UPTAKE AND ACCUMULATION OF TRICLOSAN AND TRICLOCARBAN BY FOOD CROPS IN A HYDROPONIC SYSTEM

By

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ABSTRACT

UPTAKE AND ACCUMULATION OF TRICLOSAN AND TRICLOCARBAN BY FOOD CROPS IN A HYDROPONIC SYSTEM

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Triclosan (TCS) and triclocarban (TCC) are two antimicrobial chemicals that are used in many personal care products. In this study, triclosan and triclocarban were added to hydroponic nutrient media that was used to grow different food crops. The initial triclocarban and triclosan media concentration was 500 ppb and concentrates were measured weekly. The concentration of antimicrobials in the media decreased over the 4 week testing period. After the end of the 4 weeks, the plant tissue was also tested for triclocarban and triclosan concentrations. Most of the antimicrobials in each plant was found in the root tissue. From the root concentration factor (RCF) and concentrations in the plant tissues, onion is best at taking up triclocarban, while chili appears to take up the most triclosan. Chili and cucumber seem to have high potential to take up both chemicals. Celery, broccoli, and asparagus appear to have the lowest concentrations and RCFs of both chemicals. When the exposure of the crops was calculated using national produce consumption data, the average exposure to TCC and TCS from eating contaminated crops was still much less than the NOAEL.

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CHAPTER 1: INTRODUCTION

Pharmaceuticals and personal care products have been used for decades, but have only recently emerged as a concern for water resources. Triclocarban (TCC) and triclosan (TCS) are common chlorinated aromatic compounds used as antimicrobial agents in products such as soaps and toothpastes (Aryal and Reinhold 2011; Cha and Cupples 2010; USEPA 2002; USEPA 2010) which end up in waste water treatment plants (WWTP) through water disposed of in sinks and drains. In wastewater treatment plants, triclosan and triclocarban sorb with large particles that settle out of solution into what is called "biosolids". Biosolids are often applied to agricultural fields because of the high nutrient content. While there are regulations regarding treatment of biosolids before use as a fertilizer, triclosan and triclocarban are not on the list of chemicals that are regulated in biosolids. Chemicals in biosolids can then be introduced into the soil and sorb to soil particles, where most of the initial chemical in the biosolids will remain. Crops might then be able to take up chemicals in the soil like triclosan and triclocarban.

Though the bioavailable fraction of these contaminants may be low due to low solubility in water and sorption to soil and organic particles, accumulation of both TCC and TCS in plants has been observed (Aryal and Reinhold 2011) and the fruits or vegetables from these plants may be consumed by people. Triclocarban and triclosan are not considered health hazards by the EPA or FDA, but animal studies have demonstrated that triclocarban and triclosan could result in disruption of endocrine functions and the central nervous system respectively, and hence further investigations are required (Paul and others 2010; USEPA 2010; USFDA 2010). Studies also indicate the induction of antimicrobial resistance in bacteria in biosolid applied agricultural lands (USFDA 2010). Several pathogens are known to be resistant to both TCC and TCS at high concentrations. For example, *E. coli* and *P. aeruginosa*, are resistant to up to 1024 μg/mL of

triclosan (Coulborn Rebecca and others 2010; Coulborn and others 2010). However, information on bacterial resistance to triclocarban is limited. Plants, including food crops, are capable of taking up a number of organic contaminants into its biomass (Kim and others 2005b; Loffredo and others 2010a; Murano and others 2009; Zhang and others 2009). The studies indicate higher concentration of organic contaminants such as TNT, dieldrin, dibenzodioxin, dibenzofuran and bisphenol in the roots of the plants compared to the shoot. Similarly, TCC and TCS accumulation in pumpkin and zucchini were higher in the root than the shoot (Aryal and Reinhold 2011) which emphasizes concern over the accumulation of organic contaminants in edible root crops. Based on the hypothesis that plants are capable of accumulating both TCC and TCS more in the root and shoot, the objectives of this study are to 1) screen various crops plants on the uptake and accumulation of TCC and TCS using a hydroponic system 2) understand the changes in TCC and TCS in the growth media.

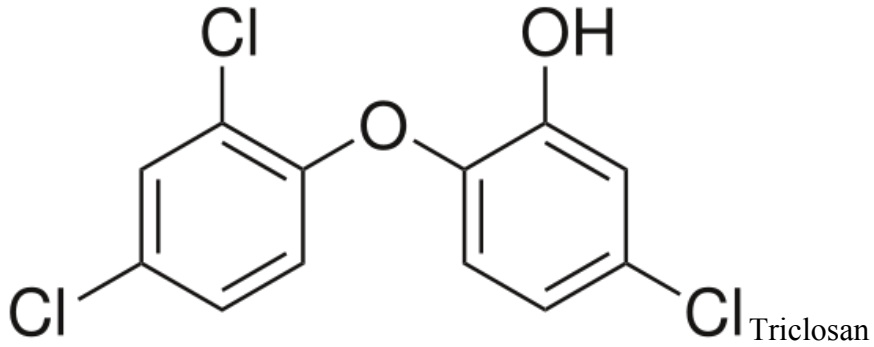
The information gained from this research will help guide policy in matters concerning these chemicals. For instance, it will help determine if the use of these chemicals should be regulated, or if the use of biosolids application is safe, and whether it should be limited to certain amounts of certain crops. It can also help determine if phytoremediation is a good method of removing these chemicals from the soil without posing a threat to public health. This research is very important since biosolids application is very common and has many benefits both to farmers and the environment. The purpose of this study is to determine if crops can take up TCC and TCS and how much.

CHAPTER 2: LITERATURE REVIEW

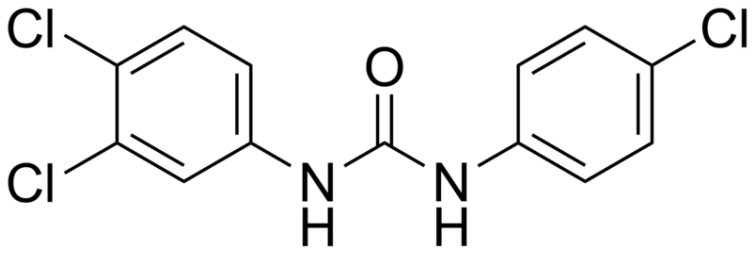
2.1 Description of chemicals

2.1.1 Type of chemical

Triclosan and triclocarban (TCS and TCC) are chemicals that are used as antimicrobial agents in personal care products such as antibacterial soaps and lotions. Triclocarban and triclosan are chlorinated aromatic compounds (USEPA 2002; USEPA 2010). The chemical formula for triclocarban is $C_{13}H_9Cl_3N_2O$, and the formula for triclosan is $C_{12}H_9Cl_3O_2$ (Halden and Paull 2005; USEPA 2002). Figure 1 shows the chemical structures of TCC and TCS (from Wikipedia.org).



Triclosan



Triclocarban

FIGURE 1. Chemical structures of TCS (top) and TCC (bottom)(images from wikipedia.org).

Both chemicals are used as antimicrobial agents and preservatives in products such as soaps and deodorants (USEPA 2002; USEPA 2010). As well as being used in antimicrobial personal care products, triclosan is also used as a commercial pesticide and as a "material preservative" in products such as adhesives, fabrics, and plastics (USEPA 2010).

2.1.2 Chemical Properties

Table 1 lists chemical properties of triclosan and triclocarban found in literature. The solubility of both TCC and TCS in water is very low, especially for TCC, due to their organic and nonpolar natures. The low vapor pressure indicates that TCC and TCS are not volatile. The aerobic half-life will be discussed in another section.

TABLE 1. Chemical properties of triclosan and triclocarban

Property	Triclosan	Triclocarban	Reference(s)
Molecular weight (g/mol)	290	315.59	(Chen and others 2009; Halden and Paull 2005)
Dispersion coefficient (L kg)	33-55	193-296	(Cha and Cupples 2010)
Log K_{OW}	4.8	3.5	(Halden and Paull 2005; Snyder and others 2010)
Aerobic half-life (d)	20-58	87-231	(Wu and others 2009)
Solubility in water (mg/L)	1.97-4.6	0.045	(Halden and Paull 2005; Snyder and others 2010)
pKa	4.5	12.77	(Chu and Metcalfe 2007; Snyder and others 2010)
Vapor pressure (Pa)	0.00062	4.81 x 10^-7	(Chen and others 2009; Snyder and others 2010)
Melting point (°C)	180	140	(Halden and Paull 2005)

2.2.3 Antimicrobials in Biosolids

Both triclosan and triclocarban are hydrophobic organic compounds and hence have high affinity for organic matter (Kwon and others 2010a; Kwon and others 2010b). The products of the WWTPs are biosolids and effluent water. Approximately 50% of biosolids are used as fertilizers for agricultural fields as it reduces the dependence on chemical fertilizer (USEPA 2007). These bio-solids comprise of a high concentration of organic matter (80%), allowing preferential partitioning of TCC and TCS (Cha and Cupples 2009; Kinney and others 2006). Studies indicate that approximately 76 and 50% of TCC and TCS respectively are sorbed into the organic fraction of biosolids from WWTPs (Kwon and others 2010b). Walters et al. (2010) reported finding triclosan in biosolids/soil mixtures in an initial concentration of 1265 ppb and triclocarban at 2715 ppb. Chu and Metcalfe (2007) reported the presence of triclosan and triclocarban in biolsolids and activated sludge in ranges of 0.68-11.55 ppm for triclosan and 2.17-5.95 ppm for triclocarban (Chu and Metcalfe 2007).

According to EPA regulation 40 CFR part 503, there are varying qualities of biosolids depending on the degree of treatment for pathogens, pollutants, and vector attraction. Treated biosolids can be applied to agricultural fields, though there are restrictions on how they can be applied and how soon crop scan be harvested depending on the quality of the biosolids. While some states may have more strict regulations, the additional regulations in Michigan mainly focus on applying at certain distances from water bodies and sources (Jacobs and others 2003). There are four different quality classifications based on how much treatment the biosolids have had. With regards to pathogens, biosolids can either be Class A or Class B. The goal of Class B is to reduce the pathogens to a low enough number that there they do not pose a threat to public health. The goal of Class A treatment is to reduce the pathogens to below detectable levels.

Class B biosolids have more restrictions on how long after application crops can be harvested. Class A biosolids have more rigorous treatments against pathogens and are less regulated. For pollutants or contaminants, biosolids are categorized as either following ceiling concentration limits (CCL) or pollutant concentration limits (PCL). PCLs are lower concentrations than CCLs, and if biosolids do not exceed the PCLs, then the cumulative pollutants throughout the lifetime of the site do not need to be tracked. The CCLs are the maximum concentrations of each pollutant that can be present in land-applied biosolids. Exceptional quality (EQ) biosolids are Class A and meet all the PCLs and CCLs and can in general be used freely as any other type of fertilizer. Pollutant concentration (PC) biosolids can be either Class A or Class B, but must meet all PCLs for pollutants. There are more restrictions on where and how these biosolids can be applied, for instance, they can only be applied in bulk and not on lawns or other lands easily accessible by the public. Cumulative Pollutant Loading Rate (CPLR) biosolids can be either Class A or Class B and can only be applied in bulk. They typically have at least one pollutant over the PCL, but not the CCL, and the cumulative pollutants must be tracked. Annual Pollutant Loading Rate (APLR) biosolids are sold in bags or other containers with instructions on safe use. They are Class A and have at least on PCL exceeded, but no CCLs. The annual loading of the pollutants must be tracked. TCS and TCC are not currently regulated under part 503, and therefore do not have a PCL or CCL for biosolids, though TCS is under review by the EPA (USEPA 2012).

2.2 Physiochemical Processes of TCC and TCS

2.2.1 Sorption to Soils

The most likely pathway for both TCC and TCS once introduced to an agricultural field is to sorb to soils, and triclocarban sorbs more strongly than triclosan (C. Wu 2009; Cha and Cupples 2010; Wu and others 2009). Wu et al. (2009) studied the adsorption and degradation of triclosan and triclocarban in sandy loam soil and silt clay soil both with and without biosolids amendments. Triclocarban had stronger sorption that triclosan in all soils tested, and sorption for both compounds was higher in sandy loam than silt clay, possibly because the large amount of clay in the silt clay inhibited interactions between the compounds and organic matter (Wu and others 2009). The study found that adding biosolids to the soil increased pH, organic matter, and cation exchange capacity; the effects were found to be able to last several years, and biosolids amended soils sorbed more antimicrobials in all cases except triclocarban in silt clay, likely because of the increased organic matter (Wu and others 2009). Sorption of triclosan decreased slightly with increasing pH (from 4 to 8), but triclocarban sorption was not significantly affected by pH; the decrease in sorption is likely due to the change in speciation for triclosan, as the study found that the amount of the neutral form of triclosan changed from 100% to 39% with the increase in pH from 4 to 8, but the sorption of the anionic form was still relatively high (Wu and others 2009). The study also found that when the two compounds coexisted in the same solution, the sorption of both compounds decreased at low concentrations, but co-solution had less of an effect with higher concentrations (Wu and others 2009). The sorption of these chemicals can change based on the properties of the soil, but regardless, triclocarban is more likely to sorb to

soils than triclosan. Cha and Cupples (2010) also found that triclocarban sorbs to soils more strongly than does triclosan in fields receiving biosolid applications.

2.2.2 Leaching

The leaching of chemicals from soils to groundwater is a common concern with any chemical that has the potential to pose health risks to the public. Triclosan typically is not thought to leach out of soil systems, but rather becomes immobilized in the soil (USEPA 2010). Cha and Cupples (2010) studied leaching potential of triclocarban and triclosan using a leaching potential model. The study found that triclocarban had a groundwater ubiquity score (GUS) of less than -0.5, and triclosan had a GUS of less than 0.7, which are both less than the 1.8 required to be leachable or transitional under the Gustafson's criteria (Cha and Cupples 2010). Therefore, both chemicals are much more likely to sorb to soils than to leach into groundwater. However, Aryal and Reinhold (2011) found in soil column studies that there were concentrations of triclosan and triclocarban in the leachate from the columns in ranges of 460-1670 µg/L for triclosan and 120-370 µg/L for triclocarban. Leaching was reduced in columns that contained plants (Aryal and Reinhold 2011). Leaching can occur, but not often, and is not much of a concern. Leaching may be a concern in certain circumstances, such as rain too soon after biosolids application.

2.2.3 Photodegradation

Triclosan can undergo photodegradation through photo-oxidation which can result in breakdown to many other products (Lawrence and others 2009; Ozaki and others 2011). Ozaki et al. (2011) studied the photodegradation of triclosan on dried loamy sand under simulated

sunlight conditions and found that the half-life of triclosan in sunlight was 17 days and that the data fit the light penetration-limited model best. Photodegradation of triclosan can form dichlorodioxins and chlorophenols, both of which can be toxic and persistent (Lawrence and others 2009). Triclosan can be degraded relatively quickly by light, but the rate of degradation is limited by light availability, and therefore by how deep within the soil the chemical is. Furthermore, even though light can cause triclosan to degrade, the products left behind could potentially have worse effects for health and the environment than the original triclosan.

2.2.4 Volatilization

Triclosan is typically not thought to volatize out of most soil or water systems (USEPA 2010). Triclocarban is non-volatile (USEPA 2002). As shown in table 1, both chemicals have very low vapor pressures, so volatilization is expected to be a very small portion of removal of the chemicals. Chen et al. (2009) found volatilization to be an insignificant portion of the removal of the chemical from soil.

2.2.5 Reactions with other chemicals

The most common degradation product of triclosan is methyl-triclosan, in which a methyl group attaches to the phenol group of triclosan (Bester 2005). Ozone is also known to react rapidly with the phenol group, either by adding oxygen or by opening the phenol ring (Suarez and others 2007). TCC can also be degraded by ozone (Tizaoui and others 2011). The phenol group of triclosan was found to be readily oxidized by manganese oxide (Zhang and Huang 2003). This reaction produced quinones of triclosan and 2,4-dichlorophenol, though the report mentioned there are most likely other products that were not detected by the methods used in the study.

2.3 Biological Processes of TCC and TCS

2.3.1 Microbial degradation

Wu et al. (2009) found that the two compounds can be degraded in soils under aerobic conditions, but not under anaerobic conditions. Aerobic degradation of triclocarban can form chloroanilines (Lawrence and others 2009). Triclocarban was degraded less than triclosan, possibly due to less bioavailability to microbes due to stronger sorption to the soil (Wu and others 2009). The half-life of triclocarban was higher than triclosan in all treatments (87-231 days and 20-58 days, respectively); half-lives were higher in silt clay soils than sandy loam soils, while there was no significant difference in amended soils and not amended soils (Wu and others 2009). Methyl-triclosan is the most commonly reported product from triclosan and stays in the environment longer than triclosan (Lawrence and others 2009). Triclocarban dechlorination products (dichloro-, monochloro, and nonchlorinated carbanilides) were found in sediments in the Chesapeake Bay area, but the investigators were unsure whether it was due to microbial activity or chemical degradation, but claim that it is an anaerobic process (Miller and others 2008).

2.3.2 Bioaccumulation

Bioaccumulation is "the accumulation of a substance, such as a toxic chemical, in various tissues of a living organism" (2002). The bioaccumulation of triclosan, methyl triclosan and triclocarban has been documented in algae growing in a stream that receive effluents from a wastewater treatment plant (Coogan and others 2007), so the chemicals are often present in aquatic ecosystems. There is some possibility of bioaccumulation of triclosan and triclocarban in

aquatic systems (USEPA 2010), but the chance is relatively low that much TCC or TCS would reach the trophic level containing fish; the bioconcentration factor (BCF) is 137 for the whole fish, but only 13 for muscle tissue (USEPA 2002), which indicates that the concentration in muscle tissue is 13 times greater than that in the water. However, since in most cases triclosan and triclocarban remain in the soil, it is more likely that these chemicals would be taken up by plants than be introduced into aquatic systems.

Plants can sometimes be used to take chemicals from contaminated soils, a practice called phytoremediation, but this is not always advisable if the plants grown in the soil are meant for human consumption, depending on how much the plant can uptake and where the contaminants are stored in the plant. Therefore, there is concern that these chemicals could contaminate food crops and cause a health risk. While there has not been an extensive amount of work done on the effects on plants by these chemicals, one study found that pumpkin and zucchini take up triclosan and triclocarban and accumulate the chemicals (Aryal and Reinhold 2011). Generally the study found that there were higher concentrations of the antimicrobials in the roots than any other part of the plants (Aryal and Reinhold 2011). For most crops that would indicate little chance of consuming contaminated parts of plants, but holds a particular concern for root crops such as carrots or onions. Another study found similar results with lettuce and radish (Pannu 2012). A study with carrots also found more TCS in the roots than the leaves, but also found that more TCS was found in the peel, or outer layer, or the root than in the core (Macherius 2012). The same study found that barley and meadow fescue accumulated TCS and other contaminants, but did not differentiate between root and shoot for those crops. Another study found that algae took up significant amounts of TCC, TCS, and methyl-triclosan from water (Coogan and others 2007). The uptake of antimicrobials by plants raised in soil was higher when applied via

irrigation water than when applied with biosolids (Wu and others 2010). When soybean plants were treated with TCC and TCS, the antimicrobials accumulated in the roots and was translocated to different parts of the plant including the bean.

Crops and other plants have been shown to take up several other organic contaminants. For instance, onions have been shown to uptake TNT in both hydroponic systems and soil systems (Kim and others 2005a; Kim and others 2004). Again, most of the contaminant was found in the roots rather than the shoots or leaves. Five forage grasses and three horticultural species (cucumber, marrow plant, and radish) were found to remove bisphenol A from water after 16 days, likely with a combination of plant uptake, biotransformation, and microbial degradation (Loffredo and others 2010b). Corn, lettuce, and potato were found to take up the veterinary antibiotic sulfamethazine from manure-amended soil (Dolliver and others 2007). Twelve different crops had dioxins in the roots and shoots after only a 4 day exposure (H. Zhang 2009). The crops tested included four from the genus *Curcurbita*, such as pumpkin and cucumber, as well as sorghum, rice, wheat, maize, tomato, soybean, Chinese cabbage, and garland chrysanthemum. Root uptake was mostly accomplished by lipophilic adsorption. The curcurbits tested appeared to have better ability to translocate the dioxins from the root to the shoot, though wheat and sorghum also had dioxins in the shoot tissue. The other crops tested had shoot concentrations close to that of the respective controls. The curcurbits also had the highest transpiration stream concentration factors (TSCF), which indicates that the main mechanism for translocation to the shoot is transpiration (H. Zhang 2009). DDT is another chlorinated aromatic, like TCC and TCS. It has been shown to be taken up by hairy root cultures of *Cichorium intybus* and *Brassica juncea* (Suresh and others 2005). DDT was also taken up by pumpkin plants, and the concentration in the stem and leaves decreased with distance from the roots, indicating that

transpiration may be a major mode of translocation for pumpkin (Aslund and others 2010). In another study, pumpkin took up polychlorinated byphenyl (PCB), another chlorinated aromatic (Aslund and others 2008). PCB-5 was taken up by pumpkin, corn, and soybean (Li and others 2011).

2.4 Possible risks and effects of TCC and TCS

2.4.1 Environmental effects and harm to wildlife

Agricultural fields are not the only places these chemicals can be introduced. They are often detected in open water systems, usually deposited there after going through the wastewater treatment plant. Triclosan has been found in 36 monitored streams in the United States that receive effluent from wastewater treatment plants (USEPA 2010). When these chemicals get into surface waters, they can have serious effects on the ecosystem. Lawrence et al. (2009) studied the effect of triclocarban and triclosan on river biofilms and found that they significantly affected the structure and functionality of the biofilms. The study found that the chemicals, triclosan especially, were toxic to algae and other phototrophs, which affected the functional groups of the biofilm to being more heterotrophic than autotrophic (Lawrence and others 2009). That kind of shift can affect symbiotic activities in biofilms. The chemicals were also found to "suppress carbon utilization" in microbes (Lawrence and others 2009). According to an EPA study, typical triclosan levels in aquatic systems are not more than the levels of concern for fish, but can be more than the levels of concern for aquatic plants, which can harm aquatic ecosystems (USEPA 2010).

2.4.2 Possible Health Hazards

Triclocarban and triclosan are not considered health hazards by the EPA or FDA, but animal studies on triclosan and triclocarban have demonstrated that they could be endocrine disrupting chemicals at 0.3 μg/ml for TCC in the presence of native androgens (only 3 times the human exposure from a whole-body shower) and 300 mg/kg/d for TCS, which the FDA and EPA plan to investigate further when reviewing the chemicals again (Chen and others 2008; Paul and others 2010; USEPA 2010; USFDA 2010). There have also been studies on bacteria indicating that triclosan may be able to lead to antibiotic resistant bacteria after being exposed to 1-4 μg/ml (Christensen and others 2011; Suller and Russell 2000; USFDA 2010). Furthermore, the FDA (2010) finds no evidence that antibacterial handsoap containing triclosan is any more effective at removing bacteria from hands than normal soap and water, which even further warrants more research into possible health hazards and decreasing the concentrations of these chemicals in soil and water or the concentration allowed in products.

2.5 Root Physiology and the Rhizosphere

Plants typically have either a taproot system or a fibrous root system. A taproot system consists of one main root that begins as the embryonic root and continues to grow downwards as well as smaller lateral roots that grow out from the taproot, whereas a fibrous system consists of many small adventitious roots growing from the stem (Campbell and others 2008). In most plants, a majority of plant uptake of water and nutrients is done near the tips of the roots, with the aid of root hairs. Each root has a root cap at its end to protect the root as it grows through the soil. There is also usually a layer of polysaccharide slime produced around the root cap to lubricate the root as it moves through the soil. Water and nutrients are transported out of the root with the xylem, and sugars and other storage materials are transported to the roots with the

phloem (Campbell and others 2008). Many plants have modified roots in order to better obtain or store water and nutrients. For instance, potatoes and beets have such large parts of their roots designed for storage. The bulbs of onions are actually not roots at all, but modified stems that grow underground. The bulb actually contains a short stem with many layers of what are the bases of the leaves that continue above ground; these leaf bases are used mainly for storage (Campbell and others 2008).

Plant roots often have mutually beneficial relationships with bacteria and fungi in the soil. About 80% of extant land plant species have some sort of symbiotic relationship with fungi and roots, called a mycorrhiae (Campbell and others 2008). Interactions between bacteria, fungi, and plants roots are often crucial to a plant's growth. Roots exude materials such as carbohydrates and other nutrients, which can attract or deter different kinds of bacteria, fungi, and nematodes (Fogel 1988). Contaminants can sometimes affect the complex rhizospohere in the soil. One study found that several pharmaceutical chemicals, including triclosan, can have a negative impact on mycorrhizal communites; however, the concentrations needed to cause such impacts were greater than is found in the environment (Hillis and others 2008). Organic contaminants such as polyaromatic hydrocarbons (PAHs) and phenols have been shown to harm mycorrhizal communities and decrease biodiversity in the rhizosphere (Cairney and Meharg 1999).

CHAPTER 3: MATERIALS AND METHODS

This study consisted of growing several different food crops in a hydroponic system spiked with the two chemicals of interest, triclosan and triclocarban. They were exposed to the chemicals for one month with weekly samples of the media taken. After the month, the plant tissue was divided into above ground and below ground sections and tested for TCC and TCS concentration.

3.1 Materials

Triclocarban [CAS 101-20-2], triclosan [CAS 3380-34-5] and C13- triclocarban were purchased from Tokyo Chemical Industry and Calbiochem. Ammonium acetate (>99.99%), acetone (>99.7%), methanol (99.8%), and methanol (>99.99% for LCMS) from were purchased from VWR. Planting materials for the hydroponic study were procured from Garden Harvest Supply, Burpee, and Tasteful Garden and plants of uniform age or size were used for the experiment. When possible the plants were raised from seeds (cucumber, tomato, cabbage, okra, chili), else tubers (potato, beet), bulbs (onion) or whole plants (celery) were used.

3.2 Experimental Setup

To avoid the effect of sorption by biosolids or soil and to understand the worst case scenario of complete bioavailability, the current study was performed hydroponically. The uptake and translocation of TCC and TCS were studied in cucumber, tomato, cabbage, okra, chili, beet, celery, onion, broccoli, asparagus, and potato. The effect of plant moisture content,

fresh weight, transpiration and media pH on TCC and TCS uptake was also investigated. Following uptake, the toxicity of these chemicals on plants was studied. The change in media TCC and TCS concentrations in the presence of the plants were studied to understand the difference in uptake.

Seeds were germinated or other propagating material were raised in a potting mix and once the roots were well established the plants were transferred to a nutrient solution hydroponic system with constant aeration in order to provide oxygen to the plant roots. After 1 week of acclimatization, plants were transferred to 1L amber glass jars with 900mL test solutions that were prepared in a nutrient medium made for aquatic plants (APHA 1999). Figure 2 shows an example of the hydroponic setup.

FIGURE 2. Hydroponic system setup. (For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis)

Standard solutions of both TCC and TCS mixtures were prepared in methanol from the respective stocks. Each of the crops was raised for one month in the test solution prepared in nutrient media having 500ppb of TCC and TCS. Each treatment had 5 replicates. No-plant controls in the TCC/TCS media and plant control in the absence of TCC/TCS in the nutrient media were also maintained. The crop plants were raised in these test solutions at constant aeration with temperature maintained at constant temperature of 23±2°C and light supply. Two mL of the media samples were collected weekly and analyzed for TCC/TCS immediately. Loss of media by evapotranspiration was made up by adding nutrient solution every week. The total amount of water lost from each bottle was calculated after adjusting the no-plant control media loss (evaporation) to give the transpiration loss.

After the test period of one month, the plants were washed in tap water and rinsed in distilled water. The plants were then separated into the above and below ground parts and flower, tubers or bulbs when available. The fresh weights of the plant parts were taken before the plants were dried in an incubator at 55°C for 2 days. The pH of the media was taken both before and after the experiment.

3.3 TCC/TCS degradation in the media

Separate sets of experiments were performed to understand the behavior of TCC and TCS under different aqueous systems without the presence of plants. Solutions of 500ppb of triclocarban and triclosan were prepared in e-pure water, nutrient solution and different components of the nutrient solution (A, B and C). Media pH was measured in each bottle both before and after the experiments to understand the effect of pH on loss. All the systems were maintained with continuous aeration. The experiments were performed in triplicates. There were

also sets of experiments conducted testing the behavior of TCC and TCS in media in the presence and absence of both light and aeration.

3.4 Sample extraction and analysis

The dried plant samples were weighed and ground in a grinder and the samples extracted using a Dionex ASE 200, as shown in figure 3. Cellulose thimbles were first placed in the extractor cells and were half filled with sand, followed by the sample and then filled with sand. One of the samples in each set of the plants was spiked with 1.2ppm of C-13 labeled triclocarban (a stable isotope) to know the extraction and analysis efficiency. The extractor specifications were as follows: temperature- 100^{o}C, pressure-1500 psi, static time-5 min and flush volume - 100%. The collected extracts in amber vials were then dried in N gas, as shown in figure 4, and then reconstituted in 3mL of 1:1 methanol: acetone mixture. The mean recoveries calculated for the method used in this study are 87.5% for triclocarban, 69.6% for triclosan, and 93.5% for the C-13 triclocarban.

FIGURE 3. Dionex ASE 200

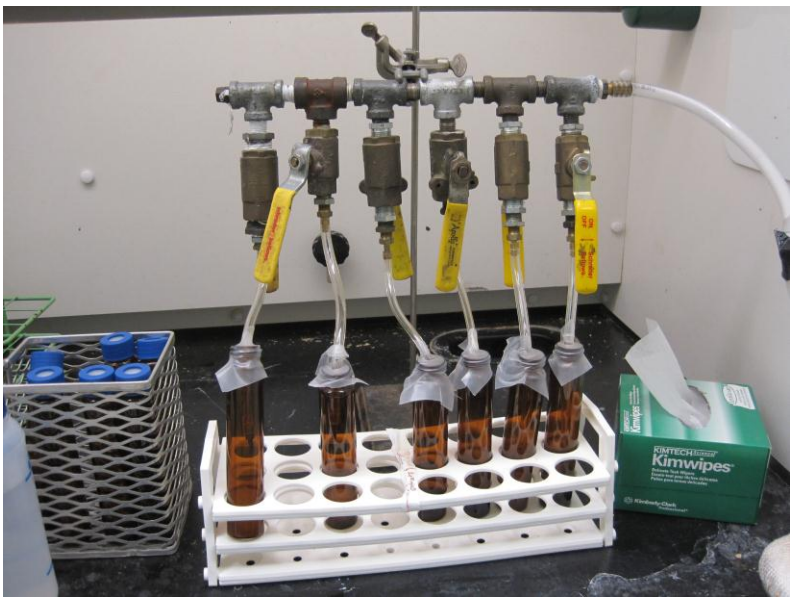


FIGURE 4. Extraction drying setup

The samples were analyzed for TCC and TCS concentrations using a Shimadzu LC-MS 2010 EV, as shown in figure 5, with an Allure biphenyl column (5 µm, 150 x 2.1 mm) from Restek using a binary gradient of 75% methanol and 25% 5-mM. Qualitative analysis was done in negative electrospray ionization with scan mode and quantification by selected ion monitoring (SIM) mode. TCC and TCS were identified by retention time ($t_R \pm 0.1$ min), specific molecular ions (m/z: 313 of triclocarban and 287 for triclosan), and reference ions (m/z 315 and 317 for triclocarban and m/z 289 and 291 for triclosan (Aryal and Reinhold 2011). Mobile phases were 5 mM ammonium acetate and methanol, for which a binary gradient from 75% to 100% methanol was used. Linear calibration for quantification of the analysis results was performed with prepared samples of at least six concentrations. Detection limits were 0.00001µg/mL for media, and 0.0001µg/g dry tissue for plants. Collected media samples from all the experiments were diluted in 2 mL methanol and the resulting 4mL diluted sample was analyzed with the LC-MS. Media samples and plant tissue extractions were both passed through a 0.2µm PTFE filter into an amber glass vial for the LC-MS that were then placed into the autosampler to be analyzed using the LC-MS.

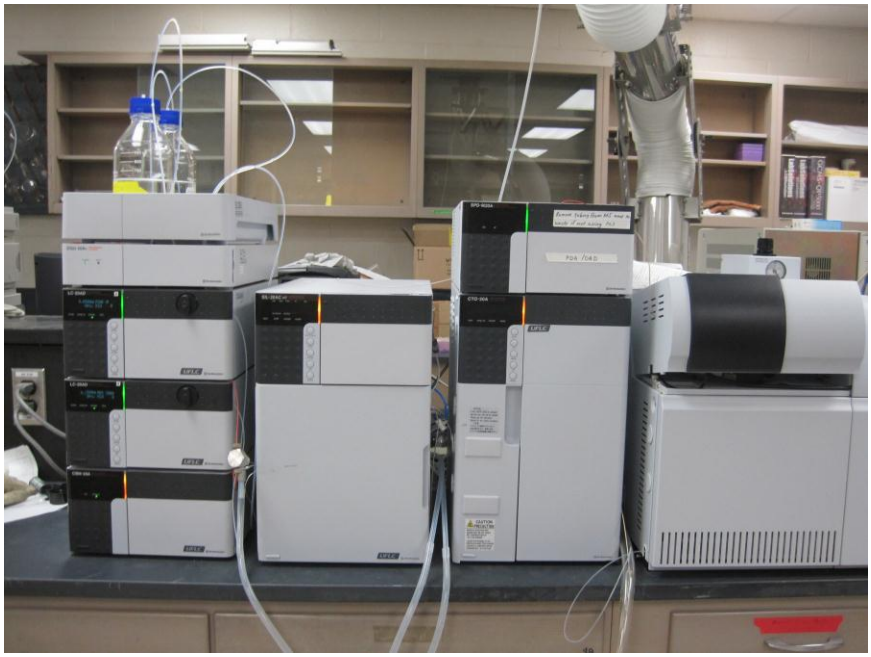


FIGURE 5. Shimadzu LC-MS

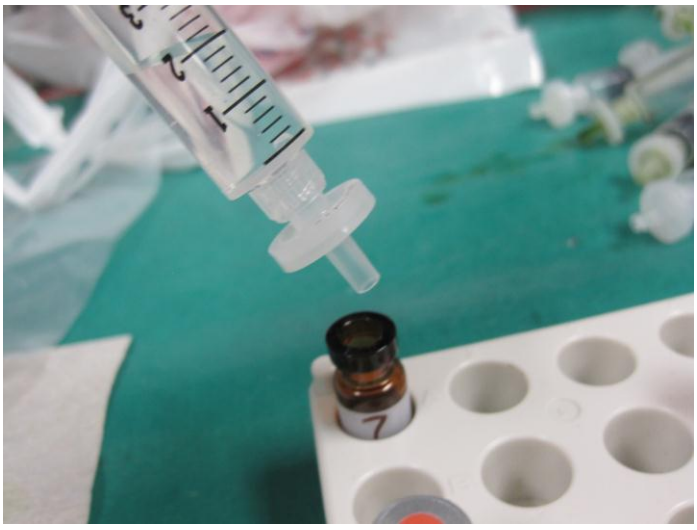


FIGURE 6. Filtering the samples

3.5 Media Statistical Analysis

Statistical analysis was performed using SigmaPlot (version 11.0). One way-ANOVA and Student-Newman-Keuls (SNK) tests were used to compare the data. Reported values are in mean ± standard error of the mean. P-values of less than 0.05 were considered statistically significant. While the data did not pass the automatic normality check for the ANOVA test, when a more detailed normality check (in the appendix) was run, only one or two sets of data failed, so the data was treated as normal.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Media TCC and TCS

4.1.1. E-pure vs. nutrient media without plants

Experiments with 500ppb of TCC and TCS mixture in e-pure and nutrient media indicated a loss of TCC from both the e-pure and nutrient media. Loss of triclocarban from e-pure water was minimal compared to nutrient media, which accounts for ~ 43% loss in 30 d/ 4 w (Figure 7). This indicates that nutrients play a major role in the loss of TCC. However, it is not clear whether this loss is by a chemical or microbial degradation process. One study found that triclosan can be oxidized by manganese oxide (Zhang and Huang 2003). While manganese oxide is not used in the making of the nutrient solution used in this study (manganese chloride is used for manganese), there are other metal oxides that could have a similar effect, and with the multitude of chemicals in the nutrient solution, there may be reactions that result in the formation of manganese oxide in the solution. Therefore, chemical degradation is a possibility, though more testing would be needed to determine exactly what causes the loss in the media. In the case of TCS, loss from both the e-pure (~30%) and nutrient media (~40%) in 30 d indicates the possibility of degradation (figure 7). Here, loss may indicate a reduction in concentration in the water soluble fraction of the media or degradation into other compounds.

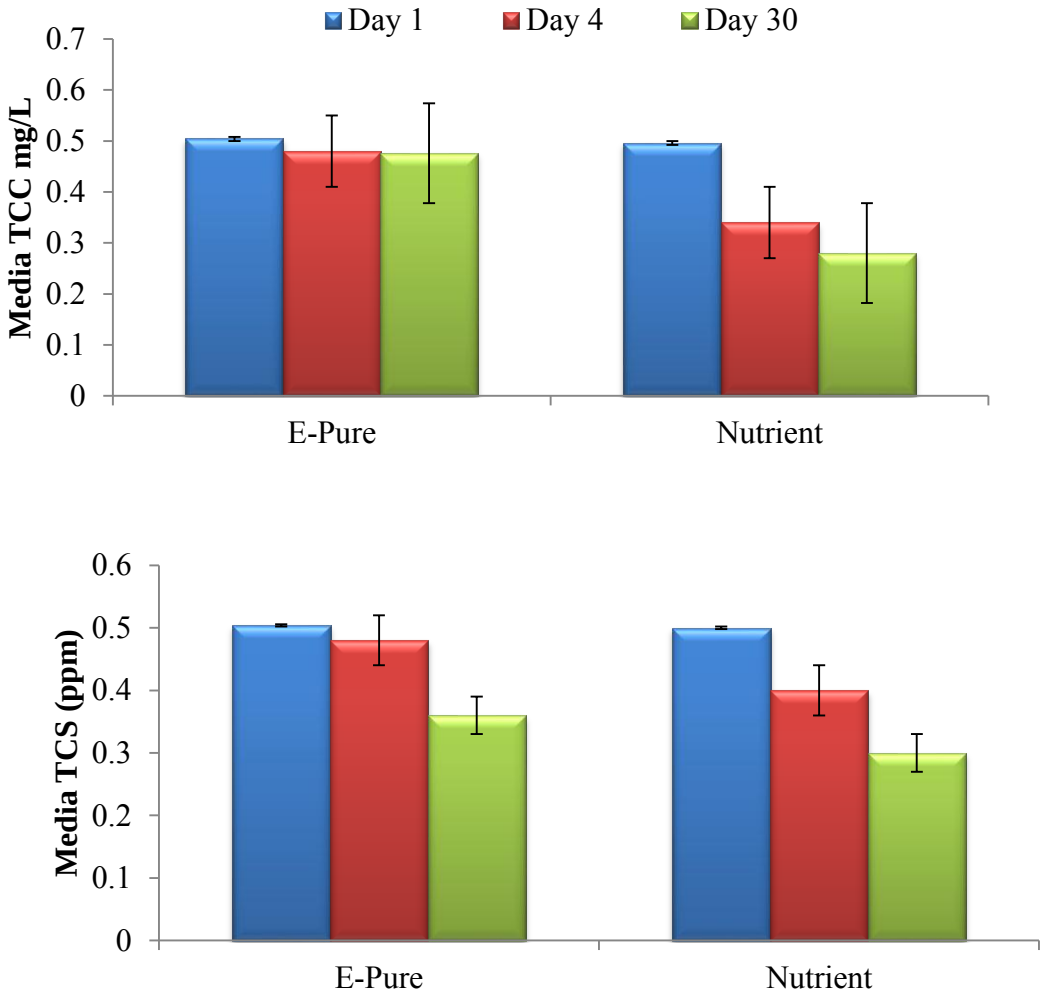


FIGURE 7. Concentration of TCC and TCS in E-pure water and Nutrient solution when treated with 500ppb of TCC and TCS for 4 weeks. Error bars represent standard error.

4.1.2. Concentration of TCC and TCS in the nutrient media with plants

The concentration of both TCC and TCS decreased with time for all the crop plants tested over the 4 week experiment (figure 8). The concentrations in the control experiment (hydroponic media with no plants) also decreased over time, showing that although the chemicals are taken up by the plants (as discussed in the next section), it is not the only mechanism for removal from solution for the chemicals.

The pH is an important determinant factor of the rate of degradation or loss of both TCC and TCS. Triclocarban can undergo direct and indirect photolysis at pH 7 (Guerard and others 2009). Rapid degradation of triclosan to 2,8-dichlorodibenzo-*p*-dioxin is occurs at higher pH (Sanches-Prado et al., 2006). Plant root exudates can alter the pH of the hydroponic system which may enhance the degradation of antimicrobials. These exudates contain low molecular weight organic acids which can house a number of microbes which may aid in antimicrobial degradation in the growth media. The pH of the media before and after the experiments was determined to understand the impact of pH on the change in concentration. The initial pH of the nutrient media ranged from 7.4 to 7.7 and the final media pH ranged from 7.3 to 7.8 and 7.3 to 8.1 in the unplanted and planted media, indicating any impact of pH on both TCC and TCS would be similar in unplanted and planted media.

With TCS, the majority of the chemical was still in the solution after 4 weeks, with the exception of a few crops. With TCC the majority of the chemical was "unaccounted for," which means that it was neither in the solution nor in the plant tissue. Presumably unaccounted for portions of the TCC and TCS were degraded, settled out of solution, reacted with one of the compounds in the nutrient media, or attached to settled particles. In many of the experiments, cloudiness was noticed in the media after the first or second week. If left without aeration after removing the plants, the cloudy gelatinous precipitate would settle to the bottom of the bottle. With the beet experiment, the bottles were left alone for several weeks after the plants were removed in order to allow the gelatinous precipitate to settle to the bottom. The precipitate was filtered out, dried, and weighed. It was then diluted and analyzed with the LC-MS. An average of 17.7 mg/L TCC and 2.63 mg/L TCS was found in the precipitate, compared to the final media concentration of 0.1-0.4 mg/L for TCC and TCS. Therefore, it was observed that a portion of the

TCC and TCS settled out of solution, though more testing would be needed to determine if this precipitate is a product of interactions between nutrients or microbes. Any samples analyzed by the LCMS were filtered, so the gelatinous precipitate and particulates would have been filtered out and would not have affected the results found for the aqueous concentrations. Since the concentrations found in the media were approximately constant after the first two weeks of exposure, especially for triclocarban, it is possible that some of the TCC and TCS from the precipitate would go back into solution whenever any was taken up by the plant, therefore keeping the concentration in the media at a constant equilibrium concentration.

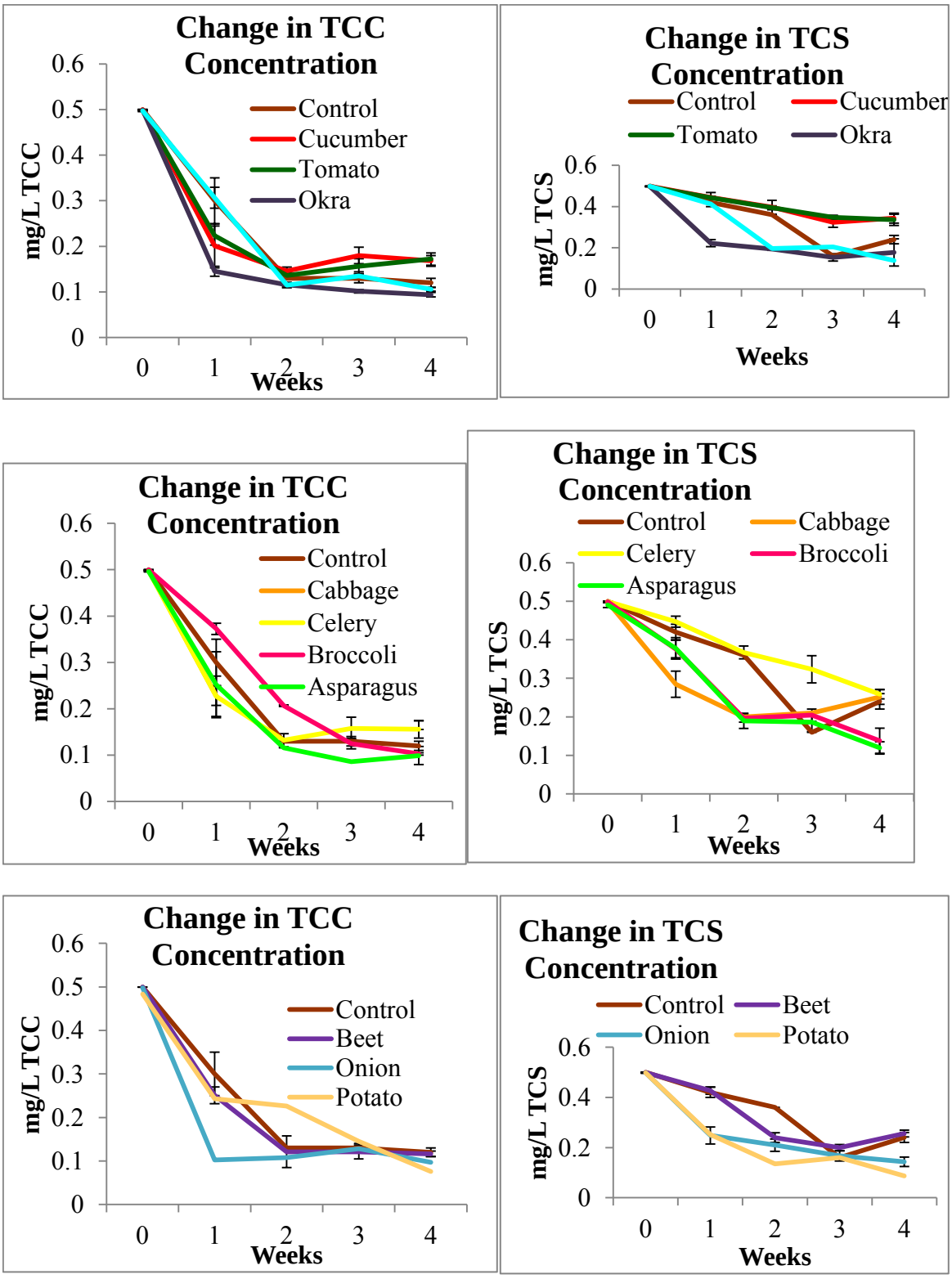


FIGURE 8. Concentration of TCC (top) and TCS (bottom) in the growth media of different crops when treated with 500ppb of TCC and TCS for 4 weeks. Error bars indicate standard error.

4.2 Plant TCC and TCS

The concentration of both TCC and TCS in the eleven plants studied was higher in the roots than the shoots (figures 9 and 10). Broccoli, asparagus, and potato had the lowest concentrations for the roots, and broccoli, asparagus, and onion had the lowest concentrations for the shoots. Chili and cabbage had the highest shoot concentration for both TCC and TCS, while root concentration varied more from the two chemicals and the different crops. Onion had the highest root concentration of TCC, but not TCS. Cucumber had the highest root concentration of TCS. Chili had relatively high concentrations for both TCC and TCS, while okra and cabbage had relatively high concentrations for TCC. According to a one-way ANOVA Student-Newman-Keuls (SNK) test, TCS shoot concentrations were not significantly different and the only value that was significantly different for the TCC shoot data was for chili. The power for the TCS shoot test was 0.731, which is relatively close to the desired 0.8. The power for the TCC shoot test was 0.758. However, while the pairwise comparison for TCS shoot data showed no differences, the ANOVA test said that there were differences. The test was run again without chili (the largest concentrations), and with that test not only was the normality test passed, but the power increased to 0.989. With the new test, a couple different statistical groups were found. For TCC root concentrations, chili and onion roots had significantly higher concentrations. Cucumber had the highest TCS concentrations in the roots, and tomato and chili also had significantly higher concentrations than the other plants. The power for the TCS root test was 1 with an alpha value of 0.05. The power for the TCC root test was 0.988. The high power values indicate that with the data provided, the test would be very likely to detect any differences in the data.

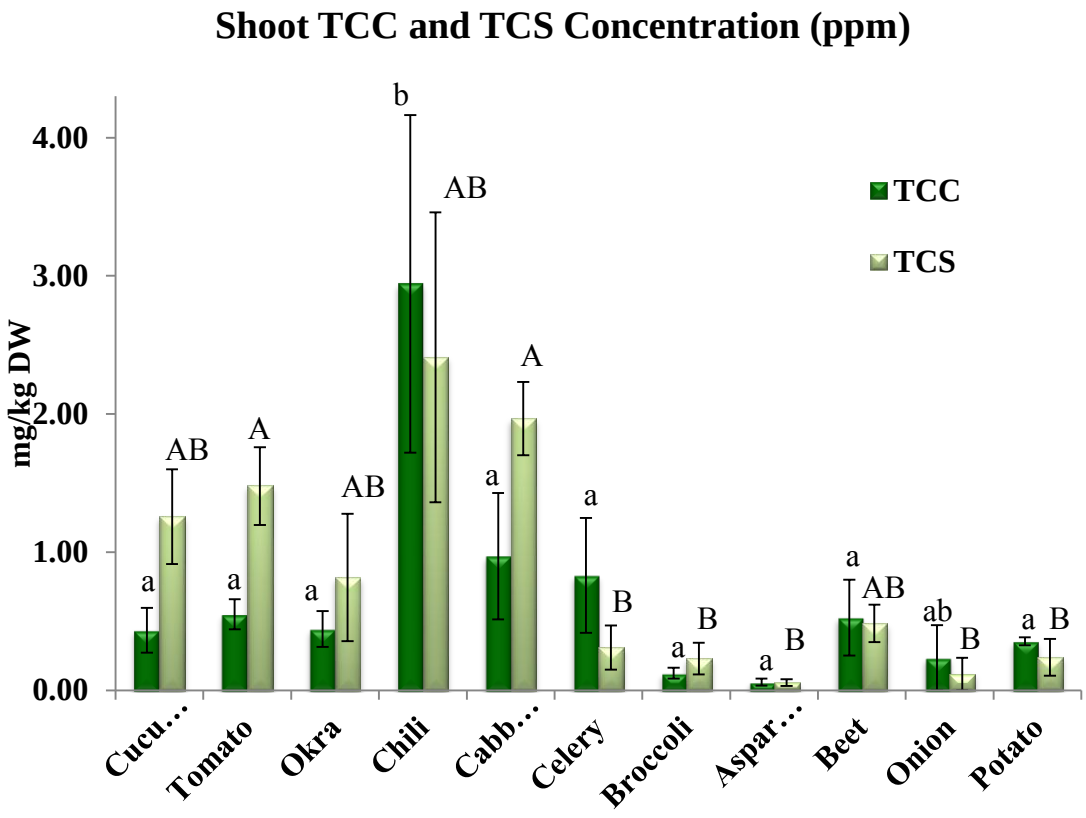


FIGURE 9. Concentration of TCC and TCS in the shoot of different crops when treated with 500ppb of TCC and TCS for 4 weeks. Error bars indicate standard error.

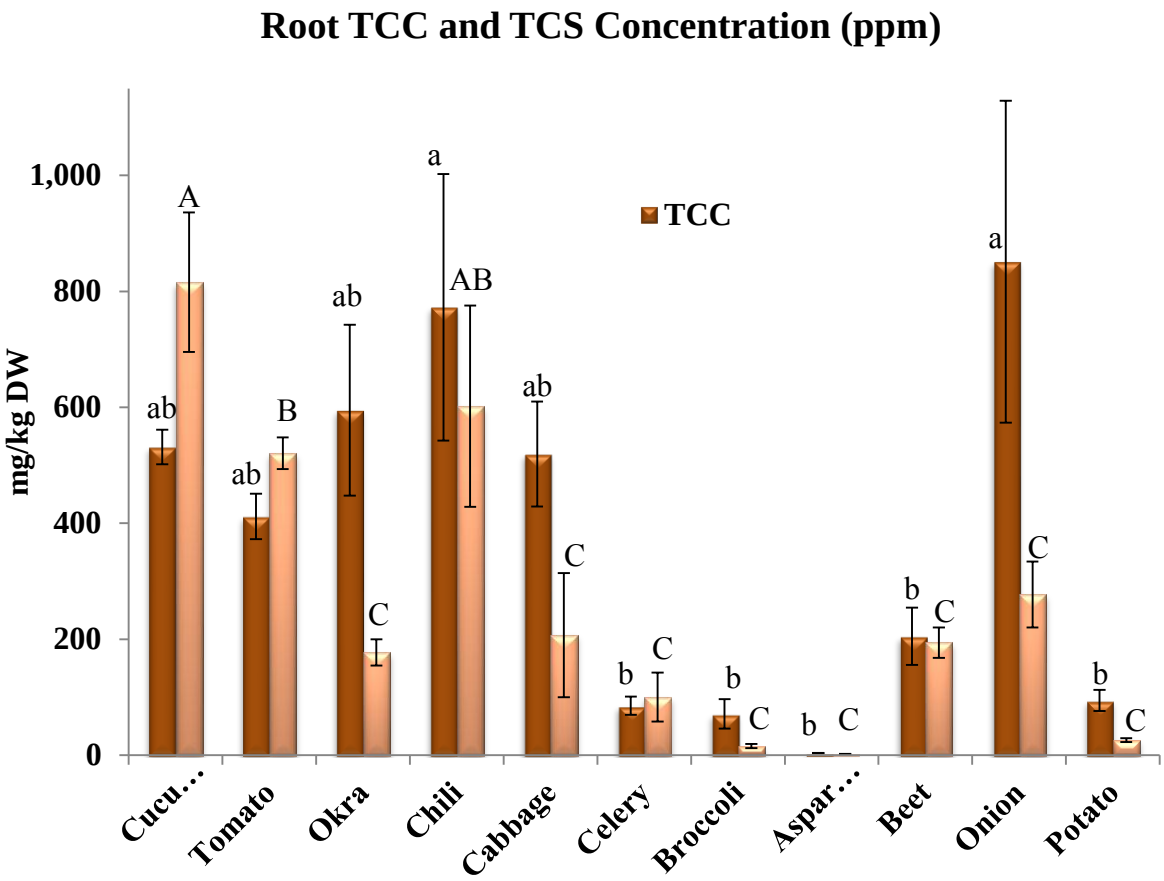


FIGURE 10. Concentration of TCC and TCS in the root of different crops when treated with 500ppb of TCC and TCS for 4 weeks. Error bars indicate standard error.

Table 3 and figure 11 both show the mass balances of TCS and TCC. Most of the plants had most of the TCC or TCS in either the solution or the "unaccounted for" portion, which means that it is either settled out of solution, in the gelatinous precipitate found in the bottles of media, degraded by light or microbes, or possibly metabolized by the plant (though more research needs to be done to determine if this is a possibility). In general TCS has higher percentages in the solution, while TCC has higher percentages unaccounted for. Since TCC is less soluble than TCS, the unaccounted for portion is likely in large part the portion of the chemical that has come out of solution.

The translocation factors (TF), ratio of the average concentration of TCC or TCS in the shoot to the corresponding concentration in the root, and the root concentration factors (RCF), the ratio of the concentration of TCC or TCS in the root to the final concentration in the hydroponic media, are shown in Table 2. A low TF indicates a low amount of TCC or TCS transported from the root to the shoot. The TF for TCC was less than that of TCS for every crop except celery and beet, implying that the translocation from the root to the shoot is more limited for TCC. There was no significant difference for the TFs for TCS of the different plants, but for TCC celery was significantly different from cucumber, potato, okra, tomato, and cabbage. The power for the test for TCC was 0.908, while the power for the test for TCS was 0.379. The low power may be due to some of the data sets having smaller sample sizes. No high correlation was found between TF and root or shoot concentration or TCC or TCS, even when potential outliers were removed. The TF found here are similar to, but somewhat smaller than, those found for pumpkin and zucchini (0.0013 for triclocarban, and 0.082 for triclosan) (Aryal 2010), but the smaller numbers are not surprising since Aryal kept plants in the hydroponic system for two months, while in this study the plants were only in the hydroponic system for one month. Higher RCFs indicate a higher potential for accumulating TCC or TCS. Aryal (2010) found RCFs of TCS for pumpkin and zucchini that were much smaller than those found in this study. Chili had the highest RCF for TCS and onion had the highest RCF for TCC.

Judging by the concentrations of the antimicrobials in the roots of the crops and the RCF, it would seem that onion is best at taking up triclocarban, while chili appears to take up the most triclosan. Chili and cucumber seem to have high potential to take up both chemicals. Celery, broccoli, and especially asparagus appear to have the lowest concentrations and RCFs of both

chemicals. From the concentrations in shoots, it appears that chili took up the highest concentrations of triclosan and triclocarban.

TABLE 2. Translocation Factors and Root Concentration Factors for various crops

TCC and TCS Translocation Factor (TF) and Root Concentration Factor (RCF) in different crops											
TCC	Cucumber	Tomato	Cabbage	Okra	Chili	Celery	Beet	Onion	Broccoli	Asparagus	Potato
Shoot	0.44	0.55	0.97	0.44	2.94	0.86	0.53	0.24	0.12	0.059	0.36
SE	1.26	1.48	1.97	0.82	2.41	0.34	0.48	0.12	0.039	0.026	0.03
Root	531	411	519	594	772	85	205	851	71	23	94
SE	29	39	90	147	229	15	49	277	25	14	18
TF	0.00082	0.0014	0.0022	0.0012	0.0054	0.015	0.0024	0.00049	0.0035	0.015	0.0014
SE	0.0003	0.0003	0.00092	0.00048	0.0028	0.0075	0.0012	0.00046	0.0021	0.0064	0.00014
RCF	3288	2403	3233	6158	7573	576	1786	8610	674	38.73	1275
SE	407	203	551	1281	2395	178	431	2566	218	5.54	258
TCS	Cucumber	Tomato	Cabbage	Okra	Chili	Celery	Beet	Onion	Broccoli	Asparagus	Potato
Shoot	1.26	1.48	1.97	0.82	2.41	0.34	0.48	0.12	0.23	0.056	0.24
SE	0.34	0.28	0.27	0.46	1.05	0.09	0.13	0.12	0.11	0.024	0.13
Root	815	520	207	177	601	100	193	277	15	120	26
SE	120	27	106	22	173	42	26	57	3	24	3
TF	0.0016	0.0029	0.032	0.0042	0.0098	0.0053	0.0029	0.0012	0.032	0.037	0.0099
SE	0.0004	0.0006	0.013	0.0019	0.0079	0.0028	0.001	0.0012	0.025	0.017	0.0055
RCF	2576	1538	732	1353	4896	327	783	1892	96	15.49	304
SE	536	122	354	303	927	101	126	267	28	3.056	45

TABLE 3. Distribution of TCC and TCS after 4 weeks

	TCS				TCC			
Crop	Solution (%)	Plant (g)	Plant (%)	Unaccounted (%)	Solution (%)	Plant (g)	Plant (%)	Unaccounted (%)
Cucumber	69.02	3.80E-05	8.44	22.54	34.57	2.46E-05	5.47	59.96
Tomato	67.63	4.71E-05	10.46	21.91	33.65	3.71E-05	8.25	58.09
Cabbage	50.44	1.75E-05	3.89	45.67	32.96	4.06E-05	9.01	58.03
Okra	35.66	6.36E-05	14.13	50.21	18.81	2.08E-04	46.25	34.95
Chili	27.67	2.34E-05	5.19	67.14	21.26	3.00E-05	6.66	72.09
Beet	51.28	3.39E-05	7.53	41.18	23.30	3.59E-05	7.97	68.73
Celery	51.80	9.44E-05	20.97	27.22	31.31	8.21E-05	18.25	50.44
Onion	28.76	2.26E-05	5.02	66.22	19.44	4.90E-05	10.89	69.67
Broccoli	27.67	0.000005	1.15	71.19	20.53	0.000023	5.01	74.46
Asparagus	24.40	0.000009	2.52	73.08	19.95	0.000018	5.20	74.85
Control	48.00			52.00	24.00			76.00

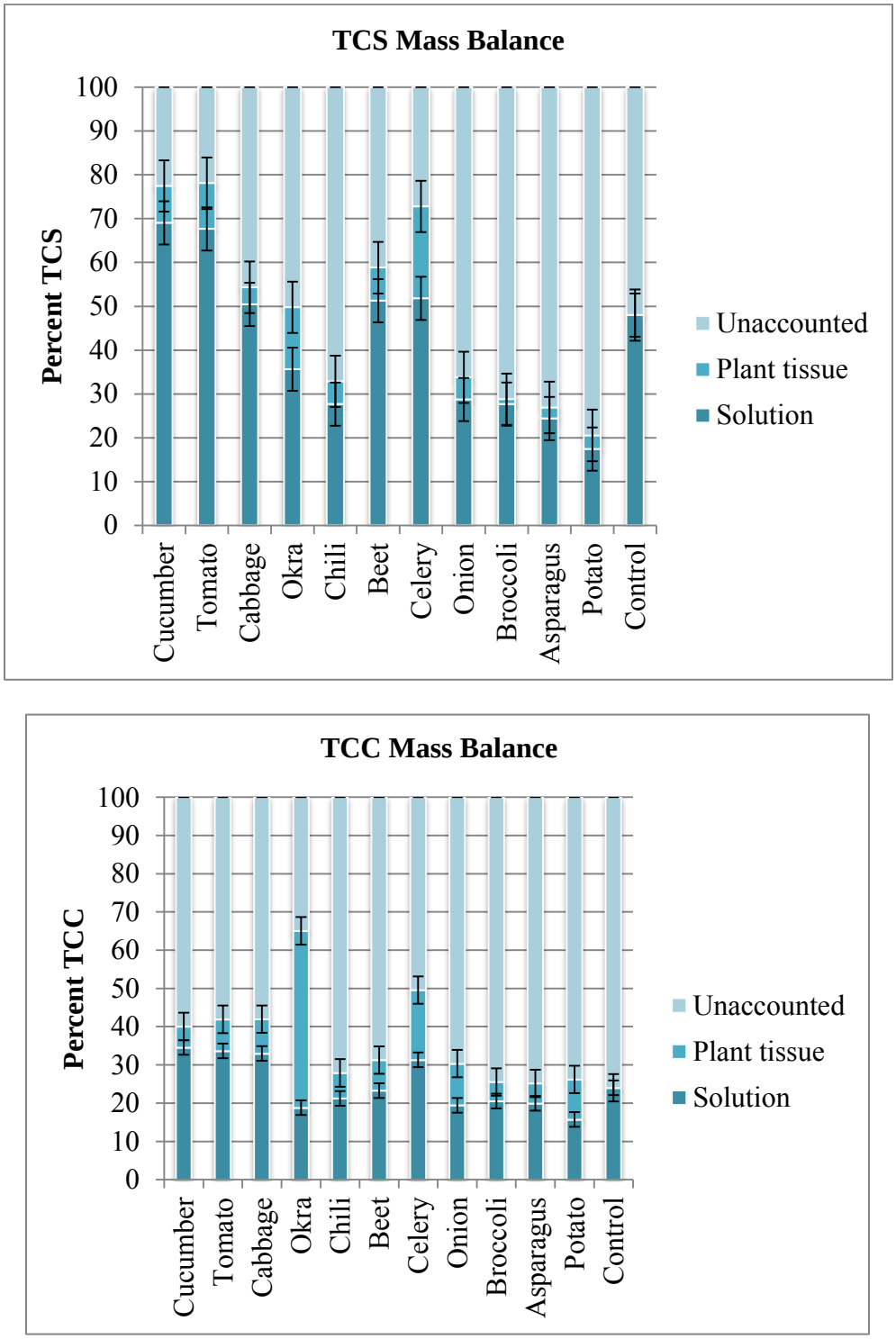


FIGURE 11. Mass balances for TCS and TCC. Error bars indicate standard error.

Table 4 and figure 12 show the data from the cucumber flowers, beet bulbs, onion bulbs, and potato tubers, including three layers of the tubers. There was no significant difference found between the miscellaneous parts of the different plants for TCC or TCS. However, the power of the test for TCS was only 0.35 and the power for the test for TCC was only 0.548, which is less than the desired power of 0.8. This may be due in part to the small sample size of some of the data sets. For instance, both the cucumber flowers and beet bulbs only had enough biomass to constitute one sample each. In general the flowers and bulbs had higher concentrations than the shoots, but still much lower concentrations that those in the roots. The exception is the potato tubers, whose concentrations were comparable to the shoot concentrations. With both onion bulbs and potato tubers, effort was taken to make sure that the bulb or tuber was not submerged in the media, though the onions were smaller and more prone to the bottom dipping in the media. Both roots and bulbs or tubers were washed before analysis in order to remove as much surface TCC and TCS as possible, but that might still be a factor in the higher concentrations. The potato tubers were large enough to separate into layers. There was the peel, a middle section of the tuber, and the core. The core had the highest concentration of the different layers. The peel had one replicate out of five with very high TCC and TCS, but it was found to be a statistical outlier and not included when making calculations.

TABLE 4. Bulb and flower TCC and TCS concentrations

Crop	Bulb or Flower TCS (ppm)	SE	Bulb or Flower TCC (ppm)	SE
Cucumber	3.80	--	1.89	--
Beet	4.81	--	5.31	--
Onion	16.37	9.75	25.60	13.16
Potato tuber peel	0.10	0.01	0.10	0.02
Potato tuber middle	0.05	0.01	0.10	0.01
Potato tuber core	0.32	0.13	0.24	0.05
Potato tuber total	0.14	0.05	0.11	0.01

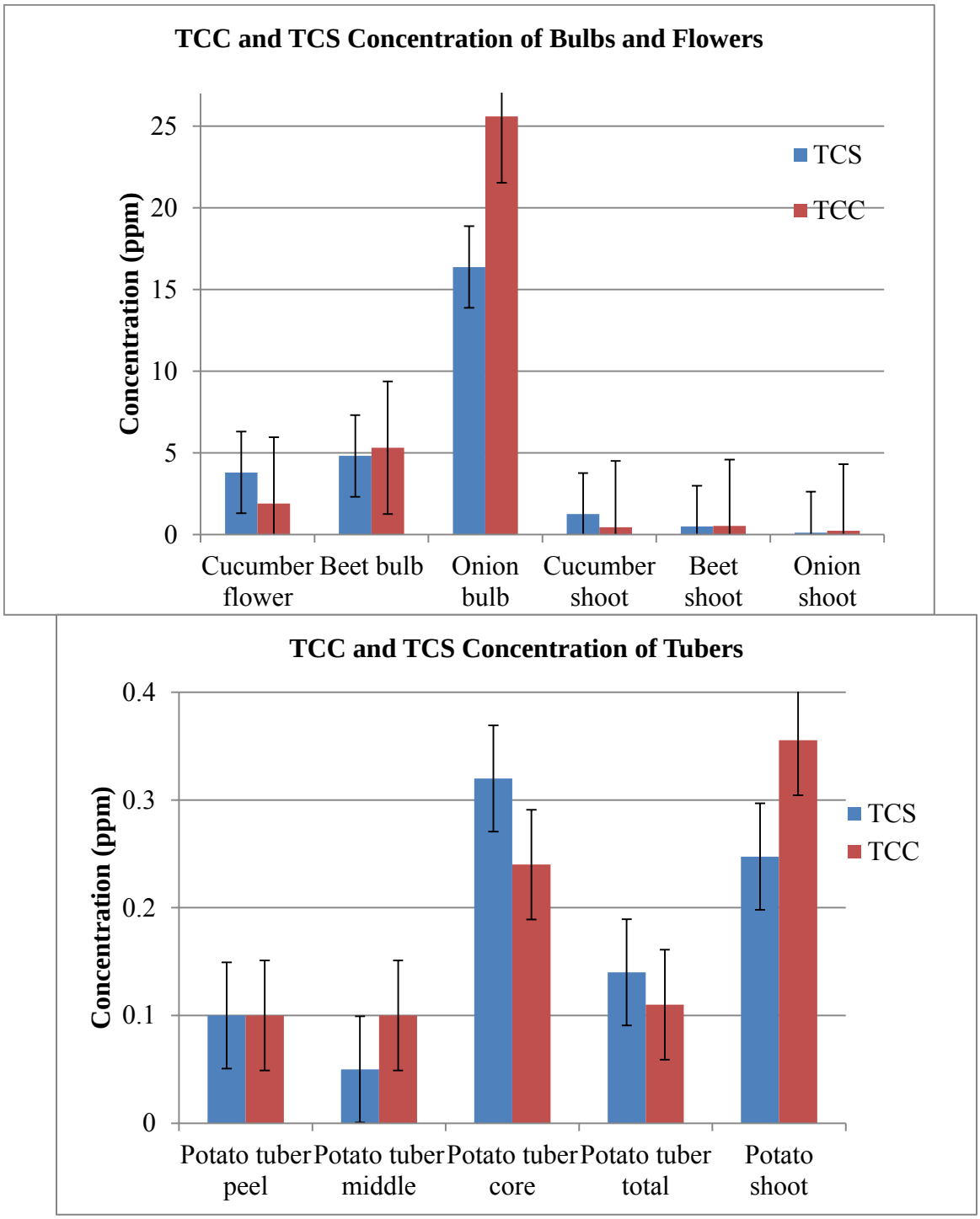


FIGURE 12. Concentrations of TCC and TCS in cucumber flowers, beet bulbs, onion bulbs, and potato tubers. Error bars indicate standard error.

Moisture content of the crops ranged from 35.2-96.3%. Measured biomass (dry weight) for the roots and shoots was highest for celery. The lowest shoot biomass was in tomato, and the

lowest root biomass was in onion (not including the weight of the bulb). When the root or shoot biomass for each crop was graphed against the corresponding antimicrobial concentration, there was not a very strong linear correlation in any case ($R^2 < 0.5$), indicating that the different concentrations are likely not merely due to larger plants having more tissue to dilute the antimicrobials. There was also no correlation over 0.6 between TCS and TCC concentration in either the shoot or the root. The fresh weights of the plants were also compared to the antimicrobial concentrations, and also had no strong correlations. Moisture content also had no strong correlation with antimicrobial concentration. When transpiration was graphed against TCC and TCS concentration, there was no strong correlation, as well as when the transpiration as graphed against the total mass of TCS and TCC found in the plant tissue. When the final pH was graphed against antimicrobial concentration in the roots and shoots of the plants, the highest R^2 value was 0.59 from shoot TCC concentration with a statistical outlier removed. With the outlier included, the R^2 value was only 0.19, and the next highest was only 0.37. Therefore, there is likely not a significant effect on TCC or TCS from pH.

TABLE 5. Lipid content and triclosan and triclocarban in multiple crops

Plant	Lipid % DW	Reference	TCC (ppm)	TCS (ppm)
Cucumber root	2.7	(Murano and others 2009; Murano and others 2010)	531.41	815.73
Cucumber leaves	0.7	(Bulder and others 1989)	0.44	1.26
Tomato root	0.12	(Gonzalez and others 2003)	411.89	520.80
Tomato stem	0.7	(Gonzalez and others 2003)	0.55	1.48
Cabbage root	0.68	(Gao and others 2005)	519.43	207.03
Cabbage leaves	0.43	(Gao and others 2005)	0.97	1.97
Okra root	1.7	(Gopalakrishnan and others 1982)	594.98	177.09
Okra stem and leaf (av)	2.9	(Gopalakrishnan and others 1982)	0.44	0.82
Pepper root	4.4	(Lyons and Lippert 1966)	772.60	601.80
Celery root	1.45	(Chiou and others 2001)	72.01	84.56
Celery leaves	1.3	(Buttkus 1978)	0.86	0.34
onion root	2.7	(Cooper and Losel 1978)	851.02	277.12
onion bulb	1	(RodrÃ-guez GaldÃ³n and others 2009)	25.60	16.37
onion leaves	0.77	(Novitskaya and others 2006)	0.24	0.12

Lipid content of plants was suggested as a possible factor in chemical accumulation in plant tissue. A literature search provided several numbers for lipid content for some of the plants tested in this study. Table 5 shows the lipid values found in literature and the TCC and TCS values found for those crops on this study. No correlation was found with lipid content and either TCC or TCS in shoots or roots. The highest R^2 value found was 0.36 (root TCC vs. lipid content). Lipid content is not likely to have a large factor in accumulation in plant tissue, at least not for the crops tested in this study.

Phytoextraction is the ability of plants to remove metals and other contaminants from soil and translocate them into above ground biomass (Robinson and others 2003). The phytoextraction (the ratio of shoot concentration to final media concentration) ranged from 0.69% (Asparagus for TCS) to 61.03% (Chili for TCS). Only half of the phytoextraction values were over 5%, and only 3 values are over 10%. Those values are the chili TCC and TCS values (28.63% and 61.03%, respectively) and onion TCC (12.02%). Broccoli and asparagus had the lowest values. A study with curcurbits and a different organic contaminant, *p*,*p*'-DDE, found phytoextraction values ranging from 0.023% to 0.78% (White and others 2003). However, the plants were grown in contaminated soil rather than hydroponic media, so it is not surprising that the phytoextraction would be so much lower in a soil study since less of the chemicals would be bioavailable. Aryal (2010) found values of 0.0014 for TCC and 0.024 for TCS in the hydroponic study, and 15.87 for TCC and 3.72 for TCS in the soil column study

The transpiration stream concentration factor (TSCF) is the mass of chemical in the shoot divided by the product of the total transpiration and the average of the initial and final media concentration (H. Zhang 2009). It represents the ratio of concentration in the sap and the nutrient media and can be used to quantify how easily chemicals can be taken up and translocated in a plant through its transpiration stream. Figure 13 shows the TSCFs for both TCS and TCC. Onion had by far the highest TSCF for both chemicals. Potato, okra, and cabbage also had high values for both chemicals. Chili had a high value for TCC, but a mid-range value for TCS. Asparagus had the lowest TSCFs for both TCC and TCS, followed closely by broccoli and tomato. Celery had a low value for TCS, but a mid-range value for TCC.. The high values indicate the plants such as onion use mainly transpiration to uptake TCC and TCS, whereas plants with lower values likely use another mechanism more than transpiration.

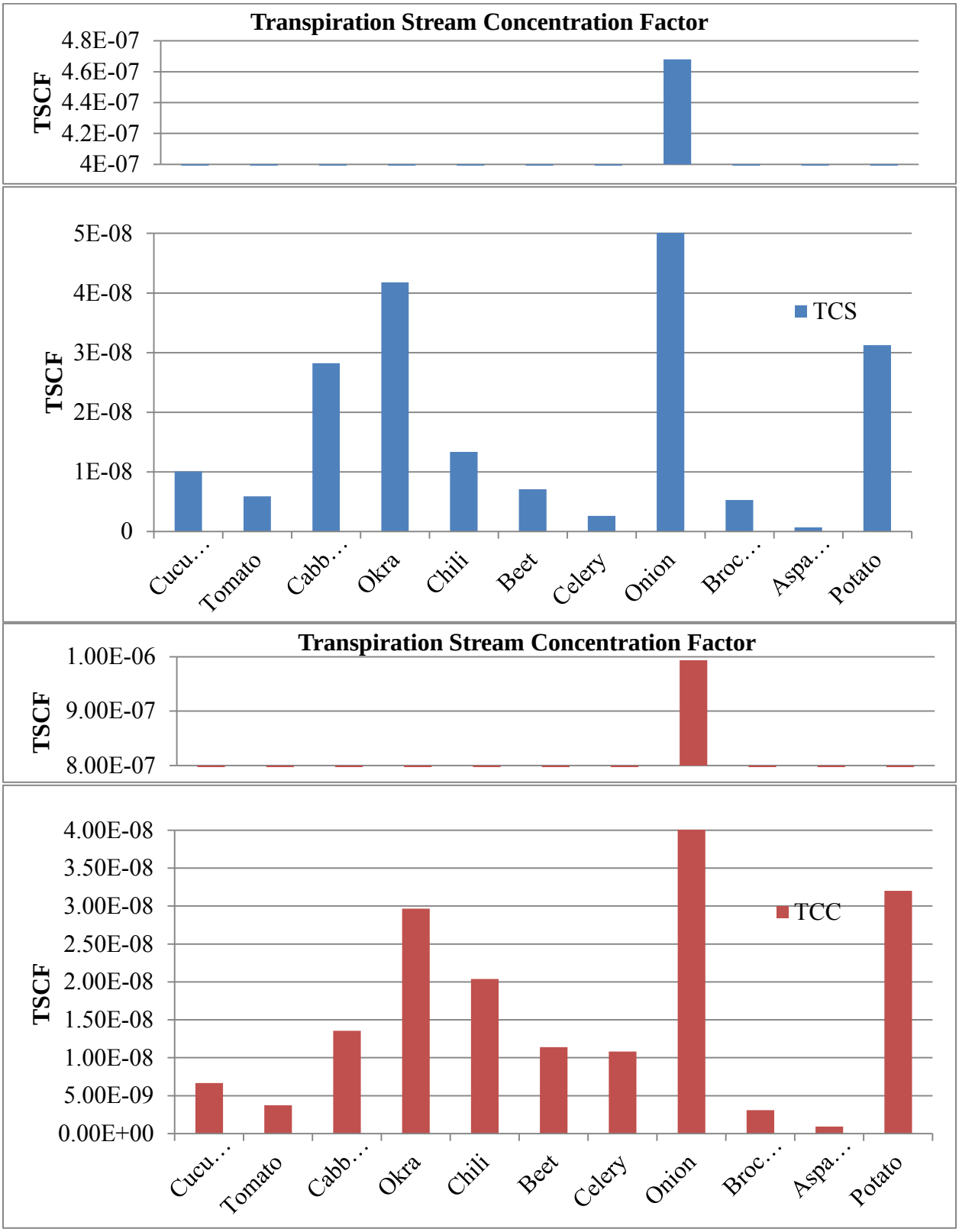


FIGURE 13. Transpiration Stream Concentration Factors for TCS and TCC. Error bars indicate standard error.

4.3 Toxicity of TCC and TCS

Table 6 shows the observations made on toxicity effects from the antimicrobials on the plants compared to the plants grown in the control solution (not containing TCC or TCS). In this study several plants had negative effects at 500ppb TCS when treated for 1 month. With okra and onion, the plant in the control media also had negative effects, presumably due to the methanol in the control media. Therefore, for those two plants the toxic effects may be due to the methanol instead of the TCC or TCS. Figure 14 shows data on cabbage toxicity as an example. The graph shows the number of dried and healthy leaves on both the control plant and the treated plants. For cabbage the control had all healthy leaves, while most of the leaves on the treated plants dried out. Figure 15 shows the comparison between the control cabbage and two of the treated cabbage plants. The dry mass of the treated cabbage shoots ranged from 0.25 to 1.34, indicating a range of health conditions. This range in health conditions possibly brought on by

TABLE 6. Observations on toxicity effects of TCC and TCS on plants compared to control plants.

Plant	Treated with TCC/TCS	Control
Cucumber	Yellowing of leaf margins in older leaves. New leaves have yellow veins	No negative effect
Cabbage	Yellowing and drying of older leaves Slower new leaf formation	No negative effect
Okra	Yellowing of leaf margins in older leaves. New leaves have yellow veins Blotching Leaf fall	Yellowing of leaf margins in older leaves. New leaves have yellow veins Blotching Leaf fall
Onion	Drying of older leaves after 2 weeks	Drying of older leaves after 2 weeks

the toxicity of the chemicals could lead to a decrease in uptake. For instance, the chili plants, some of which had a pest problem, had the widest variability in its data. Some of this variability may have been caused by the health conditions of the plants, though more testing would need to be done to make any solid conclusions.

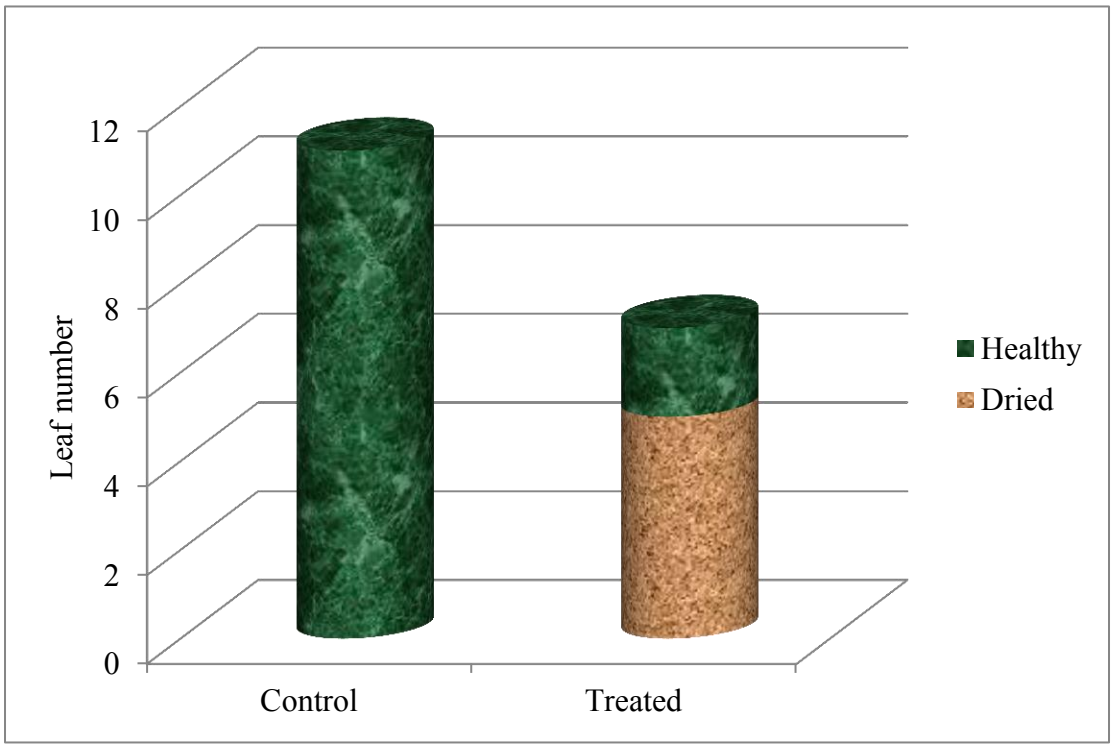


FIGURE 14. Cabbage toxicity comparison between the control and treated plants as shown by the number of healthy and dried leaves

FIGURE 15. Pictures of cabbage control (left) and two treated cabbage plants (middle and right)

4.4 Potential Exposure to antimicrobials

Phytoremediation is a good way to remove contaminants from soil, but if the contaminants are too concentrated in edible portions, food crops cannot be used for phytoremediation. Furthermore, if contaminants are concentrated enough in edible portions of food crops, the use of biosolids may need to be reconsidered. Since the hydroponic study represents a scenario of maximum bioavailability of the chemicals, it can be considered a "worst-case scenario" of the accumulation of the chemicals by crops. Therefore, if there is no health hazard from the amounts of chemicals taken up in the hydroponic study, there is not likely to be a health hazard from the use of biosolids on agricultural fields. The value of no observable adverse effect level (NOAEL) for TCS is 30 mg/kg (ppm) and the lowest observed adverse effect level (LOAEL) is 100 mg/kg (ppm) (USEPA 2008). The NOAEL for TCC is 25 mg/kg (ppm) and the LOAEL is 75 mg/kg (ppm) (USEPA 2002). However, those values do not consider the possibility of endocrine disruption, which recent studies show could be a potential risk for TCS and TCC (Chen and others 2008; USEPA 2010; USFDA 2010). Figure 16 shows the exposure in ng TCC and TCS/kg crop/day based on consumption data from the Exposure Factors Handbook from the National Center for Environmental Assessment (Assessment 2011) (pumpkin and zucchini data came from Aryal and Reinhold, 2011). The exposure was calculated by multiplying the concentration in the edible portion of the plant (shoot, root, or bulb) by the average consumption by each age group by the average dry weight content of the crop. Note that TCC has a much lower scale than TCS. Figure 16 shows that infants are exposed to higher concentrations of both TCC and TCS. It also shows that the highest exposure to TCS comes from zucchini and pumpkin, while the highest exposure to TCC comes from cabbage, potato, and

chili pepper. Figure 17 shows the estimated exposure from the mean of the crops compared to other routes of exposure. The estimated exposure from vegetables is 10^3 times greater than that from drinking water and $10^{0.5}$ to 10^2 times less than that from product use, which refers to using products containing TCC or TCS. The total estimated exposure is $10^{2.9}$ to $10^{3.3}$ times less than the acute NOAEL. The total exposure from water, product use, and exposure from vegetables (as estimated by this study) is still three orders of magnitude less than the NOAEL.

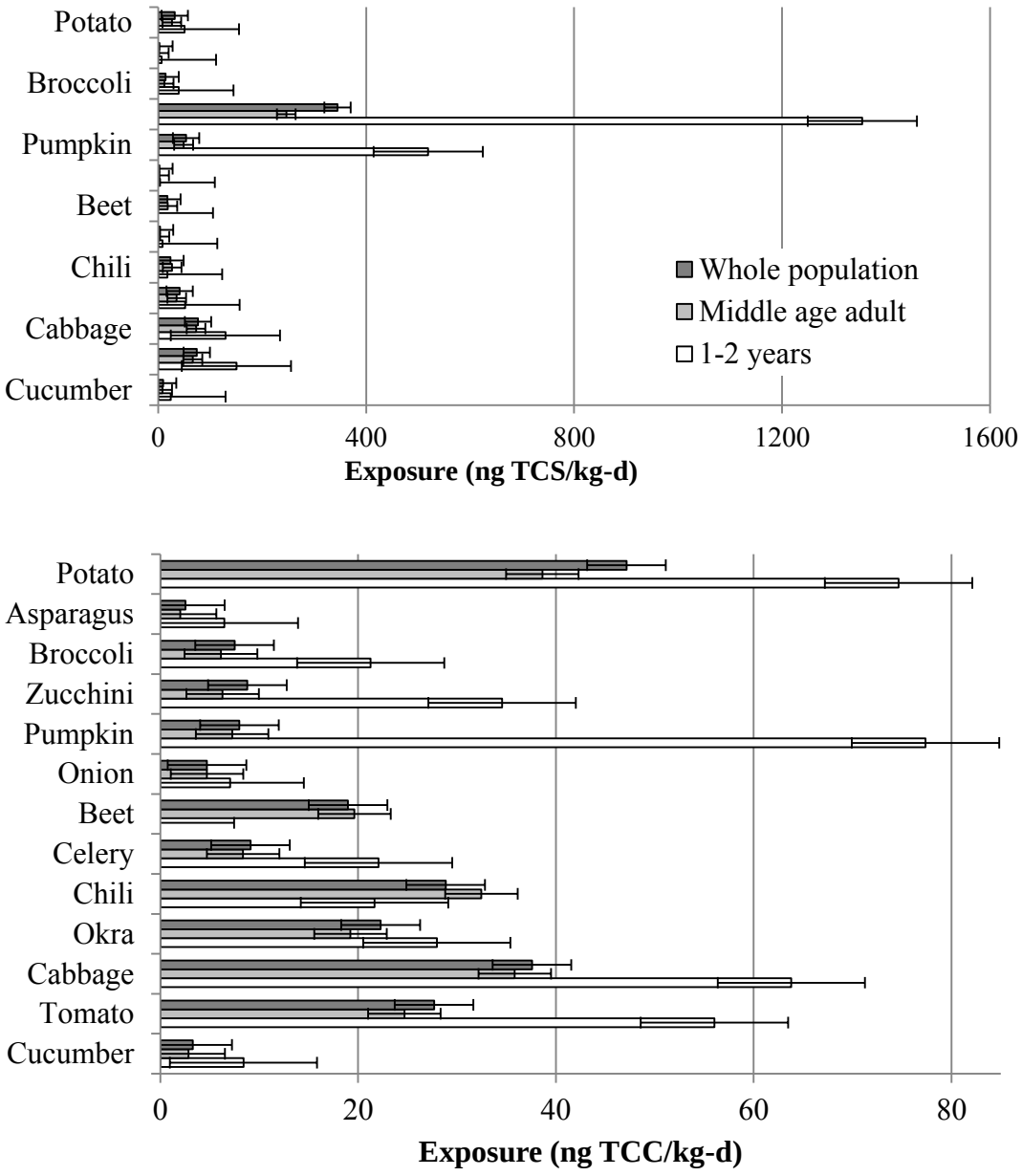


FIGURE 16. Potential exposure to antimicrobials from food crop consumption

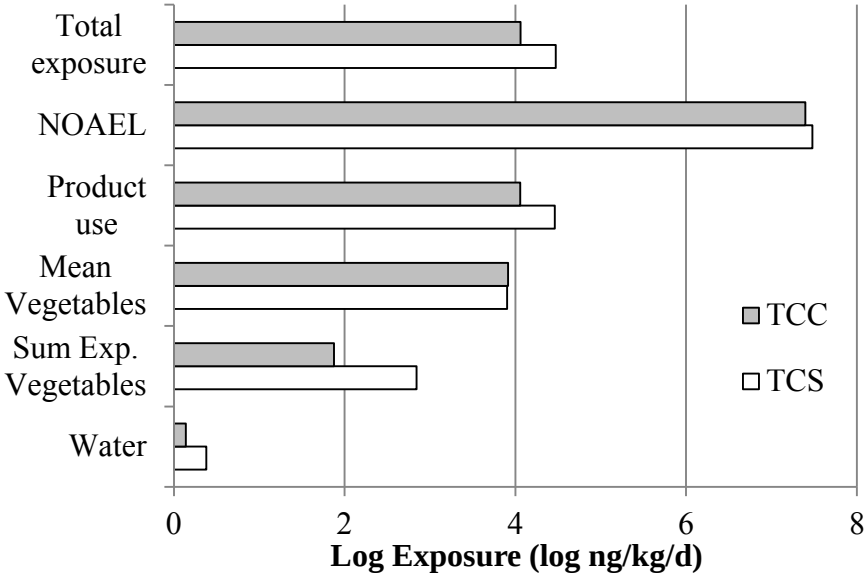


FIGURE 17. Potential exposure from crops compared to other routes

CHAPTER 5: CONCLUSIONS

5.1 Conclusions

This study found that the eleven plants tested took up TCC and TCS after one month of exposure in a hydroponic system. In all crops tested the concentration of the chemicals in the roots were much larger (two or three orders of magnitude) than the concentrations in the shoots. The translocation factors were slightly lower than those found in pumpkin and zucchini in previous research, which is likely due to the shorter exposure time. Chili and okra seem to have high potential to take up both antimicrobials because of their high RCFs. Onion had a high RCF for TCC. Celery, broccoli, and asparagus had the lowest RCFs for both TCC and TCS. Since more TCC and TCS was removed from the media than was found in the plant tissue, plant uptake was not the only mechanism responsible for removing these chemicals from solution, though more testing is needed to determine what exactly caused the TCC and TCS to be removed from the solution. TCC and TCS were found in precipitate collected from the bottom of the bottle after the beet experiment, though it is uncertain if that is from settling, microbial activities, or attachment to particulates. Based on the crops studied in this project, exposure to TCC and TCS from consumption of plants exposed to contaminants would not pose a health risk, since the sum of exposure from all the crops studied is less than estimated exposure from use of products containing TCC and TCS. The total exposure from estimated water exposure, product use exposure, and vegetable consumption exposure is still three orders of magnitude less than the reported NOAEL. However, this is not considering recent findings that indicate that TCC and TCS could both be endocrine disrupting chemicals. This study furthers research that shows that crops can uptake organic contaminants found in soil, which aides in governmental reviews of such chemicals.

5.2 Future Research

The next phase in this research involves testing crops that took up the most TCC and TCS in the hydroponic study in a soil system to have a better understanding of the real-world system. There will also be more investigation into what mechanisms are used to take up TCC and TCS and whether they are passive or active. For that, the transpiration stream and enzyme activity will need to be closely monitored during the exposure period. Furthermore, it would be beneficial to test even more types of crops. Originally this study was going to include fruits and berries in order to include several types of food crops, but the varieties selected did not thrive in a laboratory environment and therefore were not used. Comparing fruits and vegetables would be an interesting and more complete look into both phytoremediation possibilities as well as exposure potential.

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