Potential Uptake and Distribution of Relatively Water Soluble Organic Contaminants by *Acorus gramenius* and *Canna hybrida* 'Orange Punch'

Abstract

Organic contaminants are often detected in wastewater treatment effluent and surface waters receiving wastewater and reclaimed water. Conventional remediation techniques are often impractical for non-point source contaminants and for compounds with no water quality regulations. One possible remediation technique under evaluation is the use of floating islands established with ornamental wetland plants. To investigate the potential of this phytoremediation technique, mass balance studies were conducted to characterize the uptake of 14C-labelled soluble organic contaminants by ornamental wetland plants. Acetaminophen and sulfamethoxazole, two anthropogenic compounds often detected in surface waters globally were used as surrogates to determine uptake potential of *Acorus gramenius* (Japanese Sweetflag) and *Canna hybrida* 'Orange Punch'. Observed reductions of each contaminant in hydroponic solutions over a 14 day exposure period were 60 percent (sulfamethoxazole) and 100 percent (acetaminophen) for *A. gramenius*, and 40 percent (sulfamethoxazole) and 84 percent (acetaminophen) for *C. hybrida*, indicating these two wetland plants be useful candidates for removal of soluble organic contaminants ($\log k_{ow}<1$).

Introduction

Surface water ecosystems, especially in unconfined aquifer settings are susceptible to contamination by biologically active organic wastewater compounds through recharge from water sources contaminated by septic systems (Standley et al., 2008). Standley et al. (2008) found that the detection frequency and concentrations of organic wastewater contaminants

increased as the density of housing increased. Organic contaminants they studied included hormones androstenedione, estrone, and progesterone and the pharmaceuticals carbamazepine, pentoxifylline, sulfamethoxazole, and trimethoprim. Yang et al., (2015) found organic contaminants such as the pharmaceutical acetaminophen in sediments from a river bordering an urban watershed.

Organic contaminants are carbon-based chemicals that are found in environments where they should not be present. Organic contaminants of emerging concern (CECs) are not thoroughly understood in terms of environmental fate and human and environmental toxicity due to their relatively recent introduction/interests. However, the frequency of detection of CECs in the environment warrants concern. CECs may enter directly or indirectly into the environment through various pathways, including excretion following human use, flushing of expired/unused personal care products/pharmaceuticals down sinks and toilets, landscape applications of pesticides, and leakage/leaching from landfills and septic tanks.

CEC's are poorly understood because of the lack of information on their fate and potential effects to non-target organisms, including humans. Some contaminants need not be persistent to have an effect on ecosystems since their continuous introduction into the environment overshadows their removal rates (Petrovic, 2003; Richards and Cole, 2006). Pharmaceuticals and their metabolites have been detected in surface waters (Ternes, 1998; Sacher et al., 2001; Kolpin et al., 2002; Benotti et al., 2009; Bruce et al., 2010; Fram and Belitz, 2011). Most toxicity exhibited by CECs appears to be chronic rather than acute (Petrovic, 2007) and some tend to bioaccumulate in the food chain (Yang et al., 2015). However, knowledge gaps exist in terms of assessing risks associated with long-term exposure to low concentrations of pharmaceuticals and the combined effects of mixtures of pharmaceuticals (WHO, 2011).

The chemical and physical properties of organic contaminants can greatly affect their fate in the environment (Petrovic, 2007). While tempting to correlate penetration of pharmaceuticals through cell membranes with logk_{ow} relationships, these relationships may not always explain uptake since many pharmaceuticals have both lipophilic and hydrophilic characteristics (Williams, 2005). Chemicals that are soluble in water (or fractions of chemicals with limited water solubility) tend to move with water in different environmental matrices (e.g. ground water, surface water) (Chin, 2013). Organic contaminants of particular concern are acetaminophen (also known as paracetamol) and sulfamethoxazole. These pharmaceuticals are among the most highly manufactured and used compounds in the world (Kim et al., 2007; Petrovic et al., 2007; Aris et al., 2014). Acetaminophen is the analgesic active ingredient in many over-the-counter pain relievers. It has a water solubility of 14000 mg/L (Yalkowsky and Dannenfelser, 1992) and log K_{ow} of 0.46 (Sangster, 1994), indicating a relatively high affinity for water (Table 1). Sulfamethoxazole is the active ingredient in several antibiotics available by prescription. The water solubility and LogK_{ow} for sulfamethoxazole are 610 mg/L (Yalkowsky and Dannenfelser, 1992) and 0.89 (Hansch, 1995), respectively (Table 1). These values indicate a high propensity of the two compounds to remain associated with water.

Acetaminophen and sulfamethoxazole have also been detected frequently in surface and ground water (Standley et al., 2008; Yang et al., 2015; Kumar and Xagoraki, 2010; Kolpin, 2002) reported that 24 percent of surface water samples in the United States contained concentrations of over 10,000 ng/L acetaminophen (Kolpin, 2002). This analysis included over 100 samples collected from 139 streams. Acetaminophen concentrations ranged from the nanogram to microgram range, with median, minimum and maximum concentrations of 110 ng/L, 5 ng/L , and 10,000 ng/L, respectively (Kolpin 2002). Kumar and Xagoraki (2010) reported that

acetaminophen ranks 40th in occurrence in U.S. stream waters and sulfamethoxazole ranks 24th. However, in the US, NDC (National Drug Code) (2005) reported acetaminophen's rank of use as 42, with a maximum detected concentration of 10000 ng/L and sulfamethoxazole's as 119 with a maximum detected concentration of 52000 ng/L (Cahill et al., 2004). In comparison, the median concentration of sulfamethoxazole in France in surface waters was 25 ng/L and maximum 133 ng/L; and in Germany the median and maximum concentrations were 30 and 480 ng/L, respectively (Ternes et al., 2005).

Sulfamethoxazole has also been detected in surface water bodies, reclaimed water, and ground water. Oppenheimer et al. (2011) detected sulfamethoxazole at concentrations of up to 450 ng/L in drinking water source waters that receive wastewater discharges. Oppenheimer et al., 2011 detected sulfamethoxazole in 55 percent of sampled surface waters at concentrations ranging from 17 to 990 ng/L. Hughes et al. (2013) conducted a literature analysis to compare global concentrations of CECs. For the analysis, they considered surface waters that were sampled in Florida, Texas, northern and southern California, Illinois, and Michigan, Colorado, Ohio, and New York. They reported the median and maximum concentrations of sulfamethoxazole were 83 ng/L and 11920 ng/L, respectively. The median and maximum concentrations of acetaminophen were reported to be 148.2 ng/L and 15700 ng/L, respectively. One of their most important findings, was that reported environmental concentrations were within the range known to cause acute or chronic toxicity in aquatic ecosystems (Hughes et al., 2013).

Both acetaminophen and sulfamethoxazole have been shown to exhibit toxicity to non-target aquatic organisms. Acetaminophen was found to be acutely toxic to zebra fish. Paracetamol (acetaminophen) concentrations of 19 mg/L were found to harm the fish cell line BF-2 (Henschel

et al., 1997; Stuer-Lauridsen). Acetaminophen acts by blocking the cyclooxygenase enzyme (the enzyme responsible for transforming arachidonic acid to prostaglandins). Prostaglandins are responsible for cell signalling and neurotransmitter release. Adverse effects of paracetamol are mainly due to formation of hepatotoxic metabolites, primarily *N*-acetyl-*p*-benzoquinone imine, synthesized when the availability of glutathione is diminished in liver cells. Richards and Cole (2006) determined toxicity using the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) in which *Xenopus* blastulae were exposed for up to 96 hours to single concentrations of commonly detected pharmaceuticals, including acetaminophen and sulfamethoxazole. They reported no significant differences in lethality at concentrations up to 100 mg/L as compared to controls. However, all tadpoles were malformed (i.e. tail flexures, edemas, gut miscoiling) when exposed to the highest concentration (100 mg/L) (Richards and Cole, 2006).

Sulfamethoxazole has also shown toxic properties to non-target aquatic organisms. Ferrari et al. (2004) reported a "no risk concentration" for sulfamethoxazole of 10 ng/L for algae and water fleas, but sulfamethoxazole concentrations as high as 12 ng/L have been found in drinking water sources (Benotti et al., 2009). The bioavailability of sulfamethoxazole is clear from its detection in fish muscle and liver at levels as high as 80 ng/L (Ramirez et al., 2006). Hazard quotients indicate the potential ecological risk of an environmental stressor, and are estimated by dividing the PEC (predicted environmental concentration) by PNEC (predicted no effect concentration. The PNEC indicates the concentration at which no toxic effects occur, and it can be determined with acute or chronic toxicity tests(Huang et al., 2010) . When the hazard quotient value is greater than unity, adverse environmental effects may be anticipated. For sulfamethoxazole, Ferrari et al. (2004) reported hazard quotients of 11.4 in France and 59.3 in Germany, while Kim

et al. (2007) calculated a hazard quotient of 6.3. However, for acetaminophen, both literature indicating a hazard quotient less than unity (Kolpin et al., 2004; Bound and Voulvoulis, 2006) and that indicating higher than unity exists. Stuer-Lauridsen et al. (2000) derived a value of 7.1, whereas Kim et al (2007) calculated a value of 1.8. Kim et al. (2007) reported hazard quotients greater than unity for acetaminophen and sulfamethoxamzole, indicating potential ecological risks and the need for further investigation. In addition to parent acetaminophen and sulfamethoxazole, metabolites may also present ecological risks. Investigating the fate of acetaminophen in wastewater effluent, Bedner and MacCrehean (2006) reported that 27 percent of the original drug concentration transformed to N-acetyl-p-benzoquinone, a hepatotoxin produced during acetaminophen metabolism, and responsible for overdose deaths in humans (Bedner and MacCrehean, 2006). The ecotoxicity of this compound to aquatic resources is unknown, but caution should be exercised given the known toxic effects in humans. The sulfonamide antibiotic sulfamethoxazole is a bacteriostatic broad-spectrum antibiotic effective against gram positive and negative bacteria which is commonly used in human medicine (Baran et al., 2011). It affects bacteria by binding competitively to dihydropteroate synthetase, thereby inhibiting the conversion of para-aminobenzoate to dihydropteroate, the precursor of tetrahydrofolic acid which is essential for the synthesis of nucleic acids. Further, sulfonamides block the cross-membrane transport of glutamic acid, an essential component in folic acid synthesis (Baran et al., 2011). The acute bacterial toxicity of sulfamethoxazole is low, for example the EC50 of the marine bacterium Vibrio fischeri after short-term exposure is >395 nmol/L. The acute EC50 for the soil bacterium Arthrobacter globiformis is >500 000 nmol/L (Białk-Bielińska et al., 2011). The chronic toxicity towards microalgae ranges from 100 nmol/L to 9500 nmol/L (Białk-Bielińska et al., 2011; Eguchi et al., 2004; Ferrari et al., 2004; 139 Isidori

et al., 2005; Yang et al., 2008). Freshwater biofilm communities react to an exposure of 1.97 nmol/L sulfamethoxazole with changes of the transcriptional activities of genes involved in replication and transcription as well as genes related to structural elements of the cell envelope and outer membrane (Yergeau et al., 2010). In a follow-up experiment, photosynthesis-related transcripts were reduced (Yergeau et al., 2012).

Remediation of these non-traditional contaminants using conventional methods used for point sources of pollution is not be feasible on a large scale due to limited land space and economics (Arthur et al., 2005). Constructed wetlands are often used for remediation of nutrients and other pollutants from surface water with varying removal efficiencies (Zhang, 2014; White et al., 2013; Lynch et al., 2015; Tanner and Headley, 2011).). These systems provide for removal by sorption to sediments and plants, uptake into plants, and microbial communities associated with the plants and sediments (reference). Constructed wetlands are often used in areas where available land is not limited. Remediation using constructed wetlands is also not always practical due to necessary equipment, land, site-work, and costs (Arthur et al, 2005). One of the main drivers of remediation potential for constructed wetlands is vegetation and associated processes. Available research studies focused on removal of organic CECs using aquatic plant based systems are limited (Zhang, 2014). One challenge for phytoremediation of contaminants by uptake into emergent plants is that the contaminant must enter the plants through the roots, which grow within the sediments. This requires diffusion through the sediments to access the roots, which is unlikely to occur efficiently. Given their large space requirements, constructed wetlands are not a practical option for use within areas that have already been developed with hard structures, such as urban neighborhoods. Many of these developed areas have stormwater

retention ponds integrated within them. These water quality treatment systems are designed to reduce discharges of suspended materials and to reduce the flow of surface runoff water from their watersheds, and often have limited capabilities to remove other contaminants (Chin, 2013). Cost effective methods for adding ecosystem processes to existing retention ponds would be very useful for reducing exposures of aquatic resources to these non-traditional contaminants.

Lab based phytoremediation studies are necessary in order to characterize the potential uptake and sinks for contaminants within plants prior to evaluating their use in the field. Floating treatment wetlands may provide a cost-effective approach to removing contaminants from surface water. This type of wetland consists of a buoyant mat with holes to allow submersion of wetland plant roots in the water column (Lynch et al., 2015; White et al., 2013). The mat and possible inclusion of media in the mat provide surface area that may allow for biofilm formation (Lynch et al., 2015; White et al., 2013; Winston et al., 2013). As the mat is buouyant, it does not depend on the volume of the water body in which it is placed. Instead of having to enter sediments before entering the roots, contaminants in the water column have direct access to the plant roots suspended in the water column.

This study was developed to evaluate the potential uptake and distribution of acetaminophen and sulfamethoxazole by two ornamental wetland plant species (*Acorus gramineous* and *Canna hybrida* 'Orange Punch'). This information is needed to determine whether their phytoremediation by plant uptake may be feasible.

Log Kow denotes the ratio of an unionized compound's concentration between octanol and water (Reinhold et al, 2010; Zhang et al;, 2013). Acetaminophen and sulfamethoxazole were selected

as surrogate contaminants for CECs with a LogKow less than 1 (i.e. very water soluble). Table 1 summarizes chemical properties of two of the most common organic surface water contaminants we studied: acetaminophen and sulfamethoxazole.

Materials and Methods

Plant Selection

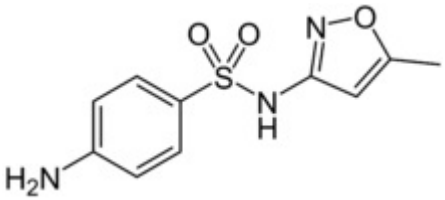
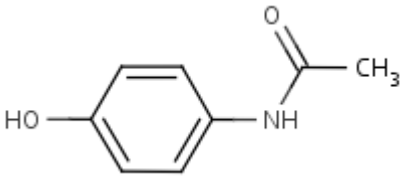
Many studies have quantified the uptake potential of organic contaminants such as pesticides by aquatic plants under hydroponic conditions. Wilson et al. (1999) reported 80% removal of radiolabeled simazine and 60% metalaxyl (2001) by *Canna hybrida* 'King Humbert'over a 7 day period. Other studies reported simazine and metalaxyl uptake by *Acorus gramenius, Myrophyllum aquaticum, Pontaderia cordata,* and *Typha latifolia* (65%, 32%) (Wilson et al., 2000 and 2001). Uptake was directly related to the cumulative volume of water transpired through the plants, and indirectly related to the water solubility and octanol:water partitioning coefficient for the pesticides. These plants are non-invasive and aesthetically pleasing, except for *T. latifolia,* making their use in urban and residential landscapes likely to be welcomed and encouraged. *A. gramenieus* and *C. hybrid* produced extensive and deep root systems in gravel-based hydroponic field studies (Wilson, 1999), making them ideal candidates for providing support to microbial communities and phytoextraction of contaminants from the water column. *A. gramenieus and C. hybrid* also have the desirable characteristics of being: 1) non-invasive, 2) robust (resistant to many pests), and 3) aesthetically pleasing ornamentals.

Plant Culturing

Acorus gramenius was purchased from Grandiflora Nursery (Gainesvile, FL) and *Canna hybrida* 'Orange Punch' was donated from Florida Aquatic Nurseries (Ft. Lauderdale, FL). One set of stock plants was located outdoors while another set is maintained within a temperature controlled

greenhouse for test-plant propagation. Stock plant rhizomes and tubers were divided using a knife for precise incisions and grown in Miracle-gro potting media (Scotts Miracle-gro, Marysville, OH). Approximately four weeks prior to start of experiments, newly propagated plants were removed from potting media in the greenhouse and transported to an indoor grow room. Roots were washed, and plants placed in 2 L containers filled with 20 percent Hoagland's nutrient solution provided with aeration. At least twice as many plants were transferred to hydroponics as needed for each experiment to ensure that uniform test plants (based on fresh weight, height/width, color, and general health conditions) were available for experiments. Upon placement in hydroponics, plants were transported to the grow room with a 12 hour photoperiod under a metal halide lamp, with relative humidity maintained between 60 to 70 percent, and temperatures between 25 to 30 °C.

Table 1. Summary of chemical properties for each target contaminant

Target compound	Structure	Chemical properties
Sulfamethoxazole		CAS number: 723-46-6 Formula: $C_{10}H_{11}N_3O_3S$ Mol. mass: 253.279 g/mol Water solubility (mg/L): 610 Log K_{ow} : 0.89
Acetaminophen		CAS number: 103-90-2 Formula: $C_8H_9NO_2$ Mol. mass: 151.16 g/mol Water solubility (mg/L): 14000 Log K_{ow} : 0.46

Contaminant Uptake and Distribution (Mass Balance)

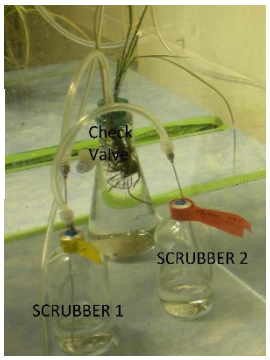
Lab studies using radiolabeled acetaminophen and sulfamethoxazole were conducted to accurately characterize uptake and distribution of contaminants within plants prior to evaluating their use in the field. For this study, mass balance chambers were constructed similar to that described by Anderson and Walton (1992). Chambers were constructed from acrylic with dimensions of 46x46x46 cm. Air flow through each chamber was adjusted to achieve 1.7 volumetric turnovers per hour, as Henderson et al. (2007) reported that was typical of natural systems. Air flow was achieved using a vacuum pump (Thermo Fisher Scientific, RAP Series, Thermo Fisher Scientific, NJ, USA.). A Flowmeter/regulator, connected between the vacuum pump and each mass balance chamber, maintained an airflow rate at 1.75 liters per minute. This flow rate amounted to one and half volumetric turnovers per hour. Each chamber was also equipped with carbon dioxide (500 mLs ethylene glycol) and volatile organic carbon (500 mLs 2N KOH) traps located between the chamber air-exit and the vacuum pump. Individual plants were sealed in the neck of 250 mL glass side-arm vacuum flasks using cored rubber stoppers and Qubitac (Qubit Systems Inc., Kingston, ON, Canada). Plants were positioned so that the roots were suspended in 260 mLs 20% Hoagland's nutrient solution spiked with 0.86 µCi acetaminophen (250 mCi/mmol) and 7.83 µCi sulfamethoxazole (77 mCi/mmol). Flasks were completely covered with aluminum foil to prevent potential photodegradation of the chemicals as well as potential growth of algae. The headspace within each flask was connected via tubing to a carbon dioxide trap (50 mLs 2N KOH), which was sequentially connected to a volatile organic carbon trap (50mLs ethylene glycol). Air within the headspace was pumped through a one-way valve on the side arm of each Erlenmeyer flask until the scrubbers began bubbling using an aquarium pump providing 1.3 L/min of air flow. Afterwards, deionized water was added to

replace water transpired and the replacement volume was recorded to keep track of cumulative transpiration (Initial water level in flask was marked on side of flask (260 mL). Placement of traps on the rhizospheres and gross mass balance chambers was necessary to account for CECs potentially mineralized to CO_2 or volatilized from the rhizosphere or shoots. Four mass-balance chambers were constructed to allow evaluation of uptake. At the beginning of each study, plants sealed in flasks were placed inside of the mass balance chambers, which was then sealed. Vacuum was started to provide air flow, and plants were left in the chamber for predetermined periods of time. Temperature and humidity inside each chamber was recorded daily.

Sampling Design

The four mass balance chambers were used to evaluate uptake and distribution over time. Three plants sealed in flasks containing radiolabeled CECs and two controls without CECs were placed in each mass balance chamber. After sealing the chambers and starting air flow, plants were allowed to grow for 2, 4, 8, or 14 d. Figure 1 illustrates an individual experimental unit. All individual plants in each respective mass balance chamber were harvested on the same day. Mass balance chamber (MBC) 1 was harvested on day 2, MBC 2 on day 4, MBC 3 on day 8, and MBC 4 on day 14. Day 0 was the day the plants were inserted into the flasks containing respective solutions. Figure 2 illustrates the mass balance chamber design used, isolating the plants from the surrounding environment and allowing measurement of mineralized and volatilized compounds in addition to uptake.

Figure 1: Individual experimental unit within mass balance chamber



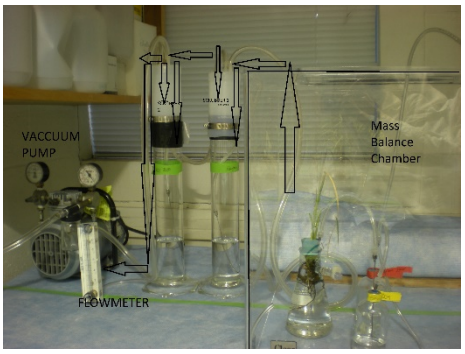


Figure 2: Arrows show direction of airflow through the system, from ambient air to mass balance chamber out to Scrubber 1, Scrubber 2, and then the flowmeter

At the time of harvest for each mass balance chamber, plants were removed for their respective flasks, roots were rinsed with deionized water, and entire plants were weighed. After weighing, plants were dissected into roots, stems, and leaves. Following dissection, individual plant parts (wrapped in Al foil) were oven-dried at 60 ^{o}C for 72 hours prior to combustion using a RJ Harvey OX-500 Biological Oxidizer (Tappan, NY, USA). The total ^{14}C-CO_2 released upon combustion was captured in R.J. Harvey ^{14}C trapping cocktail (Tappan, NY, USA) and was quantified using a Beckman LS 5801. The non-spiked controls were taken through the same procedure.

Analysis

Aliquots (0.2 mL) of solutions from the individual scrubbers (KOH and ethylene glycol) connected to the plants and the MBCs, as well as hydroponic solutions for each plant (n=5), were added to a scintillation vial, that was then filled to 15 mL with Scintiverse BD Cocktail (Fisher Scientific, Fair Lawn, NJ, USA). After storing samples in the dark overnight to reduce quenching, 14C activity in each was quantified using the Beckman LS 5801 liquid scintillation

counter programmed with a 5 min. detection window per vial. Results were output as disintegrations per minute (DPMs).

Scatterplots and regressions were used to investigate the relationships between both transpiration and uptake of chemical activity by plant, and transpiration and activity remaining in solution. ANOVAs were run for each contaminant- plant combination, using the transpiration volume values and activity remaining in solution. Acetaminophen-A. gramenius had a p value of 0.001129. The Regression statistics yielded an R value of 0.818651 and R squared value of 0.67. Acetaminophen-C. hybrid had a p value of 0.003389. The Regression Statistics yielded an R value of 0.72438 and R squared value of 0.52. Sulfamethoxazole-A. gramenius had a p, value of 0.055. The regression statistics yielded an R value of 0.5652 and an R squared value of 0.32. Sulfamethoxazole-C. hybrid had a p value of $9.9 * 10^{-6}$. The regression statistic yielded an R value of 0.8879 and an R squared value of 0.788.

Results

Acetaminophen

Concentrations in the exposure solutions decreased in the presence of the plants. Activity (DPMs) present in the solutions versus the cumulative volume transpired is shown in Figure 1a and b for each species. For acorus, concentrations in the solution decreased to below detectable levels after a cumulative transpiration volume of 11 mL. As activity in the solution decreased, activity in the plants increased proportionally (Fig. 1a). During the study, the highest cumulative transpiration volume was 16.3 mL . In contrast, acetaminophen concentrations in solution did not decrease to non-detectable levels with Canna (Figure 1). In this case, activity in the plants

also increased with cumulative transpiration volume. Mass balance recoveries for both plants ranged from 50-82% of the original activity applied, indicating significant uncertainty regarding the fate of 18-50% of the original activity. This unaccounted fraction may have resulted in inefficiencies in combustion, as well as mineralization in the rhizosphere. Based on later studies, it is likely that detectable quantities of 14C might have been detectable in the scrubbers had a larger volume of scrubber solution been used for the analysis. Given this limitation, a minimum of 65 and 20% of the original activity added was detectable in the roots of Acorus and Canna, respectively, following 14 d exposure.

Sulfamethoxazole

For acorus, solution concentrations decreased to 40 percent after a cumulative transpiration volume of 25 mL. An increased activity in plants was observed with increasing transpiration volumes. The highest transpiration volume observed for acorus was 29 mL. Despite the uncertainty of the fate of the original activity, a minimum of 5 to 13 percent of the original added activity was detected in the roots of acorus

Mass balance recoveries ranged from 65 to 82 percent for acorus, and similarly, 65 to 96 percent for canna. The maximum volume transpired by canna was 100 mL. Despite the uncertainty of the fate of the original activity, a minimum of 1 to 8 percent of the original activity was present in the roots of canna following a 14- day exposure.

Discussion

The chemicals behaved differently with respect to each plant. In the presence of acorus, acetaminophen concentrations in solution decreased to below detectable levels, whereas sulfamethoxazole concentrations were only reduced by approximately 60 percent. Interestingly, the majority of acetaminophen activity was found in the roots of the acorus throughout the studies. However, the sulfamethoxazole activity in the rhizome and leaves increased over the exposure period. Despite the similar water solubility, there were differences in reduction of the contaminants with acorus.

There were also differences between acorus and canna. On average, over the two week exposure period, canna reduced the sulfamethoxazole concentration by 40 percent and reduced acetaminophen concentrations by 84 percent. Similar, to the high removal of acetaminophen by both acorus and canna (100 percent and 84 percent respectively), Vanek et al. (2010) reported complete removal of acetaminophen by *Lupinus albus* with a starting concentration of 30 mg/L.

A high log Kow indicates high hydrophobicity of the compound. (Pilon-Smits 2005). Compounds with log Kow between 0.5 and 3.5 have high potential to accumulate in plant tissues (Briggs et al 1982).

Maharjan (2014) tested uptake of sulfamethoxazole by three plant species from a starting solution containing 100 ug/L. After a seven day exposure period, removal was negligible by *Azolla Caroliniana*, whereas 50 percent removal occurred with *L. Minor* and *P. Stratioles*. Conkle et al. (2008) reported complete removal of acetaminophen and 90 percent reduction of sulfamethoxazole in lagoons planted with *phragmites australis* in Louisiana, USA. Similarly,

Avila et al (2013) reported a 99 percent removal efficiency of acetaminophen using a constructed wetland planted with *phragmites australis*.

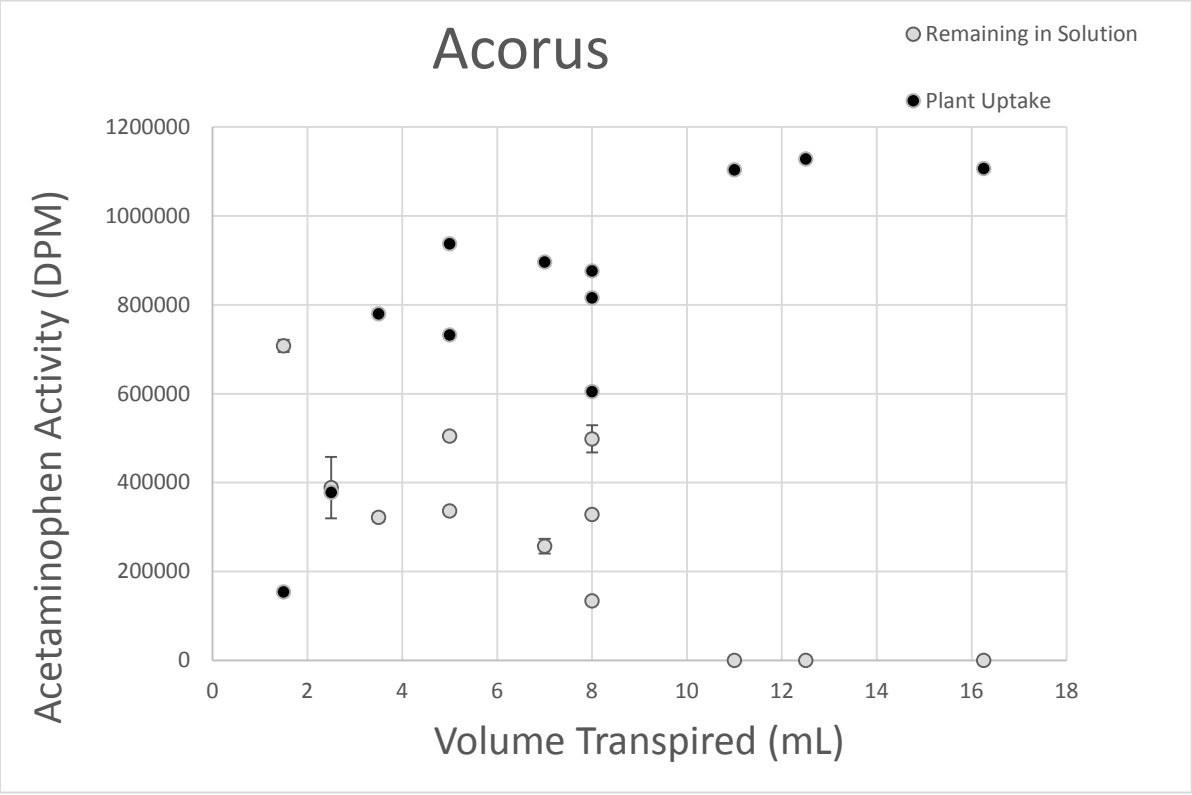
Zhang et al. (2013) reported removal efficiencies of caffeine ranging from 75 to 99 percent for mesocosms planted with Scirpus validus. Caffeine is very water soluble (2.16 E 4 mg/L) and also has a Log Kow less than 1 (-0.07) (Zhang et al., 2013).

Though the results obtained from this study showed similar removal efficiencies as those present in the literature, it is still unclear whether uptake of organic contaminants may be predicted by Log Kow. Despite earlier reports (Briggs et al, 1982; Topp et al., 1986) that plant uptake is proportional to Log Kow, more recently, Calderon-Preciado et al. (2012) and Zhang et al. (2013) indicate the two are independent. Further, the results indicate that further research on the rhizosphere and root zone may be needed for a more complete understanding as to the phytoremediation mechanism, as that was the primary contaminant sink after leaving the water column.

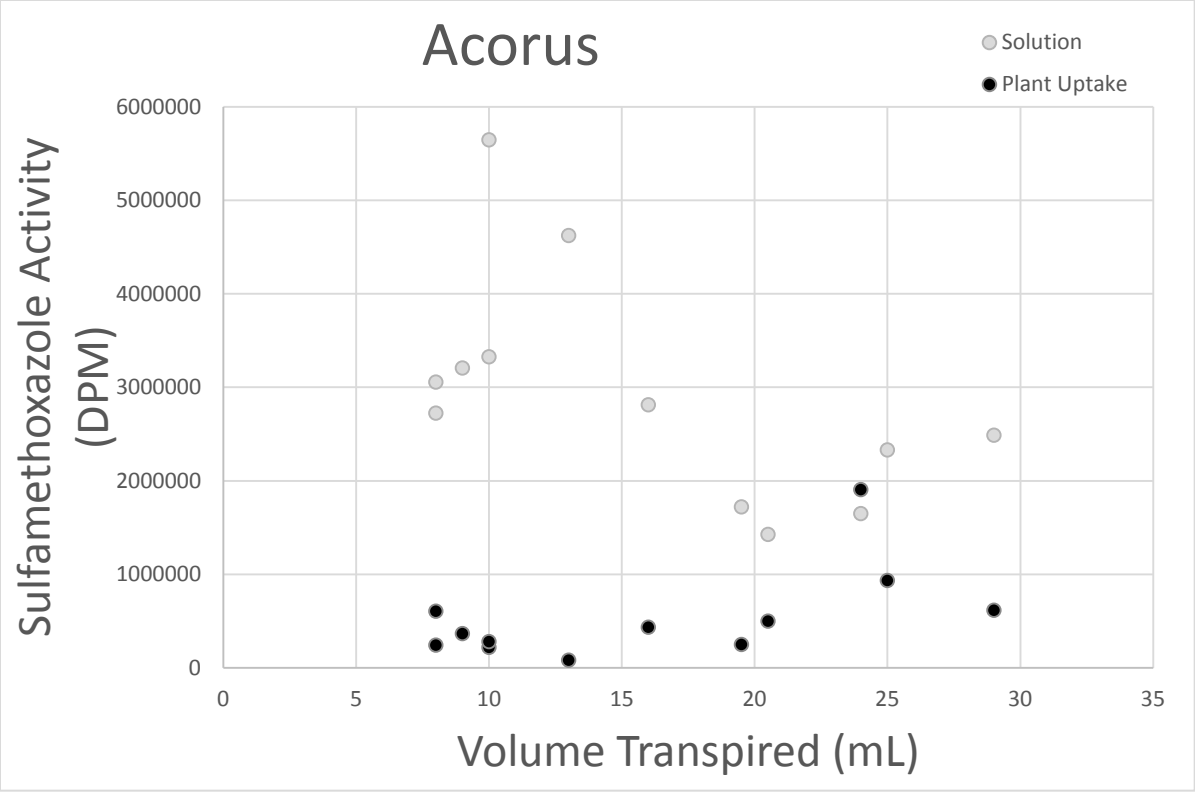
Figure 1. Acetaminophen 14C-activity in exposure solution and plants relative to the cumulative volume of water transpired by a. *Acorus gramenius* and b. *Canna hybrida* ‘Orange Punch’.

Figure 2. Sulfamethoxazole 14C-activity in exposure solution and plants relative to the cumulative volume of water transpired by a. *Acorus gramenius* and b. *Canna hybrida* ‘Orange Punch’.

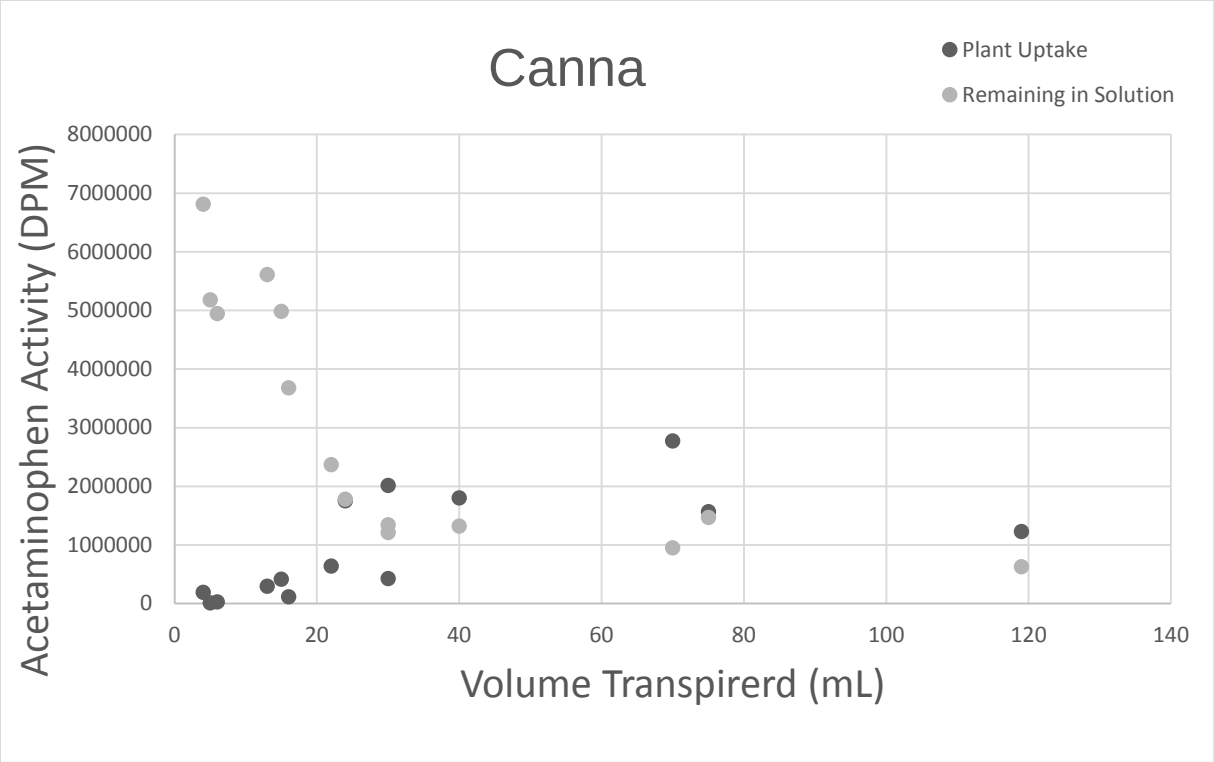
Paper 2

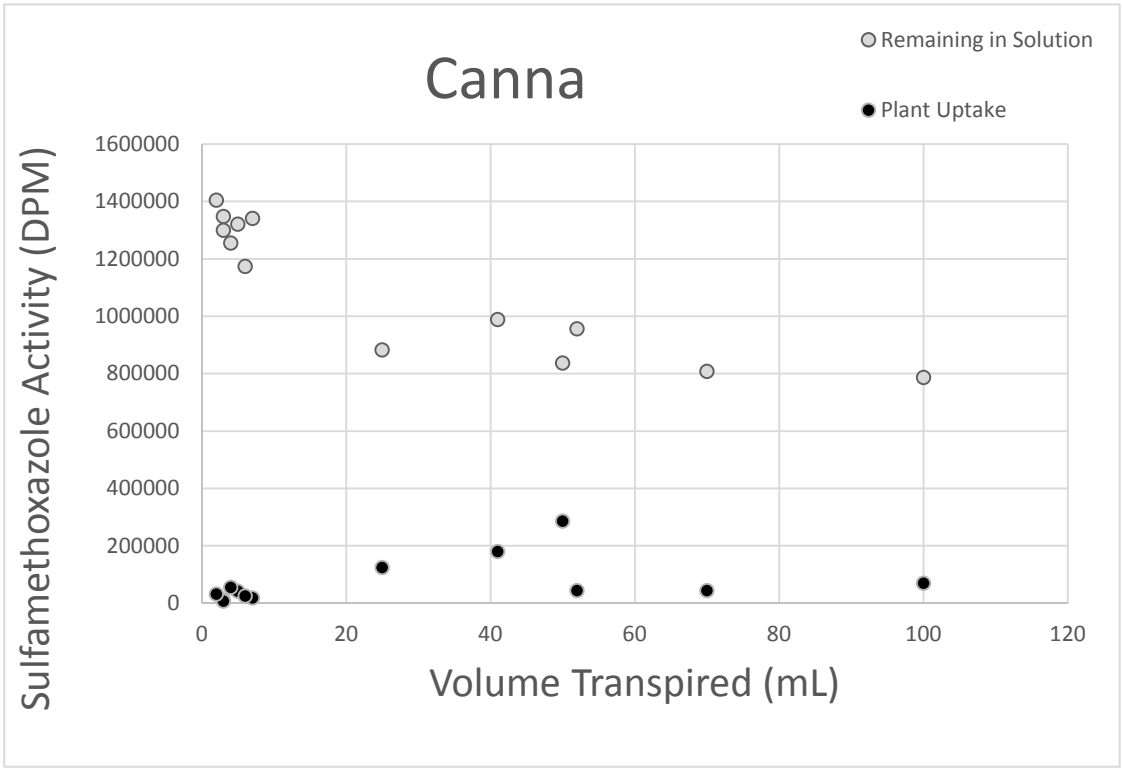

Acorus
Remaining in Solution
Plant Uptake
Acetaminophen Activity (DPM)
1200000
1000000
800000
600000
400000
200000
0
0
2
4
6
8
10
12
14
16
18
Volume Transpired (mL)

Paper 2


Acorus
Solution
Plant Uptake
Sulfamethoxazole Activity (DPM)
6000000
5000000
4000000
3000000
2000000
1000000
0
0
5
10
15
20
25
30
35
Volume Transpired (mL)

Paper 2





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