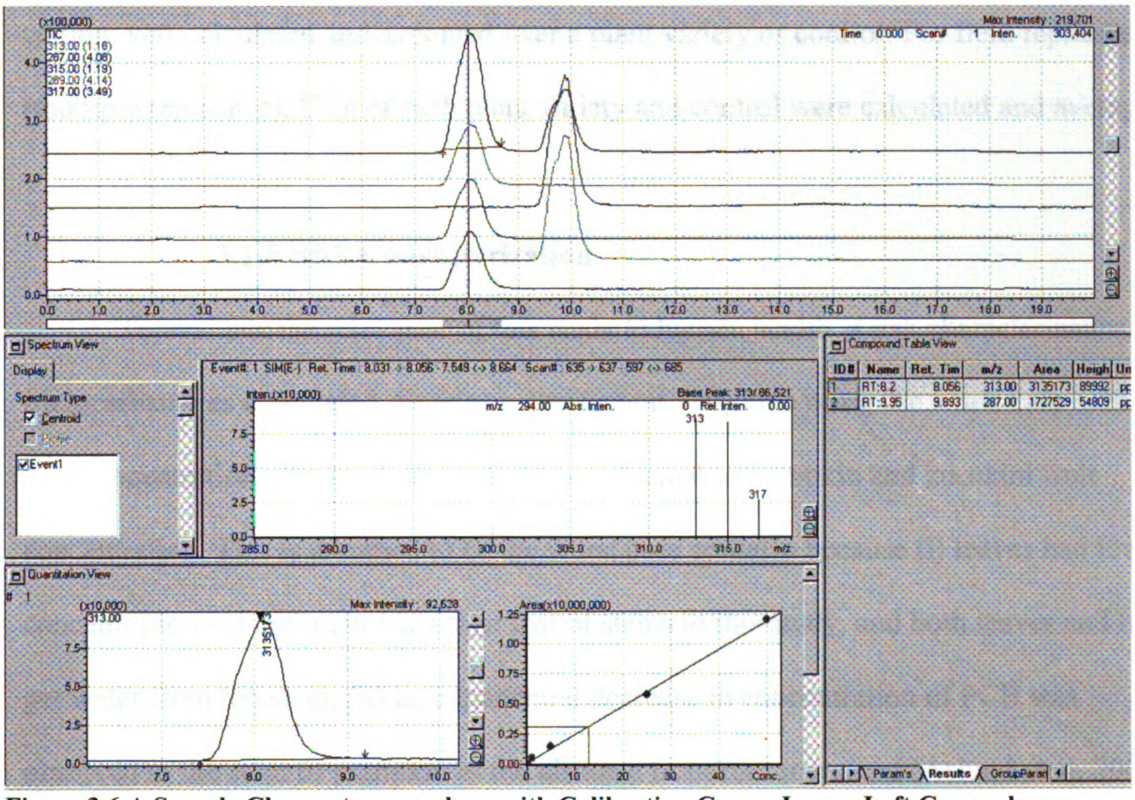


Figure 3.6 A Sample Chromatogram along with Calibration Curve. Lower Left Corner has Quantitation View for Triclocarban

3.1.7 Statistical Analysis

All statistical analysis was performed in Sigma Stat (version 11.0). A two tailed t-test was used for all comparison purposes. The reported values are in mean ± standard error of mean.

Being a biological system, variability was observed in the experimental data. An effort was made to identify the sources of variability. Coefficients of variation (CVs) were calculated to quantify sources of data variability. CVs were calculated for LC-MS analysis, accelerated solvent extraction, experimental sample replicates and field replicates. The values are reported as mean CV ± standard error of mean of CV. For experimental sample replicates (single column or reactor), CV of sub-samples for each column was calculated and averaged over a plant variety or control. For field replicates (multiple replicates), CVs of each plant variety and control were calculated and averaged.

3.1.8 Risk Characterization

To evaluate the relevance of this study to human health, a risk characterization for triclocarban was completed. Exposure assessment was done using the worst case scenario. The concentration of leaf or stem was assumed equal to pumpkin and zucchini fruit concentration. This is assumed to be the worst case scenario because (i) leaves had lower concentrations of antimicrobials than that of stems in this study, and both leaves and fruit get water from the stem, (ii) an exponential decrease in concentration of PCB was observed in the stem of pumpkin as the distance from root increased (Aslund et al. 2007), and (iii) an average bioaccumulation factor of 0.283 was observed in zucchini fruit in phytoextraction of p, p'- DDE compared to 0.87 for leaves, 5.40 for stems and 7.22 for

roots (White et al. 2003). Humans are also exposed to antimicrobials through contact while using liquid soaps, bars and body wash, and through ingestion while drinking water. Dietary, residential and non-occupational sources and their exposure were considered for the worst case scenario. Accordingly, maximum triclocarban used in bar soap (5 %), in liquid soaps (5 %) and in body wash (0.5 %) were used to calculate margin of exposure. An absorption value of 0.39% (EPA 2002a), maximum daily fruit consumption of 200 g pumpkin and/or zucchini fresh weight per person (Cook 2004), solid content of 10% in pumpkin and/or zucchini, maximum daily drinking water of 3 L and maximum leaf/stem concentration in this study to represent the fruit concentration was used to characterize risk. A no-observable adverse effect level (NOAEL) of 25 mg/kg bw/d for triclocarban and 30 mg/kg bw/d for triclosan was used to calculate margin of exposure (EPA 2002a). Aggregate margin of exposure was calculated using following equation (EPA 2001).

$$MOE_T = \frac{1}{\frac{1}{MOE_1} + \frac{1}{MOE_2} + \ldots\ldots + \frac{1}{MOE_n}}$$

Where, MOE_T = Aggregate margin of exposure

$MOE_{1,2\ldots n}$ = Margin of exposure for individual exposure route

3.2 Hydroponic Experiment

Plants were grown in hydroponic medium to expose them to higher concentrations of antimicrobials and quantify maximum accumulation of antimicrobials. Plants were grown for two months in nutrient solution spiked with triclocarban and triclosan. The concentration of antimicrobial in nutrient solutions was monitored and plant samples analyzed for presence of triclocarban and triclosan at the end of the experiment.

3.2.1 Plants and Experimental setup

The plants selected for the hydroponic study were pumpkin (*Cucurbita pepo* Howden cultivar) and zucchini (*Cucurbita pepo* cultivar Gold Rush) for the reasons mentioned in section 1.2 and successful test growth in nutrient media. Switch grass was not selected due to poor growth in preliminary hydroponic studies.

The experiment setup consisted of glass vases filled with glass marbles to support plants (Figure 3.7). Vases had 10 cm top and bottom diameter, 19.5 cm height and 7.5 cm diameter at the mid-height section. Vases were covered with aluminium foil to minimize photodegradation of antimicrobials. There were total of fifteen vases, five each for pumpkin and zucchini and controls without plants.

Figure 3.7 Vases before Wrapping in Foil, After Adding Antimicrobial Solution and Transplantation of Pumpkin and Zucchini Seedlings

3.2.2 Plants Culture in Nutrient Media

Pumpkin and zucchini seeds were germinated in antimicrobial-free soil, grown for a week and transplanted to vases filled with marbles (Figure 3.7). Triclocarban and triclosan, at 1 μM concentration each, was prepared in 280 ml nutrient solution, added to each vase and initial level of the solution was marked (Figure 3.7). The solubility of triclocarban in nutrient solution necessitated a 1 μM concentration of triclocarban. Triclosan was all spiked at 1 μM. Each week, reactors were supplemented with basal salt nutrients, the composition of which is given in Table 3.2 (American Public Health Association., American Water Works Association. and Water Pollution Control Federation. 1998). Nutrients were added so that the weekly addition was equivalent to the weekly requirements for plant growth (Rouphael and Colla 2005). The basal salt nutrient solution was selected instead of Hoagland's nutrient solution because of extremely low

triclocarban solubility in Hoagland's and to decrease microbial growth in the non-sterile systems. Plants after some weeks of growth are shown in Figure 3.8.

Figure 3.8 Experimental Vases after Some Weeks of Plants Growth

Table 3.2 Nutrient Solution Composition

Nutrient Solution	Salt	Stock Solution Concentration, g/L	Element	Concentration, mg/L
A	$NaNO_3$	25.5	N	42.0
			Na	110.0
	$NaHCO_3$	15.0	C	21.4
	K_2HPO_4	1.04	K	4.69
			P	1.86
B	$CaCl_2.2H_2O$	4.41	Ca	12.0
	$MgCl_2$	5.7	Mg	29.0
	$FeCl_3$	0.096	Fe	0.33
	$Na_2EDTA.2H_2O$	0.3		
	$MnCl_2$	0.264	Mn	1.15
C	$MgSO_4.7H_2O$	14.7	S	19.1
	H_3BO_3	0.186	B	325
	$Na_2MoO_4.2H_2O$	7.26	Mo	28.8
	$ZnCl_2$	3.27	Zn	15.7
	$CoCl_2$	0.78	Co	3.54
	$CuCl_2$	0.009	Cu	0.04

Reference: (American Public Health Association., American Water Works Association. and Water Pollution Control Federation.) 1 ml of stock solution A, B and C was added to make 100 ml of nutrient solution.

Evaporation from top surface was greater than nutrient solution to be added each week. Thus, reagent water was added each week to bring the solution level in the vase to the original level. Therefore, after adding nutrient solution and reagent water, reactor solutions were allowed to diffuse for five hours. Five hours duration was based on a prior

food dye test. In food dye test, added dye diffused homogeneously within the solution in less than an hour.

3.2.3 Sampling

Weekly sampling was done for each vase. Prior to sampling, a syringe needle (60 ml) was placed at the center of the reactor and the nutrient solution was mixed. For sampling, 0.5 mL of the solution in the vase was pipetted out and stored in an amber vial. Plants were harvested at the end of two months when pumpkin and zucchini showed slowed growth and the beginning of senescence. Plants were washed with reagent water, air dried, separated into shoots and roots, weighed and stored at 4^0C until analysis. Low plant biomass did not allow replications of samples during extraction.

3.2.4 Sample Preparation

Aqueous samples were prepared immediately after sampling on weekly basis. Samples were diluted with 1.5 ml of methanol to increase detection of triclocarban. The resulting 2 ml solution was filtered through 0.2 μm PTFE membrane filter to amber autosampler vials and analyzed in LC-MS.

For plant samples, samples at 4^0C were taken out of refrigerator, oven dried, grounded using mortar and pestle, weighed, and extracted using a Dionex ASE 200 accelerated solvent extractor. The method used was same as described in section 3.1.5 excluding the addition of triclocarban and triclosan. Triclocarban and triclosan was spiked at concentrations of 0.5 μM after extraction to make final volume to 1 mL.

3.2.5 LC- MS Analysis:

The method used for analysis in LC-MS was same as described in soil column LC-MS analysis section. External linear calibration with at least five calibration levels from 0.1 μM to 1.2 μM was used for this experiment.

Chapter 4: Results and Discussions

4.1 Soil Column Experiment

4.1.1 Leaching of Antimicrobials

Both triclocarban and triclosan were detected in leachate from the experimental soil columns. As shown in Figure 4.1, concentrations of antimicrobials increased initially for two weeks and then decreased. The lag in peak concentration was likely due to sorption of antimicrobials to the bottom 7.6 cm of soil where biosolids were not applied. Previous studies suggest strong sorption of antimicrobial to soil. For example, in saturated soil systems, sorption was the primary removal mechanism for triclocarban (Drewes et al. 2003, Essandoh et al. 2010). The maximum triclocarban and triclosan concentration in leached water from pumpkin, zucchini, switch grass and control are given in Table 4.1.

Table 4.1 Occurrence of Maximum Concentration of Triclocarban and Triclosan in Leachate Water. Values are presented in mean ± standard error.

Plants	Triclocarban maximum concentration, µg/ml	Time for maximum concentration for triclocarban, weeks	Time for maximum concentration for triclosan, weeks	Triclosan maximum concentration, µg/ml
Pumpkin	2	0.21 ± 0.16	2	0.53 ± 0.18
Zucchini	2	0.19 ± 0.11	2	1.67 ± 0.54
Switch Grass	2	0.12 ± 0.04	2	0.46 ± 0.28
Control	10	0.37 ± 0 .37	2	0.71 ± 0.45

Triclosan, despite having a lower initial concentration in the applied biosolids, was collected at higher concentrations than triclocarban in leachate water during 0-22 weeks. This phenomenon was consistent with the greater hydrophobicity and lower solubility of triclocarban than triclosan. For weeks 4-22, total triclocarban that leached

was greater than that of triclosan. Reduction in the relative leaching of triclosan was most likely due to more rapid microbial degradation of triclosan as compared to triclocarban. Degradation of triclosan in aerobic soils was more rapid with a half life of 18 days, than was degradation of triclocarban, with a half life of 108 days (Ying et al. 2007). Observed concentrations of triclosan were 1.9-8.8 times higher than those in surface runoff following land application of biosolids previously reported as 0.26 ± 0.04 μg/ml (Topp et al. 2008).

Leachate concentrations of triclocarban were 40-123 times greater than observed maximum concentrations of 0.003 ± 0.002 μg/ml triclocarban following application of dewatered municipal biosolid (Sabourin et al. 2009). Higher concentration was likely due to collection of leachate water from relatively short columns indicating a limitation to these types of studies. The second addition of biosolids, replicating field practices, increased antimicrobial concentration immediately. For triclocarban, the observed second peak was greater than first peak concentration although the increase was not statistically significant (P= 0.6). In contrast, triclosan was observed at lower concentrations after the second biosolids application (P=0.03).

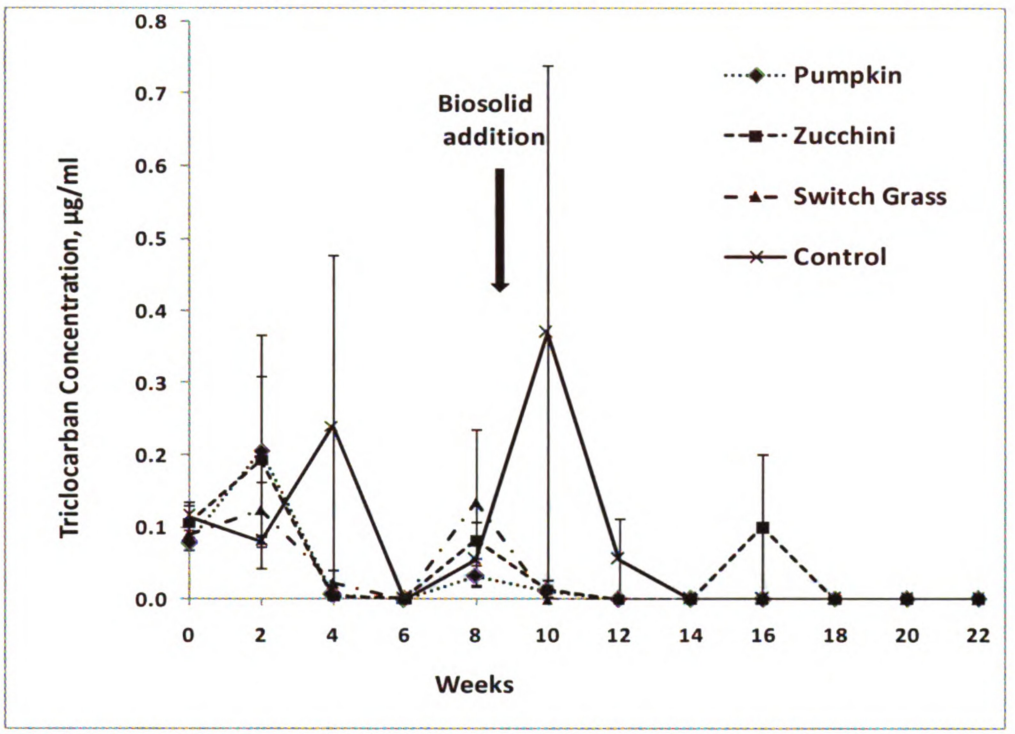


Figure 4.1(a) Triclocarban Concentrations in Leached Water with Time. Points represent mean and error bars represent standard error.

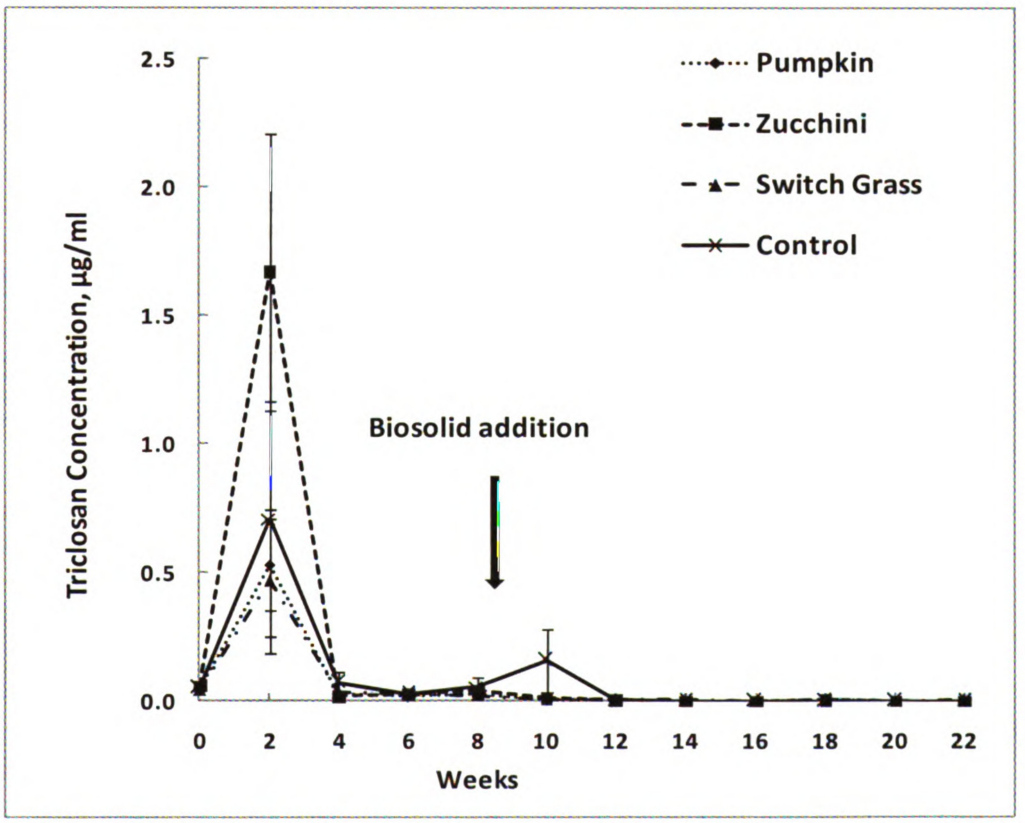


Figure 4.1(b) Triclosan Concentrations in Leached Water with Time

The total antimicrobials leached from the soil columns over 22 weeks were not significantly different than the control. However, plants were not established until week four. The total antimicrobials leached from weeks 4-22 were significantly different than that of the control, with p values of <0.01 for pumpkin, 0.01 for zucchini and 0.01 for switch grass. Consequently, the results indicated that plants play a role in reducing leaching of antimicrobials to water resources.

4.1.2 Soil Concentrations of Antimicrobials

Soil concentrations of antimicrobials at the end of 22 weeks are summarized in Table 4.2. Soil concentrations of both triclocarban and triclosan in pumpkin and zucchini columns were less than that of control columns with p-values of 0.06-0.07. However, antimicrobial concentrations in switch grass columns did not differ from that of control (p = 0.88). The observed difference in final soil concentration of antimicrobials may have resulted from difference in plant growth in the column.

Table 4.2: Soil Antimicrobial Concentrations and P-values for Comparison with Controls

	Triclocarban, µg/g	**Triclosan, µg/g**	**Sum, µg/g**
Pumpkin	0.047±0.007 *(P=0.06)*	0.000±0.000 *(P=0.07)*	0.047±0.008 *(P=0.07)*
Zucchini	0.042±0.019 *(P=0.07)*	0.00±0.00 *(P=0.07)*	0.042±0.019 *(P=0.07)*
Switch Grass	0.080±0.045 *(P=0.52)*	0.008±0. 002*(P=0.20)*	0.088±0.033 *(P=0.88)*
Control	0.175±0.063	0.021±0.009	0.196±0.070

Table 4.3 shows root and shoot biomass produced in soil column experiment along with ratio of shoot to root. Shoot mass of switch grass was 7.6 and 11 times smaller than that of pumpkin and zucchini, respectively. Root mass of switch grass was 11.3 and 9.1times greater than that of pumpkin and zucchini, respectively. Table 4.3 also shows that growths of plants were variable. Results indicated pumpkin and zucchini do

influence soil concentrations of antimicrobials after land application of biosolids. However, poor growth of switch grass did not yield conclusive result.

Table 4.3 Biomass Produced in Soil Column Experiment and Root to Shoot Ratio

Plants	Soil Column Experiment		
	Root, g	Shoot, g	Shoot/ Root
Pumpkin	0.08 ± 0.03	3.74 ± 0.93	1339.8 ± 1309.5
Zucchini	0.10 ± 0.03	5.39 ± 0.28	69.49 ± 15.86
Switch Grass	0.91 ± 0.4	0.49 ± 0.24	1.426 ± 1.19

Residual triclocarban concentration in soil was higher than residual triclosan concentration. For example, the soil concentration of triclocarban was approximately 8.3 times greater than that of triclosan in control columns. This could be due to triclocarban being i) more immobile of the two based on K_{OC} values (Table 1.1), ii) more resistant to microbial degradation, and iii) being the less soluble of the two chemicals.

4.1.3 Plant Concentrations of Antimicrobials

Both triclocarban and triclosan were observed in plant roots, stems and leaves. Antimicrobials concentrations in plant tissues (Figure 4.2 and Figure 4.3) ranged from 1.1 μg/g in leaves to 39.5 μg/g in roots. Antimicrobial concentrations in root tissues were higher than those in leaves, stem, and soil; however, they were not significantly different ($p > 0.05$). As previously observed for pumpkin and zucchini accumulation of PCBs (Aslund et al. 2007), concentration generally decreased from roots to stems to leaves. Though there were antimicrobial concentration differences, concentrations of antimicrobials in stem, leaves and root were typically not statistically different (p values from 0.07-0.98) (Table 4.4).

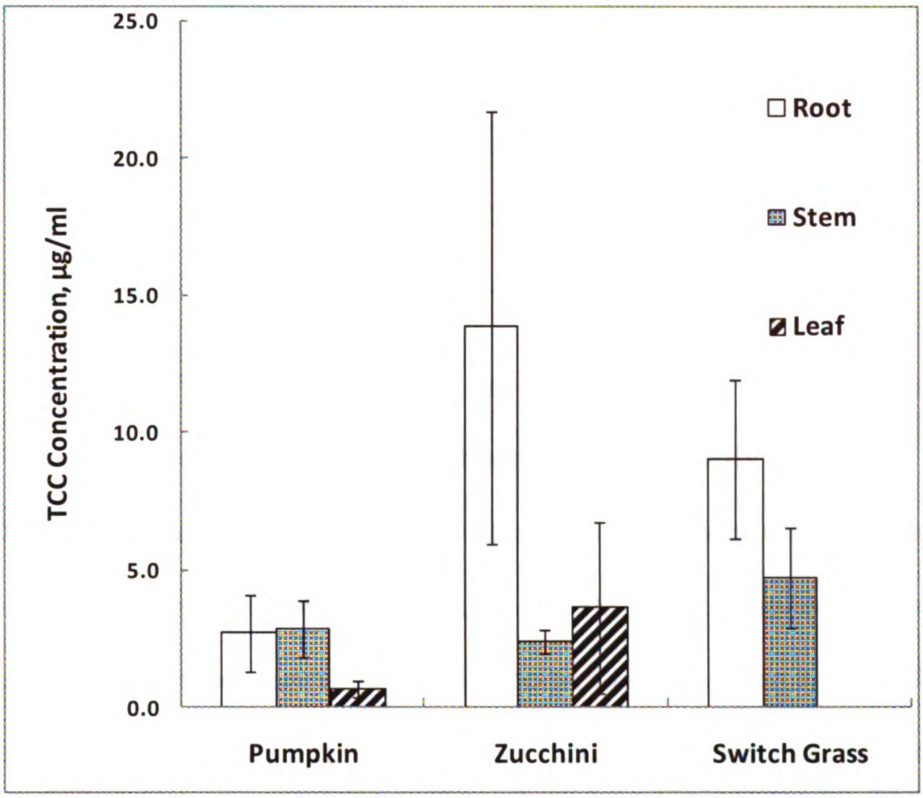


Figure 4.2 Plant Concentrations of Triclocarban

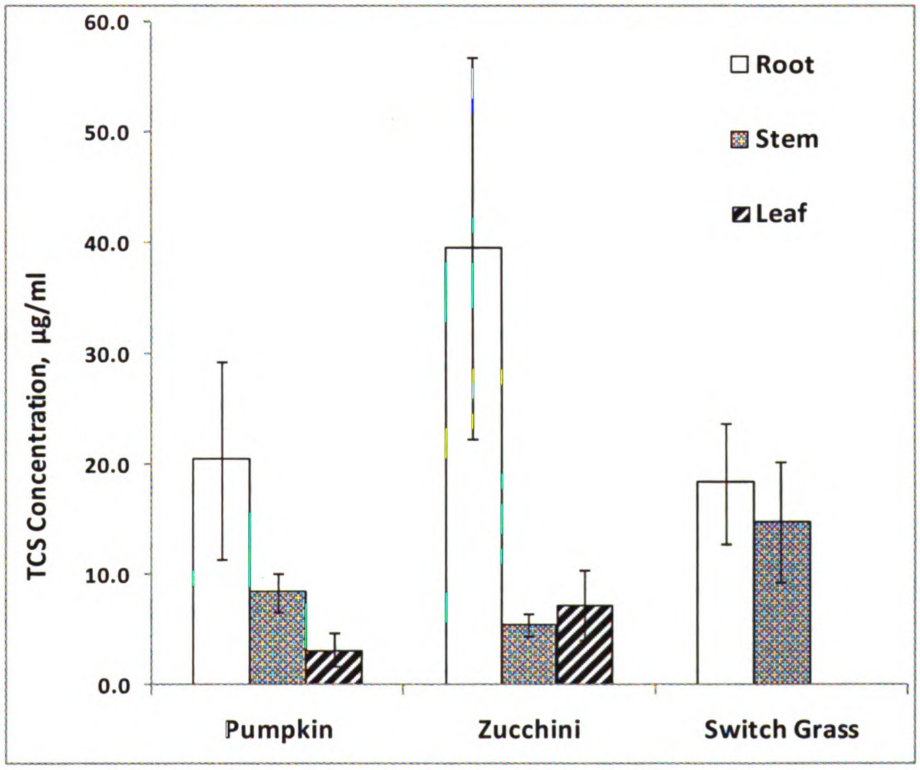


Figure 4.3 Plant Concentrations of Triclosan

Table 4.4 P-Values for Comparison of Antimicrobial Concentrations between Plant Parts

Plants		Triclocarban		Triclosan	
		Stem	Leaf	Stem	Leaf
Pumpkin	Root	0.93	0.929	0.181	0.066
	Stem	-	0.981	-	0.025
Zucchini	Root	0.196	0.24	0.095	0.082
	Stem	-	0.068	-	0.119
Switch Grass	Root	0.266		0.674	

A one way ANOVA analysis for comparison of different plant parts using Sigma Plot indicated that soil concentration of antimicrobials were significantly different (p values from <0.01 to 0.06 except for between soil and zucchini roots) from concentrations of stems, leaves, and water for all plant species (Table 4.5). Triclocarban bioaccumulation factors, the ratio of plant concentration to soil concentration, are given in Table 4.6. These values are comparably higher than those for DDT bioaccumulation by Lunney et al. (2004) for the same varieties of zucchini (*Cucurbita pepo* L. cv. Senator hybrid) and pumpkin (*Cucurbita pepo* cv. Howden), which were 3.0 and 2.0, respectively. PBT profiler modeling developed by EPA suggests that TCC and TCS are potentially bioaccumulative (log BCF 3.074 for triclocarban and 2.565 for triclosan) (Ying et al. 2007).

Table 4.5 P-Values for Comparison of Soil Antimicrobial Concentrations and Plant Antimicrobial Concentrations

Plants	Plant Parts	Triclocarban	Triclosan
Pumpkin	Root	0.068	0.041
	Stem	0.037	0.003
	Leaf	0.004	0.003
Zucchini	Root	0.13	0.061
	Stem	0.002	0.001
	Leaf	<0.001	<0.001
Switch Grass	Root	0.014	0.011
	Stem	0.027	0.024

Table 4.6 Bioaccumulation and Translocation Factors for Triclocarban and Triclosan

Plants	Bioaccumulation Factor		Translocation Factor	
	Triclocarban	Triclosan	Triclocarban	Triclosan
Pumpkin	11.0 ± 5.06	972 ± 398	0.81 ± 0.63	0.46 ± 0.08
Zucchini	40.3 ± 46.3	1822 ± 260	1.91 ± 1.83	0.35 ± 0.21
Switch Grass	30.92 ± 9.41	874 ± 706	0.82 ± 0.47	1.23 ± 0.80

Translocation factors, the ratio of shoot concentration to the root concentration are given in Table 4.6. Low translocation factor imply that the antimicrobials transport from root to shoot was limited. Triclosan gets uptaken into plant roots easier than triclocarban, but was only limitedly transported from roots to shoots. The translocation factors are comparable to those for DDT, DDD, and DDE and PCBs reported in Aslund et al. (2007) and Lunney et al. (2004) for the same plant varieties. None of the plants were triclocarban or triclosan hyperaccumulators as the accumulated concentrations were not greater than 10^6 μg/kg (dry weight) (McCutcheon and Schnoor 2003, ITRC 2001).

Mass balance for each column was assessed (Figure 4.4 and 4.5). Significant mass fraction of triclocarban remained unaccounted for most of the columns indicating other mechanisms of antimicrobial loss, such as microbial degradation, phytostimulation, or phytodegradation. The largest portion of triclocarban remained in the soil suggesting that triclocarban was sorbed to soil particles. Results are similar to previously published studies. Essandoh et al. (2010) reported that sorption and biodegradation were two primary mechanism of triclocarban removal in saturated aquifer system and attributed 56 % of total loss of triclocarban to biodegradation for the first nine days which decreased to 7 % by 16 days (Essandoh et al. 2010). For zucchini, though root concentrations were greater than shoot concentrations, total accumulation in leaves was highest followed by total stem accumulation and root accumulation. In contrast, stems

accumulated the greatest mass, followed by leaves and then by roots, in pumpkin. Accumulation of antimicrobials was greater in roots than in shoots for switch grass resulting from less shoot production. Unaccounted mass for triclosan was relatively less compared to triclocarban. This is likely due to the greater loss of soluble TCS in first four weeks of studies.

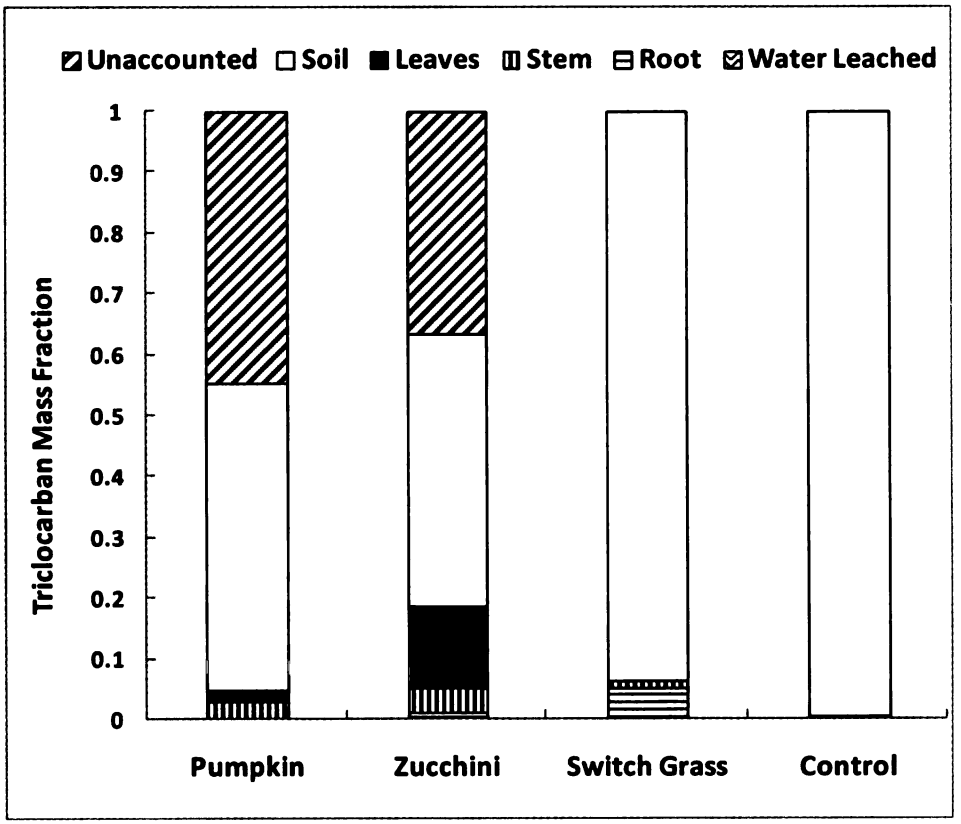


Figure 4.4: Triclocarban Mass Balance Analysis

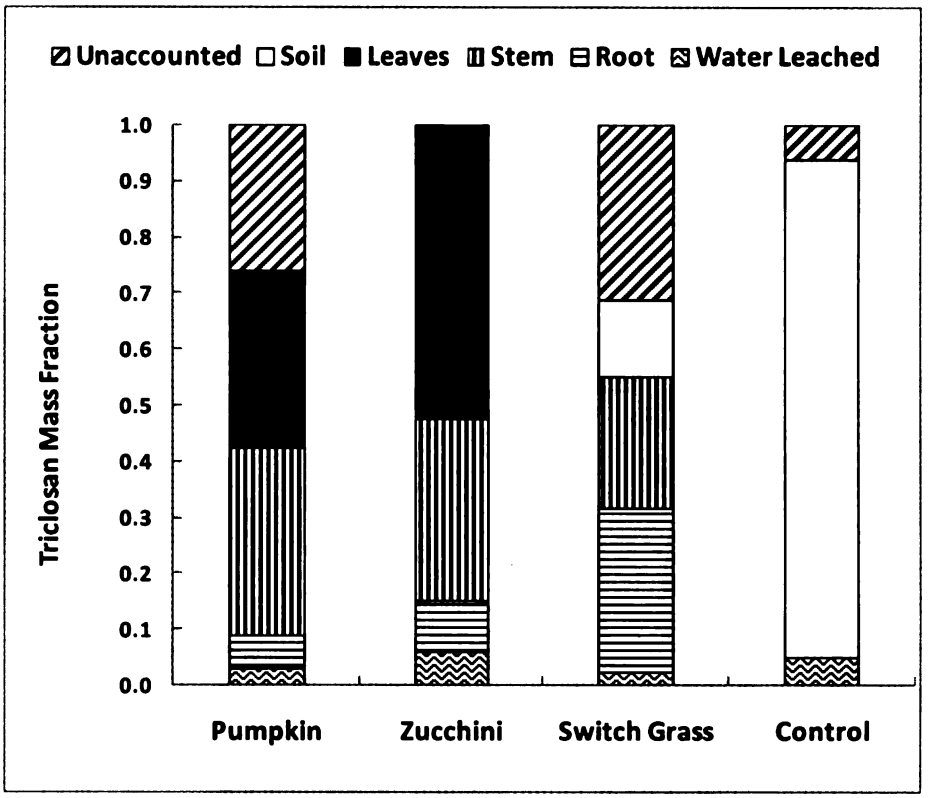


Figure 4.5: Triclosan Mass Balance Analysis

4.1.4 Risk of Land-applied Antimicrobials in Vegetated Systems

Concentrations of triclocarban and triclosan known to promote adverse impacts in soil and aquatic ecosystems, and environmental occurrences in soil and water are summarized in box plots in Figure 4.6 and 4.7.

In figure 4.6, leachate triclocarban concentrations of planted columns in this experiment (box plots 5 and 6) were higher by about an order of magnitude than water concentration of triclocarban at ambient environment (box plot 1). In contrast, soil concentrations of triclocarban in the experimental columns (box plots 9 and 10) were lower by about an order magnitude than that in environment (box plot 8).

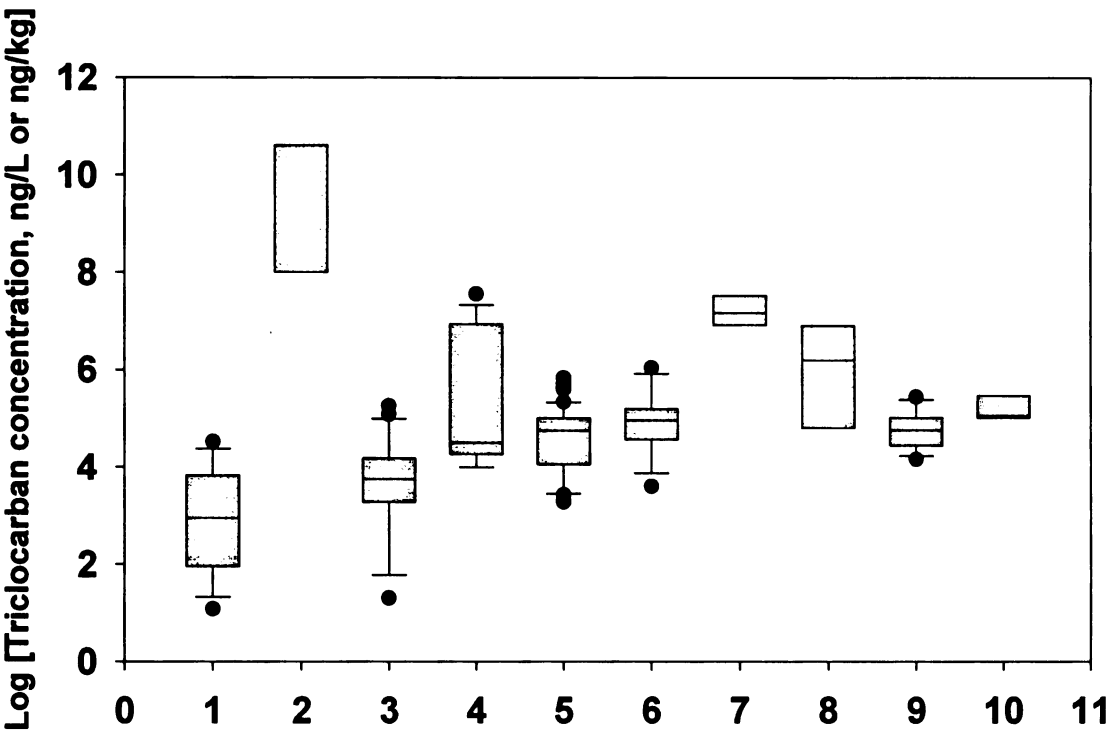


Figure 4.6 Box Plots of Triclocarban Concentrations Available in Different Literatures and Research Data 1) Occurrence in Water, 2), 3) and 4) Threshold Toxicity Values for Microorganisms, Invertebrates and Plants, 5) Experimental Water Concentrations in Planted Columns, 6) Experimental Water Concentrations in Control, 7) and 8) Occurrence in Biosolids and Soils, 9) Experimental Soil Concentration in Planted Columns and 10) Experimental Soil Concentrations in controls. Concentrations are in Logarithmic Scale in parts per trillion, or ng/L for Liquid and ng/kg for Solid Matrices. References are given in the Table in Appendix A.1 and A.2.

For triclosan, as shown in Figure 4.7, triclosan concentrations in planted columns leachate (box plots 5 and 6) had higher concentration range than environmental water concentrations (box plot 1). In contrast, concentration of triclosan in soil in the experimental columns (box plots 10 and 11) were lower than that in environment (box plot 8).

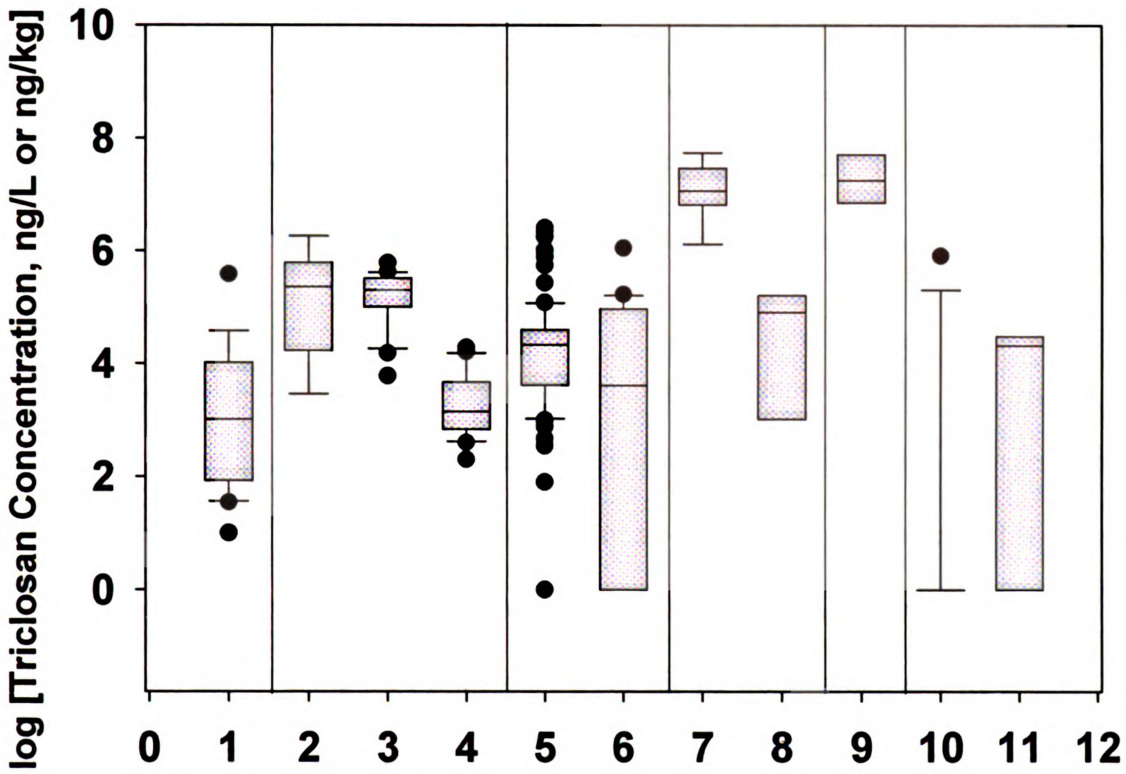


Figure 4.7 Box Plot of Triclosan Concentrations Available in Different Literatures and Research Data 1) Occurrence in Water, 2), 3) and 4) Threshold Toxicity Values for Microorganisms, Invertebrates and Plants, 5) Experimental Water Concentrations in Planted Columns, 6) Experimental Water Concentrations in Control, 7) and 8) Occurrence in Biosolids and Soils, 9) Threshold Toxicity Values for Soil Health, 10) Experimental Soil Concentration in Planted Columns and 11) Experimental Soil Concentrations in controls. Concentrations are in Logarithmic Scale in parts per trillion, or ng/L for Liquid and ng/kg for Solid Matrices. The limits of detection were 10-100 ng/L for water, 100-1000 ng/kg for plants. References are given in a Table in Appendix A.1 and A.2.

Concentration of triclosan and triclocarban detected in all matrices (plants, soil and water) in this experiment ranged from 0.00087 to 5.34 µg/ml triclocarban and 0.00072 to 6.17 µg/ml triclosan in water, 0.5 to 28.92 µg/g triclocarban and 6.73 to 81.21 µg/g triclosan in root, 0.8 to 6.6 µg/g triclocarban and 1.21 to 21.70 µg/g triclosan in shoot. The concentrations in soil and water are within the range of environmental concentrations reported in different literatures (Table 2). Concentration in plant parts

could not be directly compared to other studies due to lack of relevant studies. Comparing with the values in Figure 4.6 and 4.7, there is high risk for both soil and aquatic ecosystem health including soil respiration, enzymatic activity and microorganisms from concentrations observed in this study. Therefore, land application of biosolids is likely to adversely impact the ecosystem functioning and health of both land and water.

Uptake of antimicrobials by pumpkin and zucchini represents the first documentation of exposure of humans to antimicrobial through ingestion of vegetables. Margin of exposure (MOE), which is the ratio of no-observable adverse effect level to exposure dose, are given in Table 4.7. MOE values for pumpkin and zucchini were comparable to that of using bar or liquid soap containing triclocarban and were substantially less than that of drinking water contaminated with antimicrobials. In other words, the health risk from eating pumpkin and zucchini grown on biosolid-applied fields is likely greater than that from drinking water and is similar to that of using antimicrobial products.

Table 4.7 Risk Characterization of Triclocarban and Triclosan

Chemical	Route	Exposure mg/kg bw/d	Resulting dose*, mg/kg bw/d	NOAEL, mg/kg bw/d	MOE
TCC	Dermal-bar soap	0.10	0.005	25	5000
	Dermal-liquid soap	0.11	0.006	25	4167
	Dermal body wash	0.07	0.0004	25	62,500
	Oral-drinking water		1.38×10^{-6}	25	18,115,942
	Pumpkin/zucchini*	200 g (max)	0.011498	25	2175
	Aggregate				1092
TCS	Oral-drinking water	56 ng/L	2.4E-06	30	12500000
	Pumpkin,/zucchini		0.010896	30	2754
	Aggregate (excluding pumpkin and zucchini)			30	4700
	Aggregate				1736

References: (EPA 2002a, EPA 2008a, Cook 2004)
Resulting dose = estimated 0.39% dermal absorption of TCC (EPA 2002a)
MOE = margin of exposure; NOAEL= No-observable adverse effect level

4.2 Hydroponic Experiment

4.2.1 Aqueous Concentrations of Antimicrobials

Triclocarban concentration normalized to the initial concentration with time is shown in Figure 4.8. Triclocarban concentration rapidly decreased with time for up to two weeks and then decreased gradually for all vases. The concentration of triclocarban at the end of experiment was 0.73 ± 0.01 μM, 0.55 ± 0.01 μM and 0.61 ± 0.03 μM for pumpkin, zucchini and control, respectively. The decrease in triclocarban concentration in vases was probably due to photodegradation or chemical degradation. Sorption to marbles was unlikely as sorption of triclocarban to glass has not been reported.

Previous studies have documented phytodegradation of triclocarban. Triclocarban was removed from surface waters with direct and sensitized phytolysis with measured half life of 24 hours in sunlight (Trouts 2008). Additionally, in planted vases, the concentrations could have decreased due to phytodegradation, rhizodegradation and/or phytoaccumulation. Concentrations in pumpkin vases did not decrease as much as controls, possibly due to shading of the reactors by well-developed foliage resulting in decreased photodegradation. Concentration in zucchini reactors were less than that in the control. Group comparison between pumpkin, zucchini and control concentrations using Sigma Plot indicated that both pumpkin and zucchini were not statistically different from control (P values of 0.52-0.54). However, pumpkin plant significantly differed from zucchini (P value of 0.013).

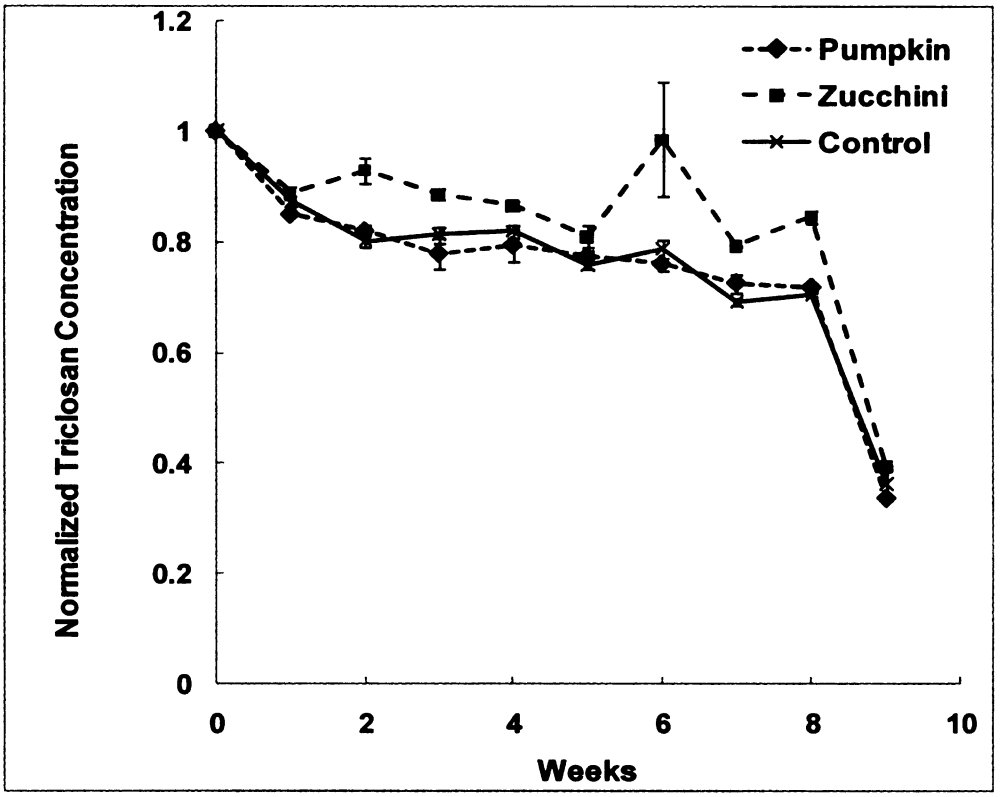


Figure 4.8 Triclocarban Concentrations with Time

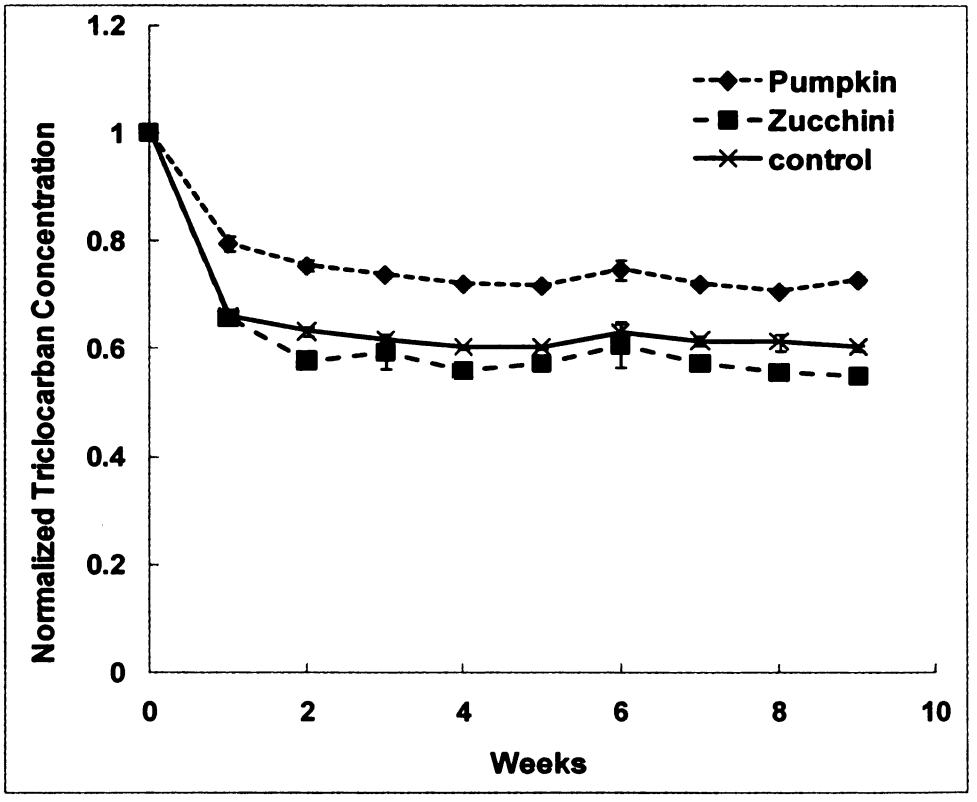


Figure 4.9 Triclosan Concentrations with Time

The plot of concentration of triclosan normalized to the initial concentration with time is shown in Figure 4.9. Concentration of triclosan gradually decreased with time for all vases. The concentration of triclosan at the end of experiment was 0.34 ± 0.01 μM, 0.39 ± 0.01 μM and 0.36 ± 0.07 μM for pumpkin, zucchini and control (respectively). The decrease in triclosan concentration in all vases was probably due to photodegradation or chemical degradation. Sorption to marbles was unlikely as no other studies have reported sorption of triclosan to glass. Previous literature on photodegradation of triclosan reported that triclosan photodegraded in fresh water with half life of 8 days (Aranami and Readman 2007). In planted vases, plants could have accumulated, or phytodegraded triclosan or have stimulated rhizodegradation. The sharp decrease in concentration of triclosan towards the end of experiment is interesting. A longer experimental period could have made it clear about whether concentration of triclosan would continue to decrease, remain the same or was an error. Group comparison between pumpkin, zucchini and control concentrations using Sigma Plot indicated that both pumpkin and zucchini were not statistically different from control (P values of 0.94 and 0.309).

4.2.2 Plant Concentrations of Antimicrobials

Though triclocarban concentrations in nutrient solutions were not significantly lower than that in control, concentration of triclocarban was detected in the plant tissues, both roots and shoots. The triclocarban concentrations in log scale in ng/kg for both pumpkin and zucchini shoots and roots are shown in Figure 4.10. Triclocarban concentration ranged from 133 - 530 μg/g in roots and from 0.08 - 0.60 μg/g in shoots.

As was observed in soil column experiment with triclocarban and triclosan, root concentration of triclocarban was higher than that of shoot. While concentration of triclocarban in pumpkin root was lower than that in zucchini roots, pumpkin shoots had higher triclocarban concentration than zucchini shoot. Since roots were well developed in hydroponic experiment, roots played a major role in total accumulation. Triclocarban concentration in nutrient solution was higher at the end of the experiment in pumpkin than in zucchini. Reduced concentration in solution of zucchini vases could have been due to more root concentration and accumulation. A statistical comparison of the concentration of triclocarban between pumpkin and zucchini roots and shoots showed that the concentrations of pumpkin are statistically different than concentrations of zucchini ($p < 0.001$). But, root did not differ statistically from shoot for both pumpkin and zucchini ($p = 0.871$ and 0.997).

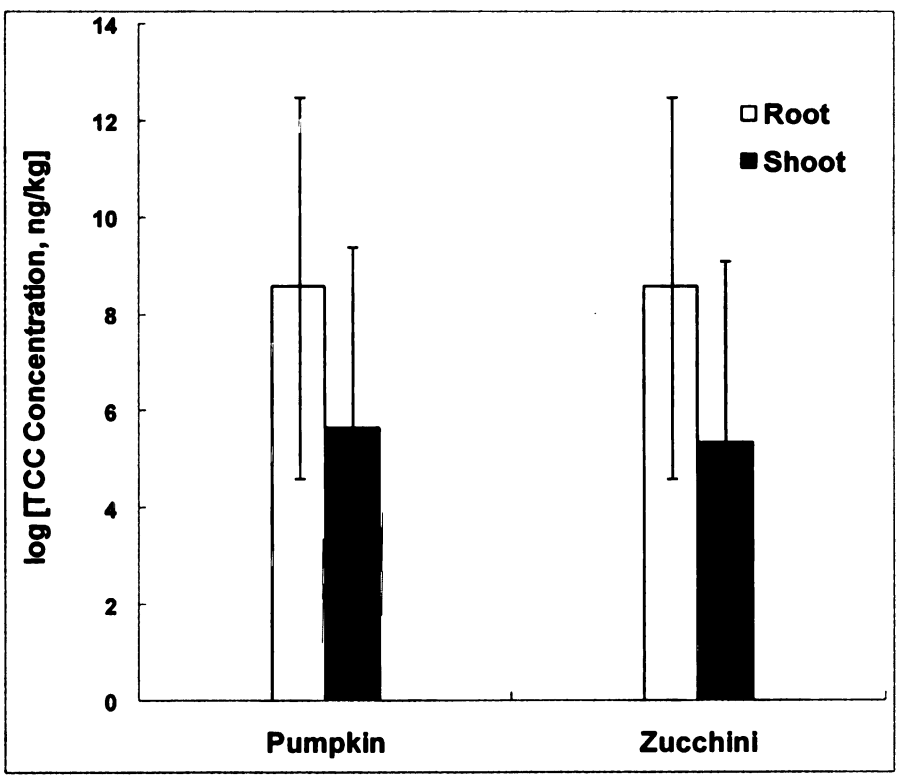


Figure 4.10 Triclocarban Concentrations in Pumpkin Roots and Shoots, log_{10} Scale for Concentration

Both roots and shoots contained detectable concentrations of triclosan (Figure 4.11). Triclosan concentration ranged from 12.07 - 130.61 μg/g in roots and from 0.56 - 25.43 μg/g in shoots. Pumpkin had lower root and shoot concentration than zucchini. As was observed in the soil column experiment with triclocarban and triclosan, concentrations of triclosan in roots were higher than that in the shoots. Since roots were well developed in hydroponic experiment, roots played major role in distribution of triclosan (Table 4.8). This is consistent with the fact that triclosan concentration in nutrient solution in pumpkin was higher than in zucchini.

Table 4.8 shows the comparison of shoot to root ratio for both soil column experiment and hydroponic experiment. Shoot to root ratio of soil column experiments is higher than that of hydroponic experiments by a factor of 224 for pumpkin and 12 for zucchini. Pumpkin shoot in soil column experiment has most growth in all. A high variability in biomass growth between columns is seen in both soil column experiment and hydroponic experiment.

Table 4.8 Comparison of Biomass Produced and Root to Shoot Ratio in Hydroponic Experiment with Soil Column Experiment

Plants	Hydroponics			Soil Column Experiment		
	Root, g	Shoot, g	Shoot/Root	Root, g	Shoot, g	Shoot/Root
Pumpkin	0.07 ± 0.02	0.43 ± 0.10	5.98 ± 0.47	0.08 ± 0.03	3.74 ± 0.93	1339.8 ± 1309.5
Zucchini	0.07 ± 0.01	0.39 ± 0.06	5.72 ± 0.82	0.10 ± 0.03	5.39 ± 0.28	69.49 ± 15.86
Switch Grass				0.91 ± 0.4	0.49 ± 0.24	1.426 ± 1.19

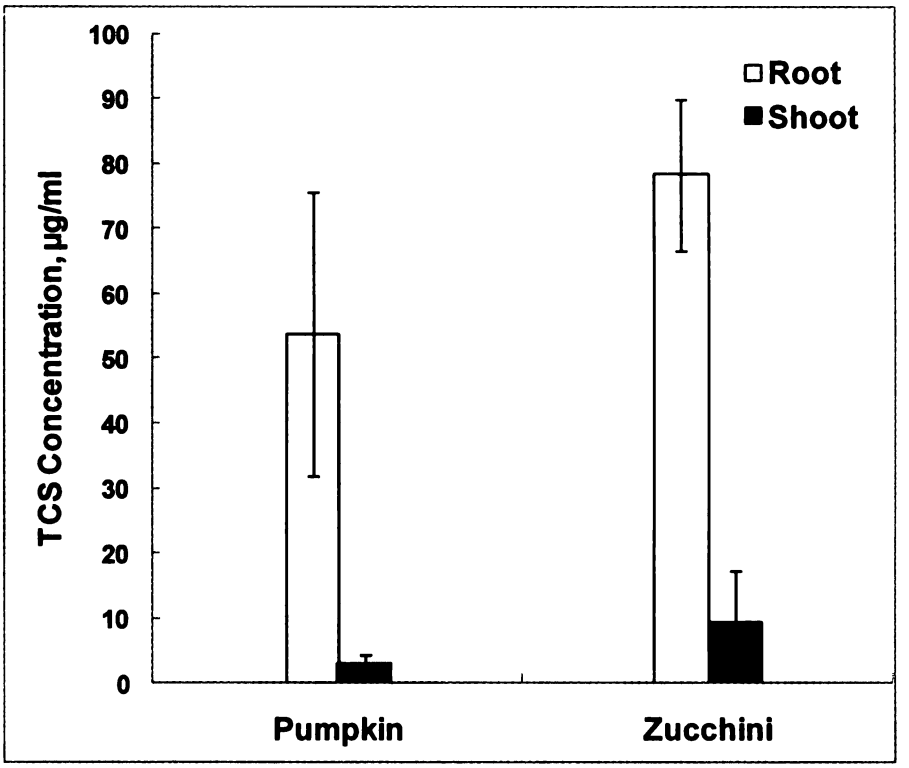


Figure 4.11 Triclosan Concentrations in Shoots and Roots of Pumpkin and Zucchini

A mass balance analysis of triclocarban in both pumpkin and zucchini is shown in Figure 4.12. A large portion of triclocarban remained in the nutrient solution and sorbed to the roots. Roots accumulated more triclocarban than shoots in both pumpkin and zucchini in contrast to soil column experiments where root had lower accumulation. This is likely due to the well developed root system in hydroponics. Moreover, difficulty in retrieving all roots in soil column also added to the discrepancy. A significant mass remained was unaccounted for in both pumpkin and zucchini indicating another mechanism of loss. The unaccounted amount is less than expected based on decrease in the concentration of triclocarban in control vases. This might have been possibly due to preferential mechanism of triclocarban to accumulate to plants over photodegradation or chemical degradation and prevention of photodegradation from shading of reactors by plant leaves.

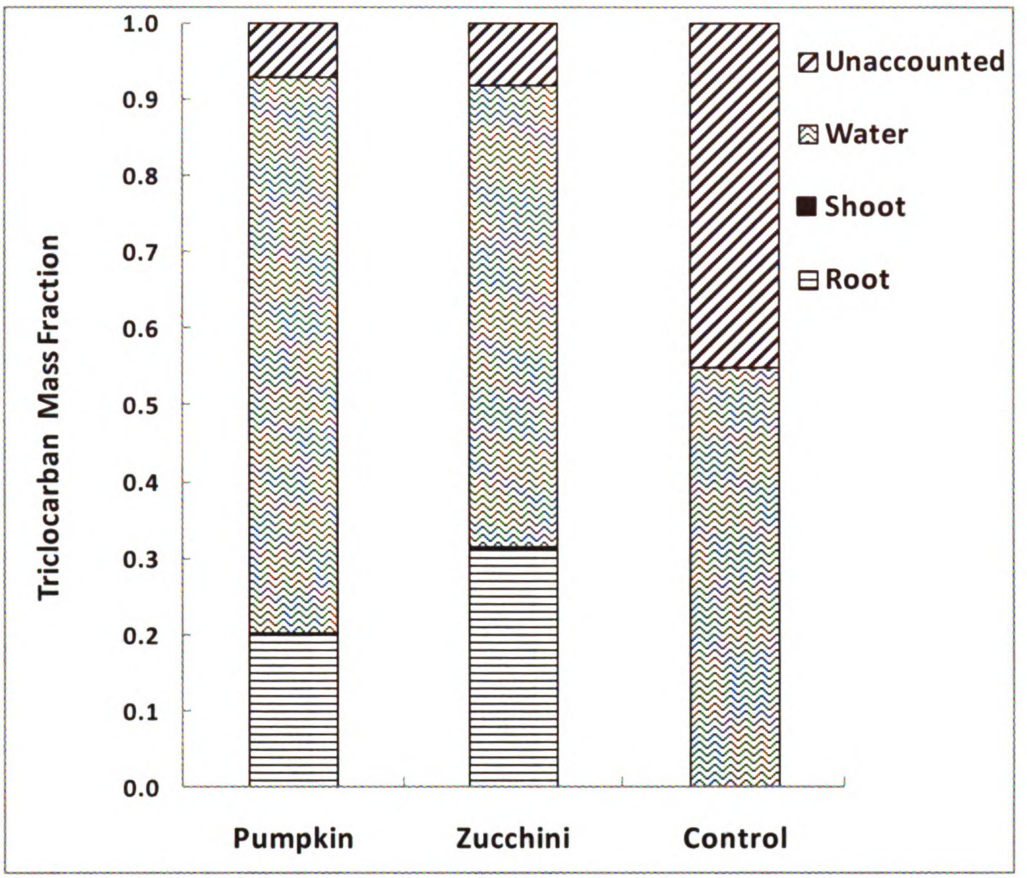


Figure 4.12 Mass Balance Analysis of Triclocarban in Hydroponic Experiment

Triclosan mass analysis was completed at the end of the experiment (fig 4.13). As opposed to triclocarban, the largest fraction was unaccounted in both pumpkin and zucchini. As was found in triclocarban mass analysis, triclosan also remained in water in substantial amount. Root accumulation was higher than shoot accumulation for both pumpkin and zucchini, though not as substantially as for triclocarban.

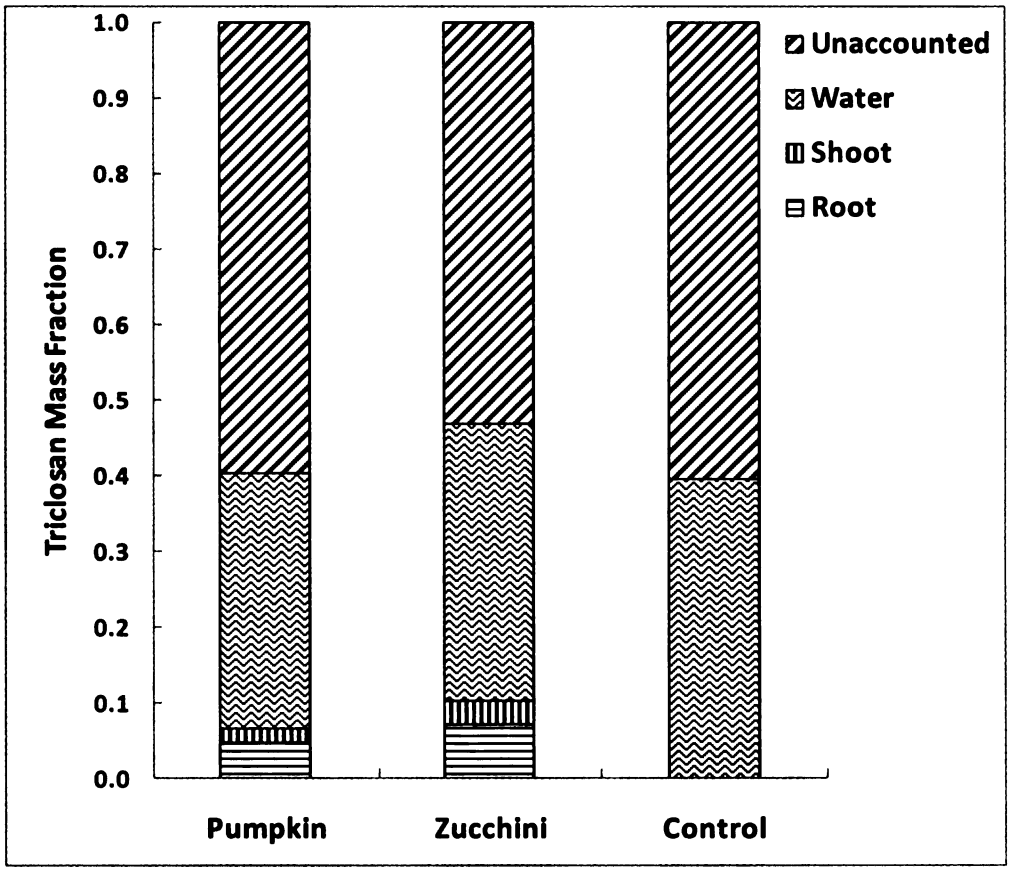


Figure 4.13 Mass Balance Analysis of Triclosan

4.2.3 Comparison of Soil Column and Hydroponics Antimicrobial Concentrations

Box plots in Figure 4.14 shows the log concentrations of all matrices in both soil column and hydroponic experiments. Box plot 3, triclocarban concentration of hydroponic solution at the end of experiment, is clearly higher than concentrations of triclocarban in water and soil in the column experiments (box plots 1 and 2). This was expected since the hydroponic solution was spiked with higher concentrations of triclocarban. The case of triclosan, shown in box plots 6, 7 and 9, was similar. Plant

triclocarban. The case of triclosan, shown in box plots 6, 7 and 9, was similar. Plant triclocarban concentration in hydroponic experiment (box plot 5) is on average is higher than that for soil column experiment (box plot 4). The same phenomenon is observed for triclosan (box plots 9 and 10). Concentration of triclocarban and triclosan in water in hydroponics was statistically different from that in soil and water in soil column studies (Table 4.9). However, plant concentrations of triclocarban and triclosan in soil column experiment were not always statistically different than that in hydroponics experiment (Table 4.9). When comparing between the soil and water concentrations with plant concentration of antimicrobials, plants in the soil column experiment were clearly more efficient in extracting antimicrobials. Translocation factors and translocation factors for both experiments are summarized in below Table 4.10.

Table 4.9 P-Values for Comparison of Hydroponic Experiment with Soil Column Experiment

			Soil Column Experiment		
			Soil	Plant	Water
Hydroponic Experiment	Triclocarban	Water	<0.01		<0.01
		Plant		0.233	
	Triclosan	Water	<0.001		<0.001
		Plant		<0.001	

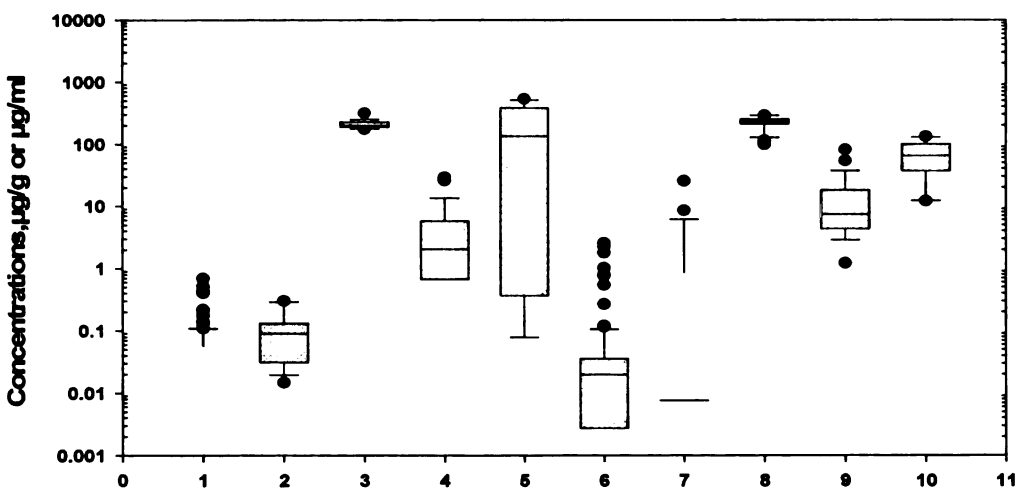


Figure 4.14 Comparisons of Soil Experiment and Hydroponic Experiment Data. Box plot Concentrations 1), 2) Water and Soil TCC in Soil Column Experiment, 3) Hydroponic Water TCC, 4), 5) Plant TCC in Soil Column and Hydroponics, 6), 7) , Water and Soil TCS in Soil Column Experiment, 8) Hydroponic Water TCS, 9), 10) Plant TCS in Soil Column and Hydroponics.

Table 4.10 Comparison of Translocation Factors and Bioaccumulation Factors for Two Experiments

Antimicrobial	Experiment	Translocation Factor	Bioaccumulation factor	
			Root	Shoot
TCC	Soil Column	0.52 ± 0.33	45.44 ± 38.02	15.87 ± 8.27
	Hydroponics	0.0013 ± 0.0010	1.78 ± 0.54	0.0014 ± 0.0010
TCS	Soil Column	0.35 ± 0.28	1242 ± 561	372 ± 208
	Hydroponics	0.082 ± 0.079	0.64 ± 0.38	0.024 ± 0.02

Roots extracted triclocarban and triclosan more efficiently from soil columns than from nutrient solutions in the hydroponic study by factors of 25.5 and 1941 respectively. Translocation of antimicrobials from root to shoot is more in soil column than in hydroponic studies by a factor of 4.3 for triclosan and 400 for triclocarban. This indicates that triclocarban is more difficult to uptake to plant roots but easier to translocate to aerial plant parts than triclosan. More liphophilicity of triclocarban might have made it easier to translocate up to shoots. The results above indicate that hydroponics can be a tool to screen if plants are phytoaccumulators or not. However, hydroponics might not represent the actual case for phytoaccumulation of antimicrobials triclosan and triclocarban by pumpkin and zucchini. Though plants in soil column experiment were efficient in removing antimicrobials from soil rather than soil, screening plants is easier in hydroponic experiment owing to its ease of experimentation with regards to cost and time.

Table 4.11 contains coefficients of variation for both hydroponic and soil column experiment. Coefficients of variation were calculated as described in Section 3.1.7. As expected, LC-MS contributed the least amount of variability. For the soil column experiment, variability of LC-MS data for all matrices was comparable. However, for hydroponic samples, variability in observed aqueous concentrations was greater than that for plant samples most likely due to directly analyzing the aqueous samples as opposed to

extracting solid phase. The lowest variability and small coefficient of variation in LC-MS data suggested that LC-MS produced good quality data for analysis of triclocarban and triclosan. As LC-MS analysis is cheaper than LC-MS-MS analysis, LC-MS analysis is an economical option for analyzing triclocarban and triclosan while producing good data quality.

Table 4.11 Coefficients of Variation in Different Units in the Experiments

	Unit	Soil Column Experiment					Hydroponics Experiment		
		Soil	Stem	Leaves	Roots	Water	Shoot	Root	Water
Triclocarban	LC-MS	0.01 ± 0.00	0.03 ± 0.05			0.02 ± 0.00	0.01 ± 0.01		0.19 ± 0.03
	ASE	0.10 ± 0.00				-	0.10 ± 0.00		-
	Single Column	0.60 ± 0.00	0.38 ± 0.12	0.95 ± 0.25	-	-	-	-	-
	Multiple Columns	0.59 ± 0.12	0.58 ± 0.12	1.26 ± 0.44	0.85 ± 0.17	0.61 ± 0.12	0.64 ± 0.12	0.28 ± 0.16	0.01± 0.00
Triclosan	LC-MS	0.00 ± 0.02	0.01 ± 0.02			0.00 ± 0.00	0.00 ± 0.01		0.15 ± 0.00
	ASE	0.22 ± 0.01				-	0.22 ± 0.01		-
	Single Reactor	0.12 ± 0.00	0.17 ± 0.12	0.28 ± 0.06	-	-	-	-	-
	Multiple Reactors	1.44 ± 0.53	0.47 ± 0.08	0.92 ± 0.03	0.71 ± 0.10	0.68 ± 0.07	1.32 ± 0.21	0.62 ± 0.28	0.08 ± 0.06

ASE also contributed to variability of data. Variability in ASE data included variability due to LC-MS as ASE extracted samples were analyzed in LC-MS for quantification of triclocarban and triclosan. Data variability in triclocarban concentration was less than that for triclosan concentration.

Single column or reactor variability stands for variability seen across the sub-samples of the same column or reactor. Single reactor variability for hydroponic experiment could not be calculated as the sample mass was not sufficient enough to sub-sample. Variability within a single column was due to variability in analytical methods,

i.e. LC-MS and ASE, and heterogeneity observed within the column. For example, previous literature has shown exponential decrease in concentration of PCBs in shoot as the distance from root increase (Aslund et al. 2007). Since stems were sampled as a unit, the variability would have been increased as compared to sampling stems on the basis of the length from root. The single column or reactor variability was higher than ASE variability by a factor of 4 to 9 for triclocarban and a factor of 0.5 to 1.3 for triclosan.

Unsurprisingly, coefficient of variation is largest for multiple columns for both hydroponic and soil column experiments. Variability in plant varieties or control is as high as 6.4 times the variability due to ASE. In addition to variability sources mentioned above, this also accounts for difference in variation among the reactors or columns. Variation of mass of plants in different columns or reactors are given in Table 4.8. Moreover, multiple column variability could also be by local micro-environment. The greatest source of variation in the experiments is due to heterogeneity within a column and variability among multiple reactors or columns. Soil and shoot had the greatest variability. LC-MS contributed the greatest variability in aqueous samples of hydroponic experiment. However, variability in multiple reactors in hydroponic experiment is less than that in soil column experiment for both aqueous and plant samples. For example, coefficient of variation of triclocarban concentration in pumpkin stem in soil column experiment is four times greater than that in hydroponic experiment. Thus, hydroponic experiment can be more accurate option to screen plants for phytoremediation studies, particularly if a more precise LC-MS method is developed.

Chapter 5 Conclusions and Future Research

Research indicated that plants influence the migration of antimicrobials from biosolids to water resources through a yet unidentified mechanism. Though pumpkin, zucchini and switch grass up took antimicrobials, they did not accumulate high enough concentrations to be considered as hyperaccumulators of triclocarban and triclosan. Roots had higher concentration than shoot, but less total mass accumulation of antimicrobials than shoots. Pumpkin and zucchini significantly reduced soil concentrations of antimicrobials. There was no or very minimal risk of antimicrobials to human due to application of biosolids to pumpkin and/or zucchini field. However, there is low to high risk to aquatic plants, microorganisms and invertebrates and soil ecosystem health. More research is needed to identify and quantify the exact mechanism that dictates the fate of antimicrobials in biosolid- applied vegetated systems. Field scale research is needed to fully represent field conditions. Additionally, study of hyperaccumulation in fruits and those plant parts which human consume is recommended along with risk of eating those fruits and vegetables.

The hydroponic experiment indicated that while there was accumulation of triclocarban and triclosan, the planted reactors did not differ significantly from the control. Root concentrations were higher than shoot concentrations for both pumpkin and zucchini in both the hydroponic and the soil column experiments. However, root mass accumulation of antimicrobials was higher than shoot accumulation for both pumpkin and zucchini, in contrast, to soil column study. Variability in both the experiments was mainly due to multiple reactors rather than analytical error. Large number of columns with longer duration could make the results better by providing more data points per

sampling event and more number of sampling events. While hydroponic experiments could be a good option for selecting the aquatic species, it might not be the best representative for the terrestrial plants as was observed in this experiment. Development of very good root system in hydroponics and a relatively undeveloped shoot system may not be representative of terrestrial plants. Further research on screening more species, terrestrial in soil column and aqueous in water column is recommended. Also, triclosan could be spiked higher due to higher solubility than triclocarban if conducted separately.

Appendices

A.1: Review of Triclocarban and Triclosan Extraction

Table A.1 Review of Extraction of Triclosan and Triclocarban from Different Matrices in Different Literatures

Sample	Chemical	Extraction method	Solvent used	Procedure	Analytical method	Reference
Marine sediment	Triclosan	PLE-solid phase extraction	Dichloromehtane	Samples homogenized, frozen, lyophilized before storage. 10 g of freeze dried samples previously sieved through 0.5 mm and mixed with hydromatrix was extracted using Dionex ASE, precleaned by evaporation and again cleaned by using extraction cartridges with silica and finally re-dissolved in acetonitrile:water (50:50).	LC-ES-MS-MS	(Aguera et al. 2003)
Wastewater	Triclosan	Liquid- liquid extraction	Toluene	Samples extracted using magnetic stirrer with 10 ml of toluene after adding an aliquot of internal standard solution (D_{15} musk xylene), cleaned by separating aqueous phase by freezing samples overnight at −20°C and concentrated to 1 ml using a rotary evaporator at 40°C and 60 mbar.	GC-MS	(Bester 2003)
Sludge, river sediment, marine sediment	Triclosan	Microwave assisted soxhlet extraction SPE	Water and dichloromethane	Sediments (3g) spiked with chemicals was placed in cellulose thimble and extracted in Soxhlet glassware fitted with a distillation flask containing 100 ml of extractant. Two sequential extractions on the same sample were carried out during 24 h and also automated microwave assisted soxhlet extraction.	GC-MS-MS	(Morales-Munoz et al. 2005)
Digested sludge	Triclocarban	PLE	Acetone	Sludge samples (10 mL) were dried, homogenized, mixed with 200 mg fractions Ottawa sand free of triclocarban contamination and extracted with acetone sung ASE	LC-ESI-MS-MS	(Heidler et al. 2006)

Table A.1 Cont'd

Biosolids, soil	Triclosan	PLE-SPE	Acetonitrile water (70:30)	Homogenized wet soil (20 g) was extracted using ASE in three cycles to give final volume of 20 ml solvent	GC-MS	(Kinney et al. 2008)
Biosolids	Triclosan	Liquid-liquid extraction (sonication) SPE	Ethyl acetate	Freeze dried biosolid (0.5 g) was spiked with $^{13}C_{12}$-triclosan (200 ng/g) and extracted thrice in a sonication bath, the extracts dried and re-dissolved in methanol and water mixture and diluted extracts re-cleaned using C_{18} cartridges	GC-MS	(Ying and Kookana 2007)
Digested sludge	Triclosan	PLE	Acetone	Oven dried digested sludge was extracted with acetone in ASE	LC-ESI-MS-MS	(Heidler and Halden 2007)
Sludge	Triclocarban	PLE	Methanol: acetone: acetic acid	Raw sewage (40ml) was diluted to 250 ml final volume and spun at 10000g for 10 min, resuspended, filtered and internal standard added	LC-ESI-MS-MS	(Sapkota et al. 2007)
Biosolids, Activated sludge	Triclocarban, triclosan	PLE-SPE	DCM	Previously centrifuged 0.1-0.2g biosolid was weighed on a aluminium foil, grounded in glass mortar with about 3g hydromatrix, spiked with internal standards and extracted by ASE, the extracts concentrated by rotary evaporator and solvent exchanged into hexane, and re-concentrated by vacuum centrifuging	LC-ESI-MS-MS	(Chu and Metcalfe 2007)
River sediment	Triclocarban, triclosan	LLE	Methanol, 0.1 M acetic acid and 5% Na_2-EDTA (2:1:1)	Lyophilized sample aliquot (0.5 g) was extracted using ultrasonication, cleaned and concentrated by solid phase extraction using strata-X	LC-ESI-MS	(Chenxi, Spongberg and Witter 2008)
Biosolids	Triclosan	Ultrasonic extraction-SPE		Freeze dried biosolid samples' aliquot (0.5 g) was centrifuged, extracted by ultrsonication for 5 min and again centrifuged, pH adjusted, cleaned by SPE	LC-ESI-MS-MS	(Chenxi et al. 2008)
Biosolids/ Soil	Triclocarban triclosan	PLE		As in (Chu and Metcalfe 2007)Chu and Metcalfe, 2007	LC-ESI-MS-MS	(Cha and Cupples, 2009)
Soil	Triclocarban, triclosan	ASE	Methanol	Freeze dried soil samples was mixed with Ottawa sand, extracted using ASE		(Wu, Spongberg and Witter 2009)

A.2 Threshold Toxicity Data of Triclocarban and Triclosan from Different Research

Table A.2 (a) Microbial Resistance (Aqueous and Soil)

Indicator	Assay	Exposure time	TCC µg/l or µg/kg	TCS µg/l or µg/kg	Susceptibility reduction factor	References	Remarks
Proteus mirabilis	MIC			200	400 (stable)	(Stickler and Jones 2008)	Urease-producing bacteria
Escherichia coli	MIC	18 h		800	2.5-100	(McMurry et al. 1998)	
	MIC			300		(Sivaraman et al. 2004)	
	MIC	75 min		100	70% (1.33)	(Escalada et al. 2005)	
Pseudomonas aeruginosa	MIC	75 min		20000	50% (2)	(Escalada et al. 2005)	
Staphylococcus aureus	MIC			25	40	(Suller and Russell 2000)	Methicillin resistant bacteria

Table A.2 (b) Ecological Health (Aqueous and Soil)

Indicator	Assay	Parameter	Exposure time	TCC µg/l or µg/kg	TCS µg/l or µg/kg	Effects	References	Remarks
Domestic sewage bacteria	HA_{50}	Acute toxicity	15 min	4×10^7			(EPA 2002a)	
	NOEC	ADI	16 d	10^5			(EPA 2002a)	
	LOEC	ADI	16 d	10^5-10^6			(EPA 2002a)	
Activated sludge microorganisms	EC_{50}	Acute toxicity	5 d		1820		(Neumegen et al. 2005)	
Heterotrophic organisms	EC_{50}	Heterotrophic respiration	5 d		38,200		(Stasinakis et al. 2008)	Nitrifiers more sensitive than heterotrophic orgs
	EC_{50}	Heterotrophic respiration	10 d		31,500		(Stasinakis et al. 2008)	
	EC_{50}	Ammonia uptake	15 d		9,970		(Stasinakis et al. 2008)	
Autotrophic organisms	EC_{50}	Ammonia uptake	15 d		6,390		(Stasinakis et al. 2008)	
Nitrifying bacteria	MIC				500		(Stasinakis et al. 2008)	
	NOEC				750		(Federle, Kaiser and Nuck 2002)	

Table A.2(b) Cont'd

Vibrio fischeri	LOEC	Bioluminescence inhibition	15 min		100		(Farre et al. 2008)	M-triclosan=75
	EC_{50}	Bioluminescence inhibition	15 min		280		(Farre et al. 2008)	M-tcs=210
	EC_{50}	Bacterial bioluminescence	30 min		220		(Stasinakis et al. 2008)	
Freshwater bacterium *Caulobacter crescentus*	Singles and double stain flow cytometric assays, IC	Growth suppression			28.95		(Johnson et al. 2009)	
Freshwater microbial community growth	Singles and double stain flow cytometric assays, IC	Growth suppression			2.895	Growth observed (tolerant microbes)	(Johnson et al. 2009)	
Composition of freshwater microbial community	Singles and double stain flow cytometric assays, IC	Changes in composition	24 h		2.895		(Johnson et al. 2009)	
Plant growth tests-rice seeds, cucumber seeeds	EC_{50}	Acute toxicity	7 days		57,000(rice); 108,000 (cucumber)	Inhibited plant growth, rice seeds more sensitive than cucumber	(Liu et al. 2009)	
Soil respiration		Direct adsorption method	4 days		10,000 dry soil	Inhibited	(Liu et al. 2009)	
Phosphatase activity			Up to 25 days		100-50,000 dry soil	Inhibited	(Liu et al. 2009)	
Pimephales promelas	LC_{50}	Acute toxicity	24h		360	Loss of equilibrium, locking of jaw, quiescence, erratic swimming of fishes	(Orvos et al. 2002)	Fish, fathead minnows, fresh water fish, Highly toxic to tcs
			48h		270		(Orvos et al. 2002)	
			72 h		270		(Orvos et al. 2002)	
			96 h		260		(Orvos et al. 2002, EPA 2008a)	
	NOEC	Hatchability of eggs, growth & survival of fry	35 d	5	100		(EPA 2002a) (EPA 2002a, EPA 2008a)	

Table A.2(b) Cont'd

Pimephales promelas	LOEC	Hatchability of eggs, growth & survival of fry	35 d	10	180		(EPA 2002a) (EPA 2002a, EPA 2008a)	
Lepomis macrochirus	LC_{50}	Acute toxicity	96 h		370		(Orvos et al. 2002)	Bluegill sunfish, freshwater fish, slightly toxic to TCS
			96 h	97			(EPA 2008c)	
	NOEC	Acute toxicity	96 h	49			(EPA 2002a)	
Oncorhynchus mykiss	NOEC/LOEC at pH 8.2	Survival, hatchability, growth	61 d		15.100/31.600		(Orvos et al. 2002)	Rainbow trout, freshwater fish, slightly toxic
	LC_{50}	Acute toxicity	96 h	180	288		(EPA 2002a, EPA 2008a)	
	NOEC	Acute toxicity	96 h		100		(EPA 2008a)	Highly toxic
Salmo gairdneri	LC_{50}	Acute toxicity	96 h	120			(EPA 2008c)	Rainbow trout, fish, estuary, fresh water
	NOEC	Acute toxicity	96 h	49			(EPA 2002a)	
Oryzias laptides	NOEC	Hatchability and time to hatching	21 d		200		(Ishibashi et al. 2004)	Madaka fish
	LC_{50}	Acute toxicity	96 h		602		(Ishibashi et al. 2004)	
	IC_{25}	Hatchability of eggs, growth and survival	9 d		290		(Tatarazako et al. 2004)	
Daphnia magna	NOEC	Acute toxicity	48 h	9.200	100		(EPA 2002a, EPA 2008a)	Crustacean, water fleas, freshwater invertebrate
	EC_0	Acute toxicity	24 h/48 h	0.020/5			(EPA 2002a)	
	EC_{50}	Acute toxicity	48h	10	390		(Orvos et al. 2002) (EPA 2002a, EPA 2008a)	
	EC_{100}	Acute toxicity	48h	40			(EPA 2002a)	
	NOEC	Survival	21 d		200		(Orvos et al. 2002)	
	NOEC/LOEC (pH 8.2-8.6)	Reproduction	21 d		40/200, <139		(Orvos et al. 2002, EPA 2008a)	

Table A.2(b) Cont'd

Daphnia magna	LOEC	Mortality, mean length, weight and reproduction	21 d	4.700			(EPA 2002a)	Crustacean, water fleas, freshwater invertebrate
	NOEC	Mortality, mean length, weight and reproduction	21 d	2.900			(EPA 2002a)	
	MATC	Mortality	21 d (100%)	0.250-0.500			(EPA 2002a)	
	LC_{50}	Acute toxicity	48 h		420		(EPA 2008a)	Highly toxic to TCS
Ceriodaphnia dubia	NOEC	Acute toxicity	48 h	1.900			(EPA 2002a)	Crustacean
	EC_{50}	Acute toxicity	48 h	3.100			(EPA 2002a)	
	NOEC at pH7/8.5	Survival	7 d		50/339		(Orvos et al. 2002	
	NOEC at pH7/8.5	Reproduction	7 d		6/182		(Orvos et al. 2002	
	NOEC	Acute toxicity	48 h	1.900			(EPA 2002a)	
	EC_{100}	Acute toxicity	48 h	6.300			(EPA 2002a)	
	IC_{25}	Hatchability of eggs, growth and survival	7 d		170		(Tatarazako et al. 2004)	
Ceriodaphnia sp.	NOEC	Survival, reproduction	8 d	2.840			(EPA 2002a)	Crustacean
	LC_{50} /EC_{50}	Survival, reproduction	8 d	4.540			(EPA 2002a)	
Mysidopsis bahia	LC_{50}	Acute toxicity	96 h	10			(EPA 2002a)	Mysid shrimp
	LC_{50}	Acute toxicity	48 h	15			(EPA 2002a)	
	EC_{50}	Acute toxicity	96 h	15			(EPA 2002a)	
	EC_{50}	Mortality	4 d	7-10			(EPA 2002a)	
	EC_{50}	Reproduction	28 d	0.210			(EPA 2002a)	
	NOEC	Reproduction	28 d	0.060			(EPA 2002a)	
	LOEC	Reproduction	28 d	0.130			(EPA 2002a)	
	MATC	Survival, time to brood, reproduction	12/28 d	0.060-0.120			(EPA 2002a)	

Table A.2(b) Cont'd

Bobwhite quail *Colinus virginianus*	NOEC	Acute oral toxicity	14 d		<147,000 body wt		(Reiss, Lewis and Griffin 2009)	Bird, slightly toxic
	LD_{50}	Acute oral toxicity	14 d		862,000 body wt		(Reiss et al. 2009)	
	NOEC	Sub-acute dietary toxicity	8 d		1250,000		(EPA 2008a)	Relatively non toxic
Mallard duck *Anas platyrhynchos*	NOEC	Acute oral toxicity	14 d		2150,000 body wt		(Reiss et al. 2009)	Bird, relatively non toxic
Scenedesmus subspicatus	NOEC/LOEC	Growth, biomass	72 h		0.5/1.2		(Orvos et al. 2002)	Green Algae
		Biomass	96 h		0.69/1.2		(Orvos et al. 2002)	
	EC_{50}	Growth	72 h		2.800		(Orvos et al. 2002, Reiss et al. 2002)	
			96 h	20-30	1.400		(Orvos et al. 2002) (EPA 2002a, Reiss et al. 2002)	
		Biomass	72 h		0.700		(Orvos et al. 2002, Reiss et al. 2002)	
			96 h	20-30			(EPA 2002a)	
Pseudokirchneriella subcapitata	EC_{50}	Growth	14 d	36000			(EPA 2002a)	Fresh water green algae
	LOEC	Growth	14 d	10000			(EPA 2002a)	
	LOEC	growth	72 h	10	0.400		(Yang et al. 2008)	
	NOEC	Growth	72 h	<10	0.200		(Yang et al. 2008)	
	IC_{50}	growth	72 h	17.000	0.530		(Yang et al. 2008)	Additive effects in mixtures of tcc & tcs
Anabaena fios-aquae	NOEC	Growth	96 h		0.810		(Reiss et al. 2002)	
	EC_{50}	growth	96 h		1.600		(Reiss et al. 2002)	

Table A.2(b) Cont'd

Microcystis aerubinosa	NOEC	growth	14 d	10			(EPA 2002a)	Algae, blue, cyanobacteria
	EC_{50}	growth	14 d	32			(EPA 2002a)	
Selenastrum capricornutum	EC_{25}	Biomass	96 h		2.440		Orvos et al. 2002)	Micro-Algae
	EC_{50}	Biomass	96 h		4.460		Orvos et al. 2002)	
	LOEC	Growth	14 d	10000			(EPA 2002a)	
	EC_{50}	Growth	14 d	Ca. 3600			(EPA 2002a)	
	IC_{25}	growth	96 h		3.400		(Tatarazako et al. 2004)	
Skeletonema costatum	EC_{25}	Biomass	96 h		>66		Orvos et al. 2002)(EPA 2008a)	Marine diatom, Algae
	NOEC	Biomass	96 h		12.6		Orvos et al. 2002)(EPA 2008a)	
Anabaena flos-aquae	EC_{25}	Biomass	96 h		0.670		Orvos et al. 2002)	Blue green Algae, cyanobacteria
	EC_{50}	Biomass	96 h		0.970		Orvos et al. 2002)	
	EC_{50}	Biomass	96 h		1.2		(EPA 2008a)	
Navicula pelliculosa	EC_{25}	Biomass	96 h		10.700		Orvos et al. 2002)	Golden brown diatom, Algae, freshwater diatom
	EC_{50}	Biomass	96 h		19.100		Orvos et al. 2002)	
	EC_{50}	Biomass	96 h		16		(EPA 2008a)	
	LOEC	Growth	14 d	1000			(EPA 2002a)	
	NOEC	Biomass	96 h		5		(EPA 2008a)	
	EC_{50}	Growth	14 d	Ca. 7800			(EPA 2002a)	

Table A.2(b) Cont'd

Lemna gibba	EC_{25}	Biomass	7d		>62		Orvos et al. 2002)	Duckweed
	EC_{50}	Biomass	7 d		>62		Orvos et al. 2002)	
	EC_{50}	NOEC	7 d		12.5		(EPA 2008a)	
	EC_{50}	Biomass	72 h	20-30			(EPA 2008c)	
Sesbania herbacea	LOEC	Seed germination	7 d		100	Root metrics affected	(Stevens et al. 2009)	Wetland macrophytes
	LOEC	Root length, root surface	28 d		0.4		(Stevens et al. 2009)	
Bidens frondosa	LOEC	Seed germination	7 d		1000	Root metrics affected	(Stevens et al. 2009)	Wetland macrophytes
	LOEC	Root length, root surface	28 d		0.4		(Stevens et al. 2009)	
Eclipta prostrata	LOEC	Root length, root surface	28 d		10		(Stevens et al. 2009)	Wetland macrophyte
Cucumber	LOEC	Shoot length, root/shoot weight			280		(Reiss et al. 2009)	
	NOEC	Shoot length, root/shoot weight			96		(Reiss et al. 2009)	
Tomato, wheat, ryegrass	LOEC	Root weight/shoot weight			930		(Reiss et al. 2009)	

ADI: anaerobic digester inhibition; EC_{50}: half maximal effective concentration; IC_{25}: growth inhibition of 25% concentration; IC_{50}: growth inhibition of 50% concentration; LC_{50}: median lethal dose; LOEC: lowest observed effects concentration; MIC: minimum inhibitory concentration; NOEC: no-observed effects concentration; TCC: triclocarban; TCS: triclosan

A.2 (c) Human Health

Indicator	Assay	Parameter	Exposure time	TCC µg/l or µg/kg	TCS µg/l or µg/kg	Effects	References
Rat	LD_{50}	Acute oral toxicity		>2 × 10^6 bw	4,334,000 body wt		(EPA 2002a, EPA 2008c, Reiss et al. 2009)
	LC_{50}	Acute inhalation			>150		(EPA 2008a)
	NOAEL	Subchronic and lifetime			4,334,000 body wt		(EPA 2002a, EPA 2008c, Reiss et al. 2009)
Rat	LD_{50}	Acute toxicity (I.p. route)		>2.1 × 10^6 bw	100,000		(EPA 2002a, EPA 2008c, Reiss et al. 2009)
New Zealand White Rabbits	LD_{50}	Acute dermal toxicity		>10^7 bw	>9,300,000		(EPA 2002a, EPA 2008c, EPA 2008a)
Guinea pig		Skin irritation	24 h	3% concentration		Irritating	(EPA 2002a, EPA 2008c)
Guinea pig		Eye irritation	24 h	20,000		Slightly irritating	(EPA 2002a, EPA 2008c)
Albino rabbit		Eye irritation	24 h	20,000		Slightly irritating	(EPA 2002a, EPA 2008c)
Sprague-Dawley rats	LOEL	Repeated chronic oral dose toxicity	24 months	75,000 bw/day		Body weight lower, anemia, organ weight increase	(EPA 2002a, EPA 2008c)
	NOAEL	Repeated chronic oral dose toxicity	24 months	25,000 bw/day			(EPA 2002a, EPA 2008c)
	LOEL	Repeated chronic oral dose toxicity	24 months	250,000 bw		Food consumption and body weight loss	(EPA 2002a, EPA 2008c)
	NOAEL	Repeated chronic oral dose toxicity	24 months	75,000 bw			(EPA 2002a, EPA 2008c)
	NOAEL	Systemic and developmental toxicity	3 generations	150,000 bw/day			(EPA 2002a, EPA 2008c)
	LOEL	Reproductive toxicity	3 generations	150,000 bw/day		Low conceive rate, low living pup at birth, clubbed legs and filamentous tail	(EPA 2002a, EPA 2008c)
	NOAEL	Reproductive toxicity	3 generations	50,000 bw/day			(EPA 2002a, EPA 2008c)
Hamster	LD_{50}	Acute oral toxicity			75,000 body wt		(EPA 2002a, EPA 2008c, Reiss et al. 2009)
	NOAEL	Subchronic and lifetime			75,000 body wt		(EPA 2002a, EPA 2008c, Reiss et al. 2009)

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