

THE EFFICIENCY OF CONSTRUCTED WETLANDS  
FOR THE REMOVAL OF THE ANTIMICROBIAL AGENT TRICLOSAN  
FROM WASTEWATER

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ELIZABETH R. ZINN

DISSERTATION ADVISOR: DR. JARMILA POPOVICOVA

BALL STATE UNIVERSITY

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To Raymond and Gail Zinn who always believed in me

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## CHAPTER 1 – REVIEW OF RELEVANT LITERATURE

### **Introduction**

Wastewater treatment is essential for healthy living conditions as well as overall environmental health. However, traditional wastewater systems are designed specifically for the removal of organic biodegradable compounds, nutrients, and pathogens (US EPA 1999). As our nation becomes increasingly industrialized, we continue to create and use a host of chemicals for cleaning and health care that are subsequently washed down drains and often never considered again. With the development of new analytical techniques and equipment, researchers have begun to find low concentrations of these chemicals in the environment, drinking water, and water bodies that receive wastewater treatment plant effluents (Daughton and Ternes, 1999). As a result, research has begun to focus on the fate of pharmaceuticals and household chemicals entering the environment through wastewater sources (Kolpin et al., 2002; Anderson et al., 2004; Johnson et al., 2004). These chemicals are entering wastewater for a variety of reasons: 1) many pharmaceuticals are not fully metabolized in the body and, therefore, are excreted into the waste stream (US CDC, 2009), 2) health care professionals, pharmacists, and hospice care organizations encourage people to flush unused or outdated medication down the

drain (Glassmeyer et al., 2009), 3) an increasing number of chemicals are manufactured and used in personal care products and household cleaners, that are consequently disposed of through household drains and into wastewater systems (Daughton, 2003). Antibiotics, analgesics, antiseptics, beta blockers, and blood-lipid regulators are only a few of the types of pharmaceuticals and personal care products (PPCPs) that have been detected in aquatic ecosystems (Jones et al., 2006). In fact, pharmaceuticals have been detected in wastewater treatment plant (WWTP) effluents and receiving waters in many parts of the world (Jones et al., 2006). Because of the dilution that occurs in the receiving waters, Kolpin et al. (2002) suggested that long term chronic effects caused by exposure to low concentrations are probably a larger concern for humans than an acute toxicity from high concentrations of PPCPs.

For urban and high population areas, wastewater is treated in WWTPs, yet research is only beginning on the efficiency of these systems for the removal of PPCPs from wastewater (Lishman et al., 2006). Rural and suburban communities traditionally employed conventional onsite septic systems that consisted of a septic tank and a drainfield for wastewater treatment, but in recent years they have started to look for alternative wastewater treatment options. Constructed wetlands are one of the new treatment alternatives that is becoming more popular. However, similarly to the centralized wastewater treatment plants, little is known about the efficiency of these wetlands to remove many non-nutrient chemicals like those of PPCPs (Huang et al., 2004; Park et al., 2009).

This chapter first addresses the problems associated with an increase in occurrence of PPCPs in the environment, and then focuses specifically on the chemical

triclosan, an antimicrobial agent that is used in soaps, cleaners, toothpaste, textiles and a host of other products. The main avenue of triclosan entry into the environment is through wastewater so the rest of this chapter will explore the fate of PPCPs and, specifically, triclosan in centralized wastewater treatment and then in onsite treatments like those used in rural areas of the country. Finally, the chapter focuses in on alternative onsite treatment with constructed wetlands, to discuss factors that influence their treatment efficiency and examine the results of recent studies about removal of non-nutrient chemicals like PPCPs and especially triclosan in these wetlands.

### **PPCPs in the Environment**

Until recently, few analytical methods were capable of detecting PPCPs at the low concentrations in which they occur in the environment (Kolpin et al., 2002). Recent improvements in analytical chemistry have resulted in an increased awareness of the growing problem of PPCPs entering the environment. Currently, the EPA does not have any specific program targeting the regulation of PPCPs, neither in the environment nor in wastewater effluent, although the agency has expressed a commitment to the investigation of this topic and the development of strategies to help protect the health of both the environment and the public (US EPA, 2012). The goal of US EPA research is to determine which PPCPs are most harmful and address unanswered questions about the fate of these chemicals in the environment (US EPA, 2005a). The United States Geological Survey (USGS) has also identified a need to study the removal rates of pharmaceuticals in wastewater treatment systems, as little is known about the effects of

low doses of these chemicals on human health and ecosystems like those found in streams receiving wastewater effluents (Buxton and Kolpin, 2002).

As these compounds enter the waste stream, they have the potential to interact and form new compounds. Similarly to a limited knowledge about the effects of PPCPs, even less is known about the effects of the complex mixtures that these PPCPs may form after they have interacted with other chemicals in the environment (Kolpin et al., 2002).

Learning more about how these chemicals interact and break down is an essential component to better understand potential health impacts these degradates may have on humans and the environment (Jones et al., 2006). Kolpin et al. (2002) frequently detected degradation byproducts of PPCPs in streams across the country and suggested that further study is needed concerning the fate of PPCPs in the environment.

### **The Antibacterial Agent Triclosan**

One of the PPCP compounds that has frequently been found in the environment is triclosan, an antimicrobial agent present in many commercial products that is used to kill bacteria on the skin and other surfaces or sometimes used to preserve products against microbial deterioration (Glaser, 2004). Triclosan is the active antimicrobial agent in many types of toothpaste, mouthwash, soaps, and many household cleaners. It is also found in textiles, sportswear, bedclothes, shoes, and carpets (Singer et al., 2002). It has been estimated to be used in over 1,500 products (Halden, 2007). Triclosan use as both a preservative and a disinfectant continues to increase despite the fact that there are no proven benefits for most of its common usages (Ferrari et al., 2002; Halden, 2007).

Triclosan is marketed under the trade name Microban® when used in plastics and

Biofresh® when used in acrylic fibers (Glaser, 2004). Triclosan degrades with an average half-life value of  $5.2 \pm 1.7$  days in the environment (US EPA, 2008)

As an antimicrobial agent, triclosan works in bacteria by blocking one of the steps in their fatty acid synthesis (Hundt et al., 2000). It does this by inhibiting the enzyme enoyl-acyl carrier protein reductase (4,5), thus blocking the active site for this enzyme and preventing the bacteria from synthesizing fatty acids that are necessary for building cell membranes and for reproducing (Singer et al., 2002; Glaser, 2004). Since humans do not have this enzyme, it has been considered safe for human use, although, it is currently under review by the U.S. Food and Drug Administration (US FDA) over concerns for human health and safety (US FDA, 2008). Murray et al., 2010 identified triclosan as a chemical of the highest priority and treatment concern because of its potential toxicity and relatively high concentration and persistence in the environment.

### **The Fate of Triclosan in the Environment and its Potential Effects**

Triclosan has been found in surface water bodies around the world. In the US, the U.S. Geological Survey (USGS) found traces of triclosan in 130 of 139 rivers tested in 2002 (Kolpin et al., 2002). It was expected that most of this triclosan entered the environment through WWTP effluent since over 95% of triclosan is used in products that are disposed of via residential drains and the selected study sites for the reconnaissance study were downstream from wastewater treatment facilities. Glaser (2004) suggested that some of this triclosan was not being removed during wastewater treatment and was, therefore, discharged into streams and rivers. Recent studies have provided evidence for

this theory. A study by Adolfsson-Erici et al. (2002) found triclosan in the bile of fish in the water receiving effluent from WWTPs. Balmer et al. (2004) found concentrations of methyl-triclosan in fish from lakes downstream of WWTPs whereas no methyl-triclosan was found in fish from remote lakes that received no WWTP effluent. In a USGS study of the Colorado River, conducted by the University of Washington, elevated concentrations of triclosan were found downstream from wastewater treatment discharge locations (Sprague and Battaglin, 2005). In summary, the results of previous research clearly demonstrate that triclosan is not completely removed by traditional wastewater treatment facilities and are entering surface waters through wastewater treatment plant effluent.

### **The Fate of Triclosan in the Environment**

To better understand the potential fate of triclosan in wastewater treatment as well as its effects on human health and the environment, it is important to understand the processes that affect its movement, accumulation, and degradation in natural systems. Triclosan has a high vapor pressure and is stable against strong acids and bases, therefore, there is little or no degradation through gas exchange with the atmosphere or through hydrolysis in water (Singer et al., 2002). On the other hand, triclosan rapidly decomposes in water solution and when exposed to sunlight (Anderson et al., 2004). A laboratory study using 18-W ultra-violet irradiation at 254 nm wavelength degraded 70% of triclosan in two minutes (Lores et al., 2005). Another study using low intensity white light found triclosan to have a half-life of eight days in freshwater samples and four days in seawater samples (Aranami and Readman, 2007).

Singer et al. (2002) conducted a study on the fate of triclosan in the aquatic environment and found that there was indeed a high removal rate in the epilimnion of lakes where photodegradation would occur. However, the pH of the surface water had an influence on how triclosan behaved and researchers concluded that more study was needed about the effects of pH on triclosan degradation rate. Further, triclosan accumulated in the lake sediment where the degradation rate was slower (Singer et al., 2002). Waltman et al. (2006) found triclosan concentrations in surface waters to be highest in summer months when photodegradation rates are higher. These findings are notable because the high concentrations of triclosan in surface waters during the summer might be the result of either elevated concentrations of triclosan in wastewater plant effluent in the summer due to an increased use of products containing triclosan or from reduced water discharge of streams and rivers that results in an increased triclosan concentration due to decreased dilution (Loraine and Pettigrove, 2006).

### **The Effects of Triclosan on the Environment**

A majority of triclosan is used in products that eventually enter the waste stream through household drains and from there move to the wastewater treatment systems, either centralized or onsite, before being discharged into the environment. Triclosan influence on aquatic systems is still unknown, but a risk assessment by Ferrari et al. (2002) suggested that, even at low concentrations, triclosan could potentially be a hazardous compound for aquatic ecosystems. In contrast, a study by Capdevielle et al. (2008) used a species sensitivity distribution index and triclosan exposure modeling to predict that risks would be limited to highly polluted freshwater ecosystems. Indeed, because algal communities are potentially impacted by triclosan, if high concentrations of triclosan

enter the environment, serious consequences to the ecological balance of these systems could result (Tatarazako et al., 2004). In fact, triclosan has already been found in the environment all over the United States: a USGS study found that triclosan was among the seven most frequently detected compounds as it was detected in 57.6% of the water samples (Kolpin et al., 2002). It seems likely, then, that triclosan is influencing aquatic ecosystems across the nation though the specific impacts on plant and animal life are still being investigated. Recent studies have begun to determine the effects of triclosan on specific plant and animal communities in aquatic ecosystems. Trace concentrations of triclosan were found in fish tissue in five rivers in Illinois, Texas, Florida, Arizona, and Pennsylvania where the wastewater treatment plant effluent was discharged into the river (Ramirez et al., 2009)

Algae and other aquatic plants may be particularly influenced by triclosan since they have a similar process of fatty acid synthesis to that of bacteria. Algae has been identified as the most sensitive species of aquatic plants studied and they have been shown to be affected by low concentrations of triclosan (Fulton et al., 2009). Because algal communities are often used as a representative community of aquatic plant systems, this research suggests that triclosan is likely to cause disruption of many aquatic plant communities that have yet to be studied (Tatarazako et al., 2004). The influence of triclosan on algae is also important in aquatic ecosystems as it serves as one of the main producers in these ecosystems and a community disruption could have a significant effect on the entire food web (Tatarazako et al., 2004). A study by Wilson et al. (2003) investigated the types of triclosan effects on algal communities and they found that, although triclosan did not result in an overall reduction in algal biomass, the exposure to

triclosan resulted in a marked shift in the structure of algal community and a decline in species richness. These shifts in community structure may also affect the nutrient processing capacity of water bodies receiving wastewater effluent, considering that algae play a key role in this process (Wilson et al., 2003).

Research has begun to show that the effects of triclosan may extend even beyond the aquatic plant communities. Triclosan may cause deleterious effects in both vertebrate and non-vertebrate animals (Balmer et al., 2004; Brown et al., 2012; Valters et al., 2005), and, because of its polarity, it also has the potential to bioaccumulate in the food chain (Thompson et al., 2005).

Evidence of triclosan effects on aquatic species raises questions about the possible effects on humans. Currently, little is known about the health effects on humans, though a growing body of evidence suggests that triclosan results in endocrine disruption in certain animals. A study by Veldhoena et al. (2006) demonstrated that North American Bullfrog tadpoles treated with low concentrations of triclosan showed an increase in abnormal limb growth and a decrease in body weight. This is thought to be the result of an endocrine disruption that affects thyroid hormone-associated gene expression. A similar endocrine disruption caused by triclosan has been demonstrated in the marine bivalve *Mytilus galloprovincialis* (Canesia et al., 2007). Of even greater concern for human populations is a recent study by Crofton et al. (2007) that demonstrated disruption of thyroid hormone homeostasis in rats given an oral exposure to triclosan.

Triclosan has a chemical structure that closely resembles some non-steroid estrogens and there has been speculation that estrogen pollution could lead to higher female populations in aquatic animals. However, a study by Foran et al. (2000) showed

neither an increased number of females in fish populations nor an increase in some female characteristics after triclosan exposure. The researchers suggested that triclosan may have a weak androgenic effect on fish populations, but more study is needed before any conclusions about androgenic effects can be drawn.

### **Triclosan and Human Health**

Direct contact with triclosan is not known to be hazardous to humans though it is under review by the FDA due to some recent animal studies that suggest it could play a role in hormone disruption (US FDA, 2010). The typical amount of triclosan used in consumer products ranges from 0.1 to 0.3 percent and, at this concentration, triclosan has been shown to not be acutely toxic, carcinogenic, teratogenic, or to irritate the eyes or skin in humans (McAvoy et al., 2002). Even though triclosan has been considered safe for use in soaps and cleaners, there is currently little information about the effects on humans from exposure to drinking water contaminated with triclosan. There is growing concern about this pathway of exposure as triclosan has been found in reclaimed water that was processed for drinking (US EPA, 2005a).

Some studies have demonstrated triclosan occurrence in human populations across the globe. A 2002 study in Sweden found high concentrations of triclosan in three out of every five randomly selected human breast milk samples (Adolfsson-Erici et al., 2002). The Australian government was so concerned by this study that it commissioned the University of Queensland's National Center of Environmental Toxicology to determine triclosan concentrations in Australian breast milk samples (NICNAS, 2006). Although these studies identified the presence of triclosan in breast milk, there is currently no

knowledge about health consequences for infants who consume milk that contains triclosan.

Another potential problem with triclosan occurrence in the environment is bacterial resistance which could also have human health implications as infectious bacteria become more resistant to antimicrobial agents (Singer et al., 2002). A study by Ledder et al. (2006) demonstrated that exposure to triclosan can lead to high concentrations of resistance in some bacterial strains such as enteric bacterial strains and, in particular, *E. coli*.

### **Implication for Triclosan Derivatives**

As triclosan breaks down in the environment, it could either undergo complete mineralization to carbon dioxide, or form a range of derivatives (Singer et al., 2002). Two of triclosan's derivative pathways are known to be of concern in aquatic environments. One is the methylation of triclosan into methyl-triclosan (5-chloro-2-(2,4-dichlorophenoxy)anisole) which has been found to accumulate in the environment. The other is the transformation of triclosan under the influence of sunlight into chlorodioxins that can be highly carcinogenic. (Lindstrom et al., 2002; Thomas and Foster, 2004). A study by Lores et al. (2005) investigated the role of photodegradation in the formation of dioxins from triclosan and found that triclosan is clearly a dioxin precursor due to a conversion via an intramolecular photochemical substitution reaction. In the presence of ultraviolet light, between 0.4% and 1.5% of triclosan may be converted to dichlorodibenzo-*p*-dioxin (DCDD). Although these results show that dioxins clearly result from photoregulation, Lores et al. (2005) pointed out the need to study this reaction in natural sunlight versus artificial ultraviolet light.

Another triclosan derivative of concern is methyl-triclosan, which forms during biodegradation through the process of microbial methylation (Boehmer et al., 2004). Methyl-triclosan is more stable and persistent than triclosan and could possibly accumulate in the aquatic environment (Boehmer et al., 2004). Lindstrom, et al. (2002) found that high concentrations of methyl-triclosan accumulated in lakes where triclosan was exposed to sunlight and warm temperatures. This is likely due to the fact that triclosan quickly degrades under ultraviolet light while methyl-triclosan is stable and accumulates in the upper level of lakes.

There is also concern that methyl-triclosan has been shown to bioaccumulate in aquatic organisms. A study by Boehmer et al. (2004) analyzed muscle samples from fish in sixteen rivers over multiple years and detected methyl-triclosan in all fish samples. A study conducted in Switzerland (Balmer et al., 2004) found methyl-triclosan in all fish tissues except those collected from two remote lakes where there was no contamination from wastewater effluent. The concentrations in the fish were higher than the concentrations in the lake water from which the fish were taken which suggests that bioaccumulation had occurred and was probably caused by direct intake of water through the gills and not from biomagnification in food sources.

There is evidence that various organisms can break down triclosan to different derivatives; this has been shown in rats, guinea pigs and white-rot fungi (Hundt et al., 2000). This may provide information not only about the fate of triclosan in the environment, but also how it might be processed within the human body and wastewater treatment systems.

## **Centralized Wastewater Treatment for PPCPs and Triclosan**

### **PPCPs and Wastewater Treatment**

The fate of PPCPs in wastewater begins after the products serve their intended use and are washed down household drains, entering the wastewater treatment system.

Conventional centralized wastewater treatment is designed to treat organic biodegradable waste and to remove harmful bacteria and suspended solids; however, they are not designed to remove PPCP chemicals (Kolpin et al., 2002). The metabolic nature of many of these chemicals often leads to their incomplete removal in wastewater treatment. As a result, treatment plants become a point source for the entry of pharmaceuticals into the aquatic environment (Jones et al., 2006). This is particularly true during the summer months when there are higher levels of PPCPs in wastewater, possibly due to an increased usage of products such as pesticides and sunscreen, and many PPCPs can be detected downstream from wastewater treatment facilities (Loraine and Pettigrove, 2006).

Although the design of the WWTPs is not favorable for PPCP removal, previous research indicates that wastewater treatment does, nevertheless, remove some of the PPCPs that are present in wastewater. The rate of removal has been shown to vary depending on the treatment methods and the individual chemical properties. For example, activated sludge systems have been shown to remove more chemicals than trickling filters, which may be due to higher oxidation rates in the activated sludge systems. Also, treatments that employ denitrification have been shown to remove more of some pharmaceutical compounds possibly because chlorine or chloramines that are used in finishing treatments, are better able to interact with these chemicals without the

presence of nitrogen (Jones et al., 2006). For many PPCPs that have hydrophobic characteristics, the primary removal mechanism may be sorption to suspended solids and removal by sedimentation to primary and secondary sludge (Ternes et al., 2004). Consequently, the presence and accumulation of these synthetic organic compounds in the sludge is of additional concern to the environment and public health when sludge is land-applied as fertilizer.

### **Concentrations of Triclosan in Wastewater**

The daily use of a wide range of antimicrobial products in the United States is having an impact on the concentrations of triclosan in wastewater. In a study of five different communities in the United States, McAvoy et al. (2002) estimated the usage of triclosan to be in the range of 3 to 5 mg/capita/day. Conn et al. (2010) studied triclosan occurrence in household wastewater in six septic systems and they estimated a daily mass contribution between 0.03 and 28.0 mg/capita/day with an estimated median of 3.7 mg/capita/day contribution of triclosan into household septic tanks. Different types of businesses and industries also use triclosan in a variety of products, which further contributes to the increase of triclosan in wastewater and, ultimately, the environment. This means that the concentrations of triclosan entering WWTPs can vary greatly depending on the nature of the wastewater it receives and the number of residences it serves.

A study of four WWTPs in the UK found that the influent concentrations ranged from 0.07 µg/L to 5.1 µg/L (Thompson et al., 2005). These concentrations were similar to the findings of another three WWTPs in the UK where triclosan influent concentrations ranged from 0.6 µg/L to 5.1 µg/L (Winkler et al., 2007) and a study of 12

WWTPs in Canada that had influent concentrations of 0.01 to 4.01 µg/L (Lishman et al., 2006). Perhaps there is a higher level of triclosan use in the United States as McAvoy et al. (2002) found influent concentrations that ranged from 3.8 µg/L to 16.6 µg/L in a study of five different WWTPs. A study of four WWTPs in Georgia by Kumar et al. (2010) found triclosan concentrations between 2.4 µg/L and 38.3 µg/L. These measured concentrations might even be low estimates because much of the triclosan found in wastewater influent is sorbed to solids and particulate matter, and, therefore, may not be measured in filtered water samples. Some have estimated that between 70-80% of the triclosan in WWTP influent is in sorbed form (Waltman, 2006). The study by Kumar et al. (2010) found that triclosan was entering the WWTP in the particulate phase at about the same concentrations as triclosan in dissolved phase. This sorbed triclosan may degrade, be transformed or become mobile again at some point in the treatment process (Wilcox et al, 2009).

Given these high concentrations of triclosan found in WWTP influent, it may not be surprising that some triclosan is released into the aquatic environment through WWTP effluent. Several studies have shown WWTP effluent concentrations of triclosan to be in the range of 0.01 µg/L to 5.4 µg/L (Singer, 2002; Winkler et al., 2007; Kumar et al., 2010; Ricart et al., 2010). Given the volume of discharge from a WWTP, however, the total volume of triclosan entering the surface water can still be dramatic. Kumar et al. (2010) estimated that one WWTP alone discharged 76 gallons of triclosan per day into the local river.

## **Triclosan Removal in Centralized Wastewater Treatment**

Although triclosan is found in wastewater effluent and in water bodies receiving this effluent, research has demonstrated that triclosan is removed at high rates through the wastewater treatment processes at WWTPs. Kumar et al. (2010) found that 94-99% of triclosan was removed in five U.S. WWTPs, but the removal rates varied depending on the method of treatment at the plant. A number of studies have shown that the removal rates can range between 58% and 99% depending on the treatment method used (McAvoy et al., 2002; Singer et al. 2002; Bester, 2003; Gryaab et al., 2004; Thompson et al., 2005; Lishman et al., 2006; Heidler and Halden, 2007). Because of this variability, it is important to investigate individual WWTP treatments to better determine the treatment processes with the highest triclosan removal. Such research will also provide insight into the removal mechanisms of triclosan that pertain to onsite treatments.

Research has shown that removal of triclosan occurs at different stages of wastewater treatment (McAvoy et al., 2002; Bester, 2005; Jones et al., 2006; Thompson et al., 2005). Although WWTP designs vary greatly, most consist of three main stages; a primary treatment, a secondary treatment, and a finishing or tertiary treatment (Figure 1.1). Wastewater contaminants that are not filtered out in primary treatment or do not biodegrade, leave the WWTP either in the effluent water after the finishing treatment or in the treated sludge. Research has been conducted on the removal of triclosan in each of these stages in activated sludge WWTPs as well as in a range of alternative treatment designs.

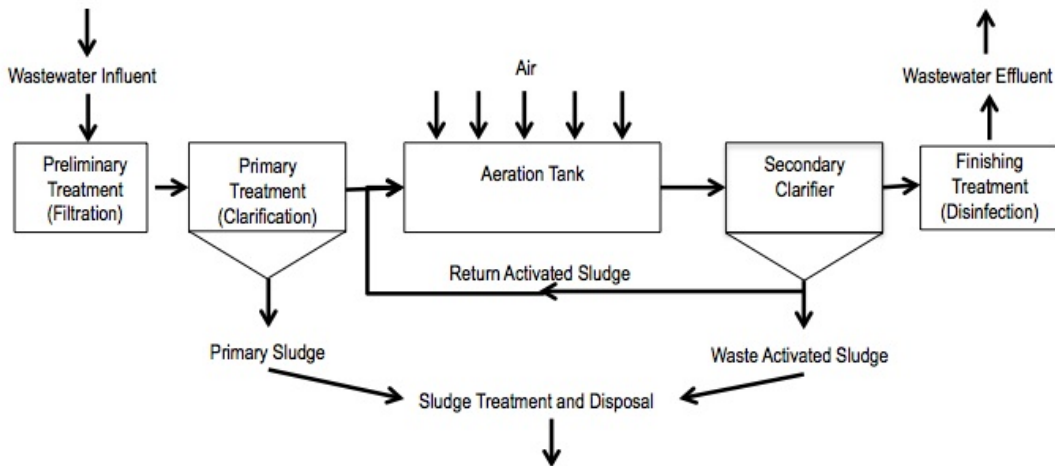


Figure 1.1 – Flow Diagram of an Activated Sludge WWTP System.

### Triclosan Removal Through Primary Treatment

With the exception of small systems that employ extended aeration processes, all centralized wastewater plants have primary treatments to physically remove large suspended organic solids. Triclosan is a hydrophobic molecule with a high octanol-water partitioning coefficient ( $\log K_{OW} = 4.8$ ), which indicates that it is likely to sorb to matter containing organic carbon and, therefore, it is more likely to accumulate in the solid phase (Jones et al., 2006; Thompson et al., 2005). Triclosan would also more readily sorb to organic particles than to fats or inorganic sediment. Thompson et al. (2005) found that primary sludge has a high concentration of organic solids and a lower fat content than secondary sludge and showed that hydrophobic compounds tend to adsorb to primary sludge. It would be expected that some triclosan would be removed through the primary clarification process when suspended solids settle out of the water.

Several studies confirm the removal of triclosan through the primary clarification process, though the removal rates are highly variable among sites. McAvoy et al. (2002) found that the triclosan removal rates by primary sedimentation ranged from 7.1 to 48.1% at five different WWTPs. Thompson et al. (2005) also found that triclosan was removed through absorption onto primary sludge and they suggest that in WWTP facilities not using activated sludge in secondary treatment, this may be the main removal mechanism for triclosan.

### **Triclosan Removal Through Secondary Treatment**

Several studies have investigated triclosan removal by secondary treatment methods. A study by Thompson et al. (2005) compared the triclosan removal efficiency in three different WWTPs in the United Kingdom that employed different types of secondary treatments. They found a removal efficiency of 58-96% in rotating biological contractors, 86-97% in trickling filters and 95-98% in activated sludge treatments. This is consistent with the findings of McAvoy et al. (2002) that also showed higher removal rates in activated sludge systems (95.4% and 96.2% ) compared to trickling filter plants (58 and 86.1%) at two WWTPs.

Further studies have begun to provide insight into why some treatments remove more triclosan than others. A study by Bester (2005) found that a two-stage activated sludge process removed more triclosan than a combination of physical and activated sludge treatments. Since the two-stage activated sludge treatment has two aeration basins, this suggests that the aeration process is a dominant mechanism for elimination, and that concentrations of dissolved oxygen (DO) may play an important role in triclosan removal. Further, Bester (2005) found that a biological filter was less effective in

triclosan removal than the activated sludge process, possibly due to lower concentrations of DO in this treatment. Triclosan is highly biodegradable under aerobic conditions with a mineralization half-life of 15-35 days (Thompson et al., 2005). A study by McAvoy et al. (2002) found that no biodegradation occurred under anaerobic conditions, although in such conditions, triclosan could still be removed through sorption to the sludge.

Similarly, Chen et al. (2011) found little triclosan removal under anoxic or anaerobic conditions.

Since triclosan needs oxygen to biodegrade and the highest removal rates for triclosan are found in aerobic, activated sludge treatments, this suggests that in activated sludge treatments, biodegradation is the primary removal mechanism, not sorption to sludge. McAvoy et al. (2002) found that 80% of the triclosan removal in activated sludge systems could be attributed to either mineralization to carbon dioxide or incorporation into the biomass. These results are quite similar to those reported by Singer et al. (2002) who found that 79% of triclosan removal was a result of biodegradation with only 15% attributable to sludge sorption. This is different, however, when the DO concentrations are low. Thompson et al. (2005) found that in processes where the dissolved oxygen concentrations were high (1.5-2.0 mg/L), biodegradation was the main removal mechanism, while in cases where dissolved oxygen concentrations were low (<0.3 mg/L), the main mechanism for removal became sorption.

As previous research indicates, most of the triclosan that is not oxidized by biodegradation is sorbed to sludge, although the mechanisms of degradation and creation of derivative chemicals have only begun to be researched. A study by Bester (2007) on one WWTP in Dortmund, Germany, using activated sludge, found that 65% of total

triclosan was metabolized. However, analysis revealed that only half of the derivatives in this metabolized portion of the total triclosan were recognizable. This means that of the total triclosan entering the system, 35% was unmetabolized, 32.5% was metabolized into known derivatives such as methyl-triclosan, and the remainder (32.5%) was metabolized into unidentifiable derivatives. A recent study by Chen et al. (2011) found that triclosan formed methyl-triclosan during aerobic treatment and the longer the duration of aerobic treatment, the more methyl-triclosan was formed. No methyl-triclosan was found to form under anaerobic conditions.

A longer treatment time results in higher triclosan removal and the formation of more triclosan derivatives (Chen et al., 2011), which has been suggested as one of the reasons why activated sludge treatments are more effective at triclosan removal than treatments such as trickling filters. Not only is the oxygen concentration higher in activated sludge treatment, but there is also a longer hydraulic retention time (Thompson et al., 2005).

Several other types of wastewater treatment processes have also been shown to remove high concentrations of triclosan. Ricart et al. (2010) examined reverse osmosis (RO) and microfiltration (MF) systems and found that the RO process removed 40% of triclosan while the removal by the MF treatment was negligible. The low performance of the MF system was confirmed by McAvoy et al. (2002) whose findings showed essentially no triclosan removal by a sand filtration system.

Since triclosan is an anti-microbial agent, there could be concern that high triclosan concentrations in wastewater may cause the mortality of microorganisms present in biofilms of the various wastewater treatment processes. Krishnakumar et al.

(2011) found that triclosan reduced number and mobility of flagellate protozoa in both anaerobic and aerobic sludges with higher trophic organisms affected most. Another study by Ricart et al. (2010) found that triclosan caused bacterial and algal death in biofilms although overall treatment rates were not affected. Triclosan does not only kill bacteria and algae within the WWTPs, it has also been shown to affect bacteria and algae in river biofilms downstream from WWTPs (Ricart et al., 2010). This has the potential to reduce the capacity of the surface water system to act as a further cleansing agent for WWTP effluent it receives.

### **Triclosan Removal Through Finishing Treatments**

One finishing treatment used in some WWTPs is ultraviolet (UV) radiation. Triclosan is known to undergo rapid photodegradation in laboratory settings (Lores et al., 2005) and it has also been shown to photodegrade in natural aquatic systems, such as lakes, with the degradation rate depending on the the time of year and pH levels (Tixier et al., 2002). However, using UV treatment for triclosan removal in WWTPs is problematic because under basic pH conditions (pH of 8 and above) UV treatment might be effective, but in acidic conditions (pH below 8) triclosan is fairly photo stable (Thompson et al., 2005). Given that the pH levels in wastewater treatment plants are usually below 8, this suggests that UV treatment would most likely be ineffective at triclosan removal. This is supported by the work of Waltman et al. (2006), which showed that the mean concentrations of triclosan before a UV treatment basin was not significantly different from the concentrations after the basin in a North Texas WWTP.

The last stage of wastewater treatment in WWTPs is called the finishing treatment or final disinfection. This often involves the addition of chlorines and chloramines, so it

has the potential to reduce triclosan because chlorine and chloramines are reactive with triclosan. Although triclosan reacts with chloramines, the reaction is too slow to provide an effective treatment given the standard residence time for final disinfection. Also, this reaction is only effective under conditions of high pH, conditions unlikely to occur under low nitrogen concentrations in wastewater effluent (Greyslock and Vikesland, 2006).

Although a high percentage of triclosan is removed from wastewater at WWTPs, it is still found in surface waters around the nation (Kolpin, 2002), and so has the potential to harm aquatic, environmental, and human health. The derivatives formed as triclosan degrades may also be harmful.

### **Triclosan Concentrations in Sludge**

Wastewater treatment plants are designed to clean the water that leaves the plant as effluent, however, many chemicals, pharmaceuticals, and antibiotics also leave the WWTPs sorbed onto sludge. Since sludge is rich in nutrients, it is valuable as a soil amendment, although this may be a pathway for PPCPs to enter the environment where they could pose a threat to both terrestrial and aquatic ecosystems and ultimately to human health (Jones et al., 2006; Halden, 2007). It is estimated that 30% of triclosan entering treatment plants can sorb to the sludge with weak or strong bonds (Bester, 2003). A study by Heidler and Halden (2007) found an even higher level of fully half of triclosan entering a U.S. WWTP still not biodegraded and detectable in the sludge. McAvoy et al. (2002) found that digested sludge contained concentrations of 0.5 to 15.6 mg/g of triclosan by dry weight. This triclosan appears to remain in the sludge even after it has been transformed into biosolids. Kinney et al. (2006) tested nine samples from different sludge-handling processes and found triclosan in all nine samples with a median

concentration of 10.2 mg/g. Heidler and Halden (2007) found a range of 30.0-110.0 mg/g of triclosan in one U.S. WWTP. Triclosan that is sorbed to sludge may eventually be applied to agricultural fields as a soil amendment, exposing plants and soil invertebrates to this antimicrobial agent (McAvoy, 2002). Research is being conducted on sludge treatments that could remove triclosan and other PPCPs. Anaerobic sludge digestion does not appear to be effective at removing triclosan from sludge. Karnjanapiboonwong et al., (2011) found triclosan concentration from 2.48 to 12.1 µg/L in sludge samples from a Texas WWTP after anaerobic digestion. Kumar et al. (2010) found that incineration of wet sludge removed 99.9% of triclosan. This may be an option for WWTPs to reduce the concentration of triclosan entering the environment through sludge, though this would be a costly addition for many WWTPs.

## **Onsite Wastewater Treatment and Triclosan Removal**

### **Use of Onsite Systems in the United States**

Although most wastewater from urban areas is treated by centralized systems, over 60 million people in the United States use decentralized, onsite wastewater treatment systems (U.S. Census Bureau, 2008) and up to one third of all new housing and commercial development disposes and treats their wastewater by use of onsite treatment systems (U.S. EPA, 2005b). Although these systems are typically in rural settings, half of the onsite treatment systems are found in suburban areas.

Wastewater treatment for rural and suburban households is a concern because the U.S. EPA (2005a) estimated that between ten and twenty percent of current onsite

systems do not adequately treat wastewater even for typically measured wastewater parameters, and half of them are at least thirty years old and beginning to fail. State water quality agencies view septic systems as the second greatest threat to our nation's water quality. Even if onsite systems are functioning well for nutrient removal and bacterial treatment, they are still a potentially significant route of entry for many PPCPs into the environment (Wilcox et al., 2009). Since PPCPs like triclosan are found in many household products and in common medications, they are prevalent in the household wastewater and are, therefore, entering these onsite treatment systems. However, traditional septic systems with drain fields may not be adequate to keep these synthetic organic chemicals from polluting the soil and groundwater.

### **Triclosan in Septic Tanks**

Ninety five percent of onsite systems in the United States are conventional septic field systems that make use of a septic for primary, i.e., physical treatment (U.S. CDC and U.S. HUD, 2006). Water flows from the residence into the septic tank where the solids settle to the bottom of the tank and the fats and oils rise to the surface (Figure 2.2). Some anaerobic decomposition may take place in this treatment tank along with the filtering of solids, fats and oils (Conn et al., 2010). Water flows from here to a disposal field, usually a series of perforated pipes in gravel trenches, where the water can seep out and percolate through the gravel before entering the soil.

Unfortunately, little research has been done on PPCP occurrence and removal from septic systems. Studies of the concentration of a range of PPCPs in septic systems have shown them to be as high as those entering centralized WWTPs (Wilcox et al., 2009). Even though these systems serve dramatically smaller numbers of people, they do

not reach dilution concentrations that are present in WWTPs. The limited research on triclosan in septic tanks shows similar results. Conn et al. (2010) found the triclosan concentrations for raw wastewater from six different households to range between 0.4 µg/L and 230.0 µg/L, with a median of 19.0 µg/L. There was a high degree of variability between sites as a result of differences in product and water use habits in the home. These concentrations are within the range of triclosan concentrations found in WWTP influent in the U.S., but the median and maximum concentrations are much higher than those in WWTPs (McAvoy et al., 2002; Kumar et al., 2010). The study of Conn et al. (2010) also showed that some triclosan is removed in septic tanks. They found the concentration of triclosan in the effluent of septic tanks to range from 0.9 µg/L to a maximum of 57.0 µg/L with a median concentration of 5.7 µg/L. This level of removal is surprising considering the anaerobic conditions in septic tanks. Conn et al. (2010) concluded that the triclosan may have sorbed onto settled solids in the septic tank.

Carrara et al. (2008) found a much lower concentration of triclosan in septic tanks at four campsite areas in Canada. Triclosan was detected in only two of the four septic tanks sampled, with concentrations of 0.001 µg/L and 0.007 µg/L. It should be noted that the authors suggested a problem with their detection method and the concentrations may have been much higher than their results indicated. Additionally, the researchers suggested that water use at campsites may differ from that of households. Despite the low concentrations of triclosan detected at these sites, Carrara et al. (2008) found significant concentrations of triclosan in plumes outside the drainage tile beds of the campsite septic system, which suggests a relatively low concentration of triclosan removal in the septic tank as well as drain field. One explanation for this occurrence may

be that a high load rate in these systems could lead to a decreased residence time for water in the septic tank. Hydraulic residence time has been suggested to be an important factor in triclosan removal in both WWTP treatments and in septic tank systems.

Temperature may also impact triclosan removal rates in septic tanks in northern climates as they are subject to a wide range of temperature fluctuations. There is some concern that their ability to remove at least some level of PPCPs from wastewater may be limited in cold winter months. However, Wilcox et al. (2009) found that temperature did not seem to be a major factor in the removal rates for a range of organic wastewater contaminants in septic systems, but more study is needed on the effects of temperature on removal processes in onsite systems.

### **Constructed Wetlands for Wastewater Treatment and Potential Triclosan Removal**

Given the limitations of traditional onsite systems, constructed wetlands are becoming an increasingly popular choice as an alternative onsite treatment option. Conventional onsite systems direct the septic tank effluent to a drain field where the water is allowed to percolate through a gravel bed and into the soil. If the water table is high or the soils are impermeable, these systems will not function correctly and dangerous wastewater backups can occur (US CDC and US HUD, 2006). Since septic systems often malfunction resulting in bacterial contamination of groundwater and surface water and the cost of sewers and centralized WWTPs is quite high, state and local governments are looking to innovative treatments and wastewater management options for onsite systems (USEPA, 2005b). There are many potential alternative treatments

including aerobic treatment units, filter systems and constructed wetlands. Many of these alternative systems, including constructed wetlands, also make use of a septic tank for the settling of solids and the separation of fats, oils, and greases (US CDC and US HUD, 2006).

When employed, constructed wetlands are considered a secondary treatment that receive the septic tank effluent before it is sent on to a disposal field (Figure 1.2). To understand the full functionality of a constructed wetland system for the removal of

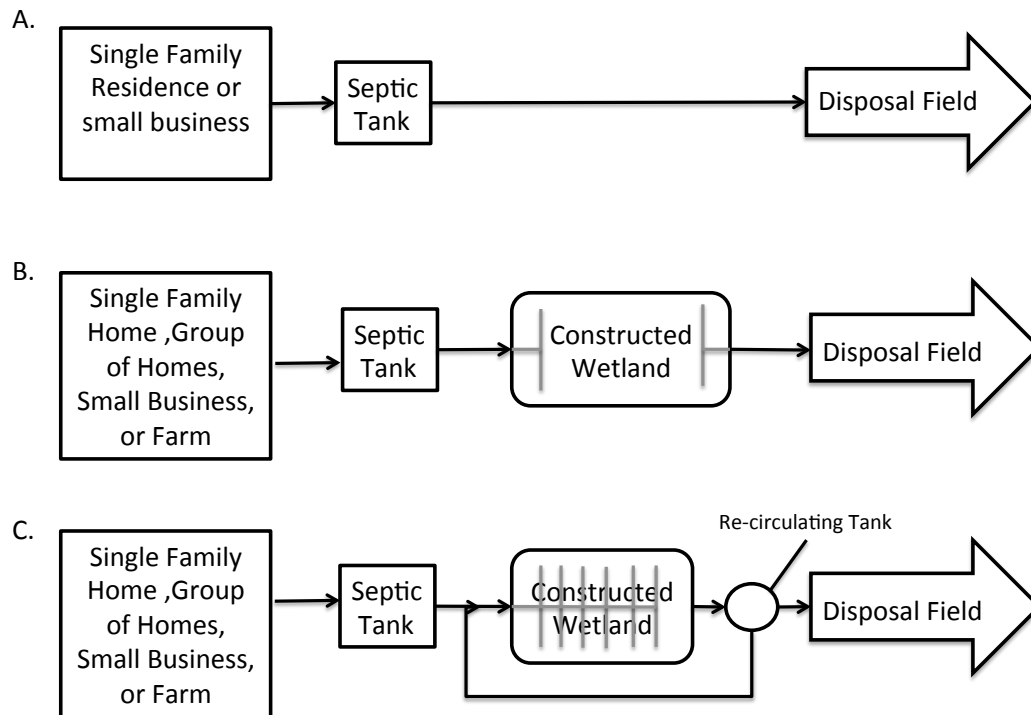


Figure 1.2 – Wastewater flow diagram for A. traditional residential septic system, B. horizontal flow (HF) constructed wetland system, and C. vertical flow (VF) recirculating wetland system.

pollutants, it is important to consider the portion that may be removed in the septic tank as well as the portion removed by the secondary treatment methods.

In the past few decades there has been an increase in the number of constructed wetlands being built to treat wastewater in small communities as well as urban storm runoff and dairy and swine farm runoff (Healy and Cawley, 2002). Constructed wetlands are shallow ponds, beds, or trenches and contain floating or emergent wetland vegetation (Matamoros et al. 2005). They make use of a natural treatment process that combines biological, chemical, and physical mechanisms for water quality treatment (Song et al., 2001). In these systems, wastewater pollutants are removed through plant uptake, sorbtion, chemical reactions, volatilization, and for many pollutants, microbial degradation is the dominant treatment process (Stearman et al., 2003).

There are two main kinds of constructed wetlands: free water surface flow wetlands and subsurface flow wetlands (Browning, 2003). Free water surface wetlands contain areas of gravel and vegetation as well as areas of open water, and wastewater flows through the constructed wetland as it would through a natural marsh. These wetlands have an aerobic zone near the surface and an anaerobic zone in the deeper water areas (US EPA, 2000). These wetlands require a larger land area and can have issues with odor and safety due to the exposed water surface, so they are used most often for treatment of mining water, farm runoff, and as a polishing treatment in WWTPs and not for individual residences and businesses.

Subsurface flow wetlands are sealed basins that contain vegetated porous gravel beds through which wastewater percolates (US EPA, 2000). The water level is designed to stay below the surface of the gravel substrate and percolates through the system either

horizontally or vertically. The subsurface flow wetland beds are confined by a waterproof liner and planted with wetland species that take up pollutants and host bacteria in their root systems that aid in the breakdown and transformation of pollutants under anaerobic conditions (Matamoros et al., 2005). The roots of the plants in subsurface wetlands increase the accumulation of organic matter and raise the sorption capacity compared to gravel alone (Matamoros, 2012).

### **BOD, TSS and Nutrient Removal in Constructed Wetlands**

It is now widely recognized that constructed wetlands can provide treatment of wastewater that is both effective and economical (Healy and Cawley, 2002). Nevertheless, most of the research on constructed wetlands has focused on common parameters such as total suspended solids (TSS), biochemical oxygen demand (BOD), nutrients, and bacterial pollutants.

Constructed wetlands are quite effective at reducing BOD and removing TSS. A study of 13 constructed wetlands by Matamoros et al. (2009) found a greater than 95% removal of both BOD and TSS in all the wetland systems. In 1993 the EPA published a technical assessment which examined the efficiency of 14 subsurface constructed wetlands from different areas of the country (US EPA, 1993). They found that the effluent in all the constructed wetlands that were studied had a BOD well below the 20mg/L, common permit requirement. They note, however, that the presence of plant matter and other naturally occurring organic materials in these systems also produce BOD, therefore these systems can never achieve a complete BOD removal and a residual concentration of 2 to 7 mg/L is often present.

TSS removal is effective in subsurface constructed wetlands. The US EPA found the effluent from all 14 wetlands studied to be below 20mg/L, regardless of the TSS levels in the influent, except for one site that was not functioning correctly (US EPA, 1993). There is contradictory research on whether plants play a role in TSS removal. A study by Manios et al. (2003) found no difference in TSS removal in two wetland systems with plants and those with only a substrate. However, a study by Karathanasis et al (2003) found a mean TSS removal rate of greater than 88% for vegetated wetlands and a range of 43 to 63% in non-vegetative systems. The substrate used in the wetland has also been shown to influence the removal rates for TSS in constructed wetland systems. Manios et al. (2003) found that gravel substrate removes a higher percentage of TSS (95%) than topsoil and sand in tests of two wetlands of each substrate type.

One common parameter often tested in constructed wetlands is total nitrogen. Subsurface constructed wetlands have been shown to remove nitrogen quite effectively from wastewater with reported removal rates often from 70 to 80% (Healy and Cawley, 2002). The primary removal process for nitrogen in a constructed wetland is through physical settling, denitrification, and plant and microbial uptake. However, plant uptake does not result in permanent removal unless the plants are harvested. Ammonia taken up by plants will return to the wetlands when the plants die and decompose (Bastviken et al., 2003). Reported removal rates for nitrogen are consistently over 80% and often over 90%, when plants are harvested. A more conservative figure of 50% is a more likely representation of a long-term removal rate without harvesting the plants (Healy and Cawley, 2002).

Although constructed wetlands are considered effective at nitrogen removal, some factors contribute to low denitrification rates, particularly in subsurface constructed wetlands. These factors include temperature, pH, anaerobic conditions, organic carbon levels, redox potential, and residence time (Browning, 2003; Healy and Cawley, 2002). Horizontal flow subsurface wetlands are less likely to engage in denitrification due to the anaerobic conditions in the sediment media. This denitrification can be limited by a few factors such as the availability of electron acceptors, which could come from either oxygen or nitrate (these are low in anaerobic systems). Another factor is that if ammonia has not been converted to nitrate because of limited oxygen, denitrification may be limited (Bastviken et al., 2003). Despite these limiting factors influencing rates of denitrification, subsurface wetlands have been shown to be effective at nitrogen removal over a wide range of temperatures, including northern climates in North America and Europe (Healy and Cawley, 2002). The three factors that appear to best ensure high nitrogen removal rates in constructed wetlands are maintaining high water levels in the wetland, a long hydraulic residence time of wastewater in the wetland, and the combination of wetland plants that support bacterial communities (Bastviken et al., 2003).

Another parameter regularly studied in constructed wetlands is the removal of phosphorus. Unlike nitrogen, the primary removal mechanism for phosphorus is adsorption onto the substrate (Healy and Cawley, 2002). Phosphorus may be taken up by the wetland plants, but it will be returned to the wetland when the plants die unless they are harvested (Bastviken et al., 2003). Also, unlike nitrogen, wetlands contain no pathway for gaseous removal of phosphorus. Often phosphorus removal will be high in

the first few years as wetland plants are growing and phosphorus is sorbing to the fresh sediment. Over time, however, plants begin to decay and the sediment becomes saturated, greatly reducing the treatment efficiency for phosphorus in the long term (US EPA, 1999; Stearman et al., 2003). A study by Vymazal (2005) found the total phosphorus removal rate to range from 41.1 to 59.5% in four constructed wetlands of different design types whereas Healy and Cawley (2002) found a mean removal rate of only 13% in a comprehensive two year study on a constructed wetland in Ireland. It is often necessary to do additional water treatment to remove phosphorus if the water is going to be released into a surface water body to meet the state water quality parameters (US EPA, 1999).

#### **The Effects of Conditions and Design on Removal Efficiencies**

In addition to testing nutrient removal, wetlands are usually monitored for the presence of dissolved oxygen, pH, and temperature. There is still much debate over the role these factors play in removal efficiencies. Temperature, hydraulic loading rates, and substrate composition had no effect on treatment efficiency for lead and zinc removal in a constructed wetland (Song et al., 2001). In addition, Stearman et al. (2003) examined pesticide removal in constructed wetlands and found that loading rate was not an important factor. However, it is generally accepted that both temperature and residence time play an important role in treatment efficiencies (Healy and Cawley, 2002).

Residence time is probably the most important design element that influences removal efficiencies, but different contaminants require different hydraulic residence times for their removal (Stearman et al., 2003). Research suggests that there is an optimum time for hydraulic residence time beyond which there is little additional

removal of pollutants. For example, Vidales et al. (2003) showed a 99% removal rate of viruses after five days of treatment, and Stearman et al. (2003) showed removal rates for pesticides did not increase after five days. Early wetland design specifications recommended four days as a sufficient residence time for effective treatment of TSS and BOD as longer times do not significantly improve efficiency for these parameters (US EPA, 1993). Because flow rates vary depending on the design of the constructed wetland, some hydraulic residence times may be much longer than four days, and some shorter. Hydrological limitations can also influence the effective functioning of a constructed wetland. If the water level falls too low below the surface in these wetlands, the removal efficiency dramatically decreases (Healy and Cawley, 2002).

#### **Comparison of Horizontal Flow (HF) and Vertical Flow (VF) Wetlands**

Most wetland research has been conducted on HF gravity-fed systems (Figure 1.3) in which water percolates through the saturated gravel bed. These systems are quite

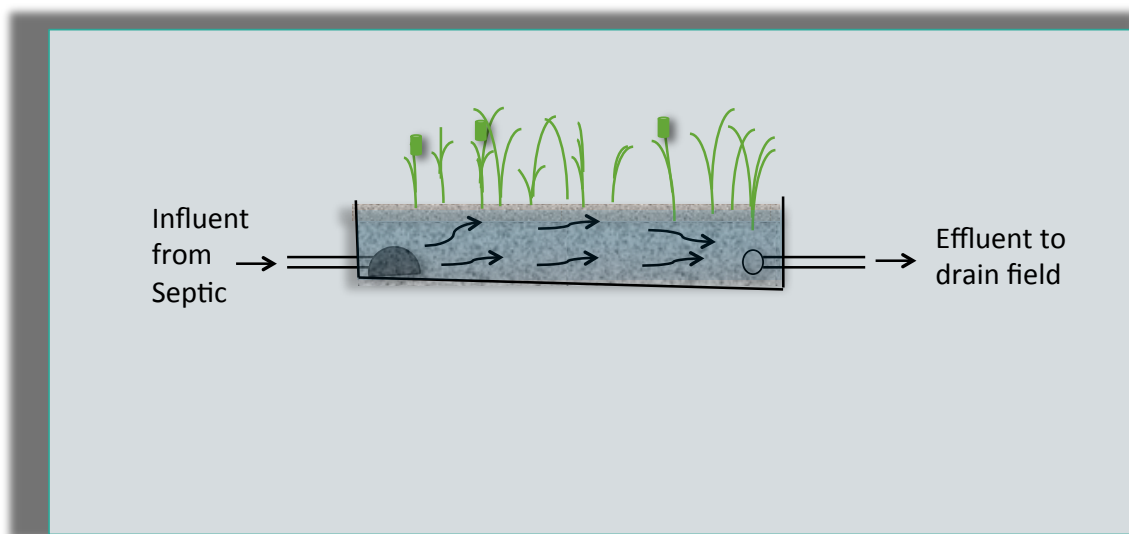


Figure 1.3 – Schematic of a Horizontal Flow Constructed Wetland

effective at nutrient removal, reducing the biochemical oxygen demand, and removal of fecal bacteria. Recent research on VF wetland designs (Figure 1.4), in which water is recirculated to the top of the gravel bed, has shown these systems to be even more effective than HF systems at bacterial and nutrient removal. Well-designed recirculating VF systems have been shown to remove up to 99% of fecal bacteria and 80% of other commonly measured contaminants (Garcia-Perez et al., 2008). These VF systems also have a smaller footprint, so they are good for spaces with small land area, such as single-family residences or small businesses.

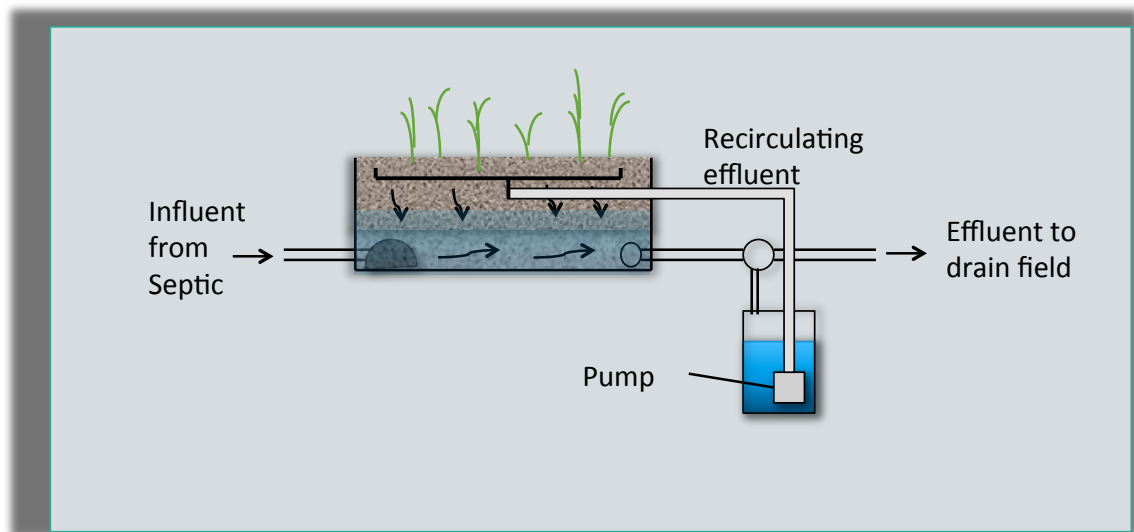


Figure 1.4 – Schematic of a Vertical Flow Constructed Wetland

The HF constructed wetlands are considered anaerobic treatment systems (D.O. ~ 1.5 mg/L) while the VF wetlands are considered more aerobic (D.O. ~ 1.8-4.0 mg/L). Because VF systems have a portion of unsaturated gravel and higher DO levels they are better at reducing BOD and ammonia than HF systems (Matamoros et al., 2009). VF systems are limited in denitrification, however, because there are no anaerobic conditions

(Vymazal, 2007). HF systems have anaerobic conditions for denitrification but they do not have the aerobic conditions to facilitate ammonia nitrification. Since the concentrations of DO are not as low in HF wetlands as they are in WWTP anaerobic treatments, dissolved oxygen may not be quite as limiting in these wetlands as it is in WWTPs. In centralized plants the longer retention times were positively correlated with triclosan removal. Horizontal Flow (HF) constructed wetlands are designed to have a longer hydraulic residence time than Vertical Flow (VF) wetlands which can have HRTs as low as a few hours in some cases (Matamoros et al., 2009). Hydraulic retention time may prove to be even more important for triclosan removal in these wetlands than the difference in DO concentrations, since the DO concentrations even in HF wetlands can be comparable to those in activated sludge treatments in WWTPs (~1.5-2.0 mg/L) (Thompson et al., 2005). Zhang et al. (2012) examined removal rates for eight different PPCPs (not triclosan) in mesocosm scale subsurface wetlands at different HRTs. They found a higher removal percentage for all eight compounds at an HRT of four days compared with an HRT of two days. Perhaps the high DO levels in VF systems is countered by the longer HRT in HF systems in regards to removal efficiencies. In a study of 13 PPCPs in five HF wetlands and four VF wetlands Matamoros et al. (2009) found no significant difference in removal efficiencies between the two systems. The same may be true of triclosan in these systems and both DO levels and HRT have been shown to be important factors in removal rates.

It should be noted that both of HF and VF systems are secondary treatments that receive influent that comes from a septic tank and then release effluent into a drainfield or some other approved soil adsorption system for further treatment (Garcia-Perez et al.,

2008). Therefore, the removal rates for the complete onsite systems would have even higher removal rates than those cited for the wetlands alone.

### **Research on Non-Nutrient Chemicals in Constructed Wetlands**

Even though most research on constructed wetlands has focused on the common parameters mentioned above, research has now begun to investigate the removal rates for other organic and inorganic chemicals that are found in wastewater. Particularly, as there is rising concern about environmental contamination and the occurrence of pharmaceuticals and personal care products in the environment, it is important to investigate the efficiency of these wetland systems for their removal (Matamoros et al., 2005). Most studies show significant, but not complete, removal of these pollutants from the wastewater. A study by Keefe et al. (2004) showed a removal efficiency between 63% and 87% for four different volatile organic compounds. Matamoros et al (2005) found a removal rate between 48% and 81% for ibuprofen in a 2000 study conducted on two subsurface wetlands in Spain. Song et al. (2001) used lab scale experimental wetlands to examine the removal of lead and zinc and they found removal rates of 90% and 72%, respectively. Selenium removal from subsurface test wetlands has been shown to vary from 48 to 76% (Gao et al., 2003). Stearman, et al. (2003) found the mean removal of 60% for the pesticide metolachor at several greenhouse wetlands with a maximum of 79%. These studies do show that constructed wetlands are able to at least partially remove some non-nutrient compounds and other organic chemicals.

The actual removal mechanisms that results in the reduction of these chemicals is not completely understood, and more research is needed. One potential mechanism could be the filtration in gravel beds; chemicals sorb to the particles which are suspended in the

water that are then filtered out when they enter gravel bed (Thomas and Foster, 2004).

The pollutants may also sorb to the gravel itself, or undergo biodegradation (Thompson et al., 2005). If the contaminants are filtered by the gravel bed or sorbed to the surface of the gravel, this is still not necessarily a complete removal from the environment. At some point the gravel may be removed or changed, and the ultimate fate of the pollutants would be unknown, which could still pose a threat to the environment (Wilcox et al., 2009).

### **Mechanisms for Removal of Triclosan in Constructed Wetlands**

Some predictions regarding the fate and removal of triclosan in a constructed wetland can be made based on the results of studies that have investigated the fate of triclosan in the environment and in WWTPs. Because triclosan is a hydrophobic compound and it sorbs easily to particles, it has been found in high concentration in the sludge of wastewater treatment plants (Singer et al., 2002). This means that triclosan probably also attaches to the sediment in a subsurface constructed wetlands. If the medium of the constructed wetland is not changed, the sediment could become saturated and removal rates would be reduced. The biological degradation of the triclosan compound seems to happen under aerobic conditions and also in the presence of light, but not in anaerobic conditions (McAvoy et al., 2002). Because wetland conditions are primarily anaerobic, this may suggest that the removal of triclosan compound may not occur via these physical and biological processes. A free water surface wetland may allow for some photodegradation and biodegradation to occur due to a higher concentration of dissolved oxygen in this type of wetland. Lishman et al. (2006) found that finishing treatment lagoons reduced triclosan levels to an undetectable level at three WWTPs with lagoons as finishing

treatments. They attribute this to a combination of photodegradation and a long HRT in these free surface water treatments. However, biodegradation is less likely to occur in the subsurface wetlands,. A laboratory study by Reinhold et al. (2010) found that microbial degradation does not significantly contribute to the reduction of triclosan in water and that sorption was the dominant mechanism when organic matter was present.

Hydraulic residence time has been suggested to be an important factor in triclosan removal in both WWTP treatments and also in onsite systems. This may be important for this study, as HF constructed wetlands have a much longer residence time than VF wetlands.

Since constructed wetlands are similar to sand filters in many ways, this suggests that the removal rates in constructed wetlands could be as low as those of WWTP sand filters. One distinct difference is that the constructed wetlands have aquatic plants that may change the bioflora and provide additional biodegradation compared to a simple sand filter.

### **Triclosan Removal in Pilot Scale Studies and Constructed Wetlands**

Little research has been conducted on the removal of triclosan in constructed wetlands systems. Most of the studies on these systems were conducted on finishing treatments or pilot study treatments at WWTPs where the wetlands were receiving WWTP effluent. These systems are larger and may have much longer hydraulic residence times than the small systems for single family residences (Reyes-Contreras et al., 2011). Preliminary studies on these WWTP finishing wetlands showed that they can significantly reduce triclosan concentrations from the WWTP effluent they receive (Waltman et al., 2006; Park et al., 2009; Reyes-Contreras et al., 2011; Matamoros, 2012). Studies of the free

surface wetlands have shown that a wide range of variability in the reduction concentrations of triclosan was related to seasonality, but the reasons for this seasonal variation is still debated. Thompson et al. (2005) found the removal rates in a reed bed to range from 0 to 71% and they concluded that the low percent removals are most likely due to a low water temperature in the winter months resulting in low biodegradation rates. Matamoros (2012) found a greater than 80% triclosan removal rate across all seasons in a finishing treatment system that included open water polishing ponds and free surface water constructed wetlands. The finishing pond that did not have wetland vegetation had the highest removal rates, which was attributed to the direct exposure to sunlight without interference from plants. Similarly to Thomspson, et al. (2010), Matamoros et al. (2012) found a significant reduction in the removal rate during winter months. He suggested, however, that this is due more to the shorter day length and decreased sun exposure than to temperature reductions. This same study also found that subsurface removal rates remained stable at different temperature conditions. Matamoros et al. (2012) conclude that photodegradation is most likely an important removal mechanism in free surface water wetlands. Reyes-Contreras et al. (2011) also examined a pilot treatment system of constructed wetlands receiving effluent water from a WWTP and found high fluctuations in triclosan removal rates during the year (very high levels in the summer and almost zero removal in winter) when the free surface flow constructed wetland was employed, while the removal rate for the subsurface constructed wetland remained relatively constant throughout the year. This difference was attributed to the oxygen exchange at the water surface that allowed for oxidation and photo-oxidation to occur in the free surface flow wetland during summer. Overall, these studies suggest that

hybrid systems which contain both free surface flow constructed wetlands and subsurface wetlands perform better for triclosan removal than one type of wetland alone (Reyes-Contreras et al., 2001; Matamoros, 2012).

Onsite constructed wetlands systems, however, consist of a single subsurface treatment cell that receives the septic tank effluent. Research on this type of system is limited. Although the subsurface constructed wetlands in the above studies showed a significant level of triclosan removal, the triclosan concentrations were already lower in these systems than the concentrations in raw wastewater because the water had already been treated at the WWTP. Park et al. (2009) studied a subsurface constructed wetland receiving raw wastewater and found a removal rate greater than 60% although the mechanism of removal was not determined.

### **Conclusion**

There is a growing awareness of the problem of pharmaceuticals and personal care products reaching the environment through the waste stream. Very little is known about the fate of these chemicals in the environment and how a complex mixture of PPCPs will interact and combine in wastewater. Even less is known about the effects these chemicals may have on the ecosystems.

Triclosan has been found in lakes and streams in the U.S. as well as many countries around the world. One of the primary avenues for triclosan to enter the environment is through wastewater, as it is found in soaps, detergents and other personal care products that are washed down the drain. Very little is understood about how

triclosan will affect aquatic systems, but research is beginning to show that it has the potential to deplete the species diversity of aquatic plant communities. It also has been shown to interfere with the metabolism of animal species, both vertebrate and invertebrate. Triclosan has also been found in samples of drinking water from reclaimed water sources as well as in a large percentage of breast milk samples from around the United States. This indicates that triclosan contamination may have human health implications in addition to any environmental impacts.

It is important to consider not only triclosan, but also the derivatives that form during the degradation process, which are of great concern. Methyl-triclosan has been found to bioaccumulate in fish and may have negative effects. In the presence of ultraviolet light, triclosan has been shown to form a carcinogenic dioxin derivative. There are also many unknown metabolites of triclosan with unknown health or ecosystem effects (Bester, 2007).

Traditional wastewater treatment removes varying amounts of PPCPs from wastewater. For triclosan, centralized wastewater treatment in WWTPs remove a high percentage of triclosan from the wastewater. Studies have shown removal as high as 95-99% for some plants (Bester, 2003; Gryaab, 2004; McAvoy, 2002; Singer, 2002). However, triclosan still reaches surface waters at levels that have the potential to negatively affect aquatic systems.

Much of the treatment of wastewater in rural and even some suburban communities in the United States is performed with onsite treatments. Constructed wetlands are one alternative onsite treatment option. They are often used as a secondary treatment to enhance the total system function over a simple septic tank and drainage

field. It is important to understand how these wetlands function and to explore how they remove non-nutrient chemicals such as pharmaceuticals and personal care products.

Although no study has investigated the fate of triclosan in full-scale, operational onsite constructed wetlands, a few studies have examined the removal rates for other chemicals and found a wide range of removal rates, depending on the nature of the chemicals.

There is much research yet to be done on constructed wetlands and the removal of PPCPs in these systems. It is also important to examine how different designs of constructed wetland may play a role in the removal rates of triclosan and other PPCPs.

The subsequent chapters describe a number of investigations that were performed to explore the removal of triclosan in constructed wetlands. The second chapter details two periods of triclosan testing performed on functioning onsite wetlands. Data was used to determine the removal percentages and to estimate the overall removal efficiency of constructed wetlands for the removal of triclosan from wastewater. This research also investigates two different constructed wetland designs, vertical flow (VF) and horizontal flow (HF), and compares their triclosan removal rates. The third portion of chapter 2 details an injection study at one HF wetland to determine the percent removal at that wetland. Chapter three discusses a microcosm-scale study of the role of wetland plants in triclosan removal to help understand the mechanisms for triclosan removal from wastewater in constructed wetlands. Both, Chapter 2 and 3 are manuscripts that will be submitted for publication. Chapter four and five discuss results of experiments and data that were used in support of experiments in Chapter 2 and 3. Specifically, Chapter 4 describes the determination of the hydraulic retention time (HRT) in one HF subsurface wetland and Chapter five describes the methods used to collect background data on a

constructed wetland to support the research in Chapter two. Table 1.1 shows the timeline of data collection and the pertinent chapters where the details of the research can be found.

Table 1.1 – Research summary of data collection times and chapter locations.

<b>Data Collection Period</b>	<b>Description</b>	<b>Chapter</b>
June-July, 2007	Background data collected on Site C constructed wetland (at Rieth Village). Influent and effluent sampled for iron phosphorus, ammonia, nitrite, nitrate, and <i>E. coli</i>	Methods - Chapter 5; Results - Chapter 2 and Appendix A
June - July, 2008	Background data collected at Site C constructed wetland. Influent and effluent sampled for pH and DO	Methods - Chapter 5; Results - Chapter 2 and Appendix A
June - July, 2008	Triclosan removal study of three operational HF constructed wetlands.	Chapter 2
September-October, 2008	Triclosan removal study of six operational constructed wetlands (three VF and three HF wetlands)	Chapter 2
June-July, 2009	Dye tracer study conducted of Site C constructed wetland to determine hydraulic retention time.	Chapter 4 (results mentioned in Chapter 2)
September-November, 2009	Triclosan injection study conducted at Site C to determine percent removal with a breakthrough curve.	Chapter 2
July-August, 2011	Laboratory scale study of the removal of triclosan with four species of wetland plants.	Chapter 3

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## CHAPTER 2 – EFFICIENCY OF CONSTRUCTED WETLANDS TO REMOVE AN ANTIMICROBIAL AGENT TRICLOSAN FROM WASTEWATER

### **Abstract**

Triclosan is a common anti-bacterial agent found in a wide range of medical and personal care products and today is frequently used as an active ingredient in most anti-bacterial soaps sold commercially. Triclosan has been identified as a chemical of concern because of its potential as an endocrine disrupter and the carcinogenic nature of some of its chemical derivatives (US FDA, 2008). The primary route for triclosan to enter the environment is through wastewater; therefore, it is important to understand how efficient wastewater treatment is in its removal. Previous research has focused on centralized wastewater treatment facilities and triclosan removal while research for onsite wastewater treatments, like constructed wetlands, has focused on pilot-scale and laboratory studies. The goal of this study was to determine the efficiency of operational subsurface constructed wetlands to remove triclosan, and to compare the removal efficiencies of two different constructed wetland designs. This study investigated a total of eight constructed wetlands in two different study periods. Five horizontal-flow gravity and three recirculating vertical flow systems were studied in LaGrange, LaPorte, and Noble counties

of Indiana in 2008. Water samples were collected from the influent and effluent pipes of the wetlands and analyzed for triclosan weekly over seven weeks in June and July of 2008 in three HF wetlands for the first study, and twice a week for 10 weeks from August through October of 2008 in three HF wetlands and three VF wetlands for the second study. Concentrations of triclosan were measured using a magnetic particle enzyme immunoassay method (Abraxis Testing Solutions, Warminster, PA). The percent removal of triclosan in the wetland cells ranged from 15.6 to 56.2%. A Type III test for Fixed Effects model showed no significant differences in triclosan removal rates between the two different wetland designs. There was no correlation between the rainfall, water depth, or temperature, and the removal rate of triclosan.

## **Introduction**

Triclosan is a polychlorinated aromatic antimicrobial agent that has become widely used in a host of consumer products such as soaps, detergents, toothpaste, clothing, and deodorants. In 2002 the United States Geological Survey conducted the first large-scale study to examine the occurrence of Pharmaceuticals and Personal Care

Products (PPCPs) in surface water bodies across the country in which they monitored 95 organic waste compounds in 139 streams across 30 states (Kolpin et al., 2002). Triclosan, one of the seven most frequently detected compounds, was detected in 57.6% of the water samples.

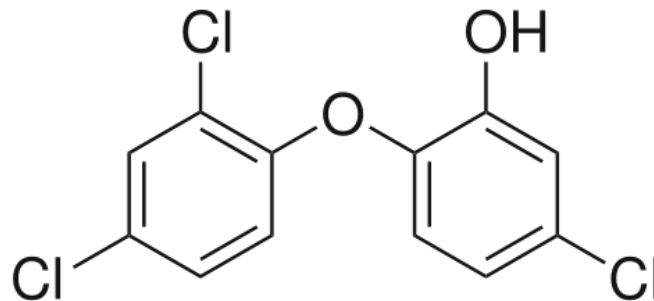


Figure 2.1 – Model of the chemical structure of triclosan.

Triclosan occurrence in the aquatic ecosystem is not only problematic as a potential contaminant, but it is also a concern for human populations. Although triclosan has been considered safe for use in soaps and cleaning products (US FDA, 2008), there is very little information about the health effects on humans exposed to triclosan via drinking water and it is currently under review by the US FDA and the EPA for its potential to act as an endocrine disrupter. This is a concern because there is evidence that triclosan is already in human populations as several studies found concentrations as high as 300 µg/kg lipid weight in human breast milk (Adolfsson-Erici et al., 2002; Toms et al., 2011).

Much of the effects of triclosan on aquatic ecosystems are still unknown but a growing body of evidence suggests that even at low concentrations it may be hazardous in aquatic environments (Ferrari et al., 2002) and, if high concentrations of triclosan enter the environment, there could be serious impacts on the ecological balance of these systems (Tatarazako et al., 2004). Triclosan has been shown to cause endocrine disruption and abnormal growth in tadpoles and bivalves grown in water spiked with low

concentrations of triclosan (Veldhoena et al., 2006; Canesia, 2007). Of a greater concern for human health is a recent study on rats that demonstrated the role of triclosan in thyroid hormone homeostasis (Crofton et al., 2007). Even low concentrations of triclosan in wastewater may have an impact due to its accumulation and concentration in sewage sludge, its persistence in the environment, and its potential to bioaccumulate in animal tissues (Balmer et al., 2004; Boehmer et al., 2004).

The main path for triclosan to enter the environment is through wastewater. Centralized wastewater treatment plants (WWTPs) remove triclosan at rates between 58% and 99% depending on the treatment methods (McAvoy et al., 2002; Singer et al., 2002; Bester, 2003). Research suggests that the two main mechanisms for its removal in wastewater treatment are sorption to the substrate and biodegradation (McAvoy et al. 2002; Singer et al. 2002; Thompson et al., 2005; Reinhold et al., 2010). Singer et al. (2002) found that 79% of triclosan degraded during wastewater treatment, and another 15% was removed through sorption to sludge. Similarly, Bester (2003) found that 5% of triclosan entering the wastewater treatment plant was dissolved in the effluent water.

The aeration process was identified as the dominant mechanism for triclosan elimination from wastewater with higher removal rates obtained by activated sludge processes (96%) than by a trickling filter (58-86%) (McAvoy et al., 2002). Additionally, a two-stage activated sludge process removed more triclosan than a combination of physical treatment and an activated sludge process (Bester, 2003).

Although most wastewater from urban areas is treated by centralized WWTP systems, around 21% of households in the United States use decentralized wastewater

treatment systems (U.S. Census Bureau, 2008) and up to one third of all new housing and commercial development employ onsite wastewater treatment systems (US EPA, 1993). Since triclosan is found in many household products that are used daily, such as soaps and toothpaste, it is prevalent in household wastewater. Conn et al. (2010) found triclosan in all of the septic systems they tested with concentrations ranging from 0.4 to 230.0 µg/L and they estimated a median triclosan contribution of 3.7 mg/capita/day into household septic tanks.

The fate of triclosan in rural wastewater is a concern because the US EPA estimates that between 10 and 20% of current onsite systems do not adequately treat wastewater even for typically measured wastewater parameters, and about 50% of those systems are at least 30 years old and beginning to fail (US EPA, 2005). State water quality agencies view septic systems as the second greatest threat to the quality of our nations' water sources (US EPA, 1993). As a result, many communities and developers are considering the use of alternative onsite systems, such as constructed wetlands or onsite filters, to ensure an efficient treatment of wastewater in rural communities.

Constructed wetlands are one of the alternative on-site wastewater treatment methods and they are becoming an increasingly popular method of wastewater treatment for small communities as well as animal farm operations. As with most wastewater treatment systems, constructed wetlands were designed and monitored for their efficacy to remove harmful bacteria, biodegradable organic compounds and, to some extent, nutrients (US EPA, 1993; Bastviken et al., 2006; Bester, 2005; Seabloom and Hanson, 2004). A few studies have investigated the effectiveness of constructed wetland systems

in their removal of various synthetic organic pollutants. Keefe et al. (2004) showed that a subsurface constructed wetland removed 63 to 87% of four different volatile organic compounds, while Matamoros et al. (2005) found the removal rates for Ibuprofen to be in the range of 48% and 81% in two subsurface constructed wetlands.

Very little research has been done to study the efficiency of these constructed wetland systems to remove emerging contaminants, such as triclosan. Several studies have examined constructed wetlands as finishing treatments in centralized systems. Waltman et al. (2006) showed a 67.9 % reduction in triclosan concentrations in a pilot scale constructed wetland that received effluent water from a wastewater treatment plant. Thompson et al. (2005) found that a removal of triclosan in a reed bed receiving effluent water from a WWTP was highly variable and ranged from 0 to 71%. The authors suggested that low temperatures might have contributed to some of the lowest removal rates. Matamoros and Salvadó (2012) studied a similar reclamation pond and a surface flow constructed wetland that was receiving effluent from a WWTP. They found high rates of triclosan removal (>85%) and suggested photodegradation as the primary mechanism for its removal in the systems with surface water flow, however, subsurface wetlands do not allow for photodegradation to occur.

One important additional factor that may play a role in the removal rate of triclosan in these wetlands is the plant community and its composition. Zhang et al. (2012) found that planted HF wetlands removed significantly more PPCP compounds than unplanted wetlands, though triclosan was not included in the study. In a review of 35 published studies on the role of plants in the performance of constructed wetlands for

the removal of PPCPs, Brisson and Charzarenc (2009) found that in a majority of the studies' wetland plants did contribute significantly to pollutant removal. However, preliminary investigations have shown that the uptake of triclosan by plants may not be sufficient to significantly reduce triclosan levels in a constructed wetland. Although different plants varied in the amount of triclosan in their tissues, the researchers concluded that further study is needed to investigate the uptake or facilitated removal by particular plant species (Chen et al., 2009; Wu et al., 2010; Zarate Jr. et al., 2012).

Thus far, however, no studies have examined the effectiveness of onsite subsurface constructed wetlands systems to remove triclosan in full-scale onsite operational wastewater treatment systems. Park et al. (2009) examined subsurface wetlands used as a finishing treatment at a WWTP and found removal rates in the range of 60 to 100%. This suggests that onsite subsurface constructed wetlands may be quite effective at triclosan removal but it is important to investigate onsite systems to see if the results compare with the results from the Park et al. study.

The goal of this study is to increase an understanding of the effectiveness of constructed wetlands to remove triclosan from wastewater. Specifically, the aim of this research is to determine the occurrence and fate of triclosan in subsurface constructed wetlands and to compare two different constructed wetland designs, horizontal flow (HF) and vertical flow (VF) wetlands, for their removal efficiencies. As of 2004 there were at least 5,000 constructed wetlands in Europe and over 1,000 constructed wetlands in North America used for onsite wastewater treatment (US EPA, 2004), and the number continues to increase. It is important to understand how well these wetlands function not only for

commonly measured wastewater parameters but also for emerging contaminants such as triclosan and other PPCPs.

## **Materials and Methods**

### **Studies**

There were three study components to this research: 1) a study on three operational HF wetlands in LaPorte and Noble counties in Indiana to determine triclosan removal rates in HF wetlands (aka The HF study), 2) a study of six operational constructed wetlands to compare HF and VF wetlands and their triclosan removal rates (aka The wetland design comparison study), and 3) a triclosan injection study at one HF constructed wetland to further investigate triclosan removal under controlled conditions (aka The triclosan injection study). Both The HF study and The wetland design comparison study were conducted in operational wetlands, and all three studies were conducted during summer months to control the variability of environmental conditions on treatment efficiency.

### **Study Sites**

The efficiency of triclosan removal was studied at eight subsurface constructed wetlands in LaGrange, Noble, and LaPorte counties in Northern Indiana (Figure 2.2). Five wetlands have a horizontal-flow (HF) design where the water is gravity-fed, and the other three wetlands have a recirculating vertical flow (VF) design where the water is actively circulated through the system five or six times, on average, before exiting (Table 2.1).

Two of the HF wetlands, sites A and B, are located at Multiplex site, a small auto parts manufacturing plant with 200 employees. These wetlands operate in parallel, meaning they receive portions of the same septical effluent. Each of these wetlands was designed to receive 2250 Gallons per Day (GPD). At these sites, water samples were taken both before the water was partitioned into the two wetlands and then from the separate effluent pipes. Site C, a HF wetland, is located at Rieth Village (Merry Lea Environmental Learning Center of Goshen College), an ecological field station with one



Figure 2.2 – Location of the eight studied constructed wetlands in northern Indiana.

office building and two small student residences. This site was used as a comparison site, so data were collected from this site during both study periods. This site has a capacity to serve 32 residents and 5 employees at peak flow, and the wetland was designed to receive

Table 2.1 – Summary of wetland sites investigated by this study.

<b>Site Name</b>	<b>Location</b>	<b>Description</b>	<b>Wetland Design Type</b>	<b>Design flow (GPD)</b>
Site A	Howe, IN	Small manufacturing plant	Horizontal Flow (HF)	2250
Site B	Howe, IN	Small manufacturing plant	Horizontal Flow	2250
Site C	Wolf Lake, IN	Offices and student cottages	Horizontal Flow	2300
Site D	LaGrange, IN	Single-family home	Vertical Flow (VF)	450
Site E	LaGrange, IN	Single-family home	Vertical Flow	450
Site F	LaGrange, IN	County animal shelter	Vertical Flow	480
Site G	Michigan City, IN	Cluster of single-family homes	Horizontal Flow	975
Site H	Michigan City, IN	Cluster of single-family homes	Horizontal Flow	975

2300 GPD; however, during the two study data collection periods, the buildings were not capacity. Based on the occupancy at the time of data collection, the estimated flow was 500 GPD during the first study of three HF wetlands and only 150 GDP during the time of the wetland design comparison study.

Two of the VF wetland systems (sites D and E) are located at single-family residences and both systems were designed to receive 450 GPD. The third VF system

(site F) services an animal shelter and was designed to treat 480 GPD. Sites G and H are located at Tryon Farms, a small housing community receiving the combined wastewater from 10 residential units with a total of 13 bedrooms. The system is designed to handle a peak flow of 1950 GPD, with each wetland receiving half (975 GPD).

### **Triclosan Injection Study**

The constructed wetland at Rieth Village (Site C) was selected for a controlled study of triclosan removal to help verify the removal rates found during the monitoring study of six working wetlands. The injection tracing was conducted twice, once on September 12, 2009 and again on October 15, 2009, by injecting triclosan solution into the wetland influent and then monitoring the subsequent triclosan concentrations in the wetland effluent. First, the hydraulic residence time (HRT) of wastewater for this wetland was determined using a tracing dye to predict the time of triclosan breakthrough in this wetland and to assist in the logistics of the study. At two different times, a total volume of 473 ml of fluorescent yellow/green liquid tracing dye (Bright Dyes, Miamisburg, OH) was added to the central opening in the influent pipe, and subsequently, the effluent water was sampled every day for three weeks. The concentration of dye in water samples was analyzed using an HACH DR/890 colorimeter (HACH Company, Loveland, CO). The mean residence time between the injection and peak dye concentrations in the effluent was calculated from the two runs to establish the estimated HRT for the wetland (see Chapter 4 for more details).

To determine the removal rate of triclosan at Site C, 150 mg of triclosan (5-chloro-2-(2,4-dichlorophenoxy) (Thermo Fisher Scientific, Pittsburg, PA) was dissolved in 500

ml of methanol and then added to the influent pipe where the water collects before percolating into the wetland cell. After three hours of mixing in the influent pipe, the influent concentration of triclosan was 2.20 $\mu$ g/L; this concentration was higher than any ambient concentration of triclosan that had been recorded for this wetland previously. It was important to measure the concentration of triclosan in the influent after the triclosan was injected to obtain accurate concentration data, as there was a potential for an additional contribution of triclosan to enter the wetland from the septic tank. The concentrations of triclosan in both the influent and effluent were subsequently monitored every other day for an 18-day period, until the peak triclosan concentration was detected. A breakthrough curve was plotted as a relative concentration of triclosan versus time. The percent removal was then calculated as a ratio of the triclosan concentration at the time of injection and the mean concentration of triclosan at the peak breakthrough found in the effluent.

### **Triclosan Removal Study – Sample Collection**

Two triclosan removal studies were conducted: the first study (HF study) was performed at three HF constructed wetlands (Sites C, G, and H, Figure 2.3). These three sites were visited every Monday from June 16, 2008 to July 25, 2008, and grab samples were taken from the influent and effluent of each wetland at each visit for a total of seven sampling events. The wetland design comparison study was performed on six constructed wetlands to compare HF and VF wetland design for triclosan removal.



Figure 2.3 – Sites C, G, and H and the building from which they are receiving wastewater.

Site C was included in both studies as a comparison. In the second study, each wetland was sampled bi-weekly from August 15 through October 21, 2008 for a total of 20 sampling events.

Grab samples with a volume of eight ml each were collected from both influent and effluent of each wetland in both studies. Sample collection and handling was performed according to the method recommended by the test manufacturer. Two mL of methanol were added to each sample to prevent triclosan adsorption to the glass walls of a sampling bottle. Preserved samples were stored on ice for transportation, and then refrigerated at 7°C until analysis.

### **Analysis of triclosan**

To determine the removal rate of triclosan by a wetland system, the concentration of triclosan was determined by a magnetic particle-based immunoassay (ELISA) (Abraxis

Testing Solutions, Warminster, PA), which detects combined triclosan and triclosan methyl in the sample water. Abraxis-supplied standards of triclosan solutions (0 µg/L, 0.025 µg/L, 0.1 µg/L, and 1.0 µg/L) were used to construct a standard calibration curve; each standard solution was analyzed in duplicate. The standard curve was constructed using Microsoft Excel (2007) by plotting the %B/B<sub>0</sub> (absorbance value for each standard/absorbance value for the zero standard) against the corresponding known triclosan concentrations for the standards. For the HF wetland study at sites C, G, and H in June and July, one analysis was performed for each sample and for the wetland design comparison study and the triclosan injection study, all collected samples were analyzed in duplicate. The triclosan concentration of collected samples was determined by use of the standard calibration curve. The Abraxis triclosan assay has an estimated minimum detectable concentration of 0.1 µg/L based on a 90% B/B<sub>0</sub> (Shelver et al., 2007). An analysis of a laboratory blank and a methanol solution blank was performed every time the samples were analyzed together with the control sample and eight standard samples (Appendix F). The results of these quality control samples showed no potential quality control problems during any of analyses performed in this study.

### **Recording Environmental Variables**

pH, water temperature, and concentrations of dissolved oxygen (DO) were measured at both influent and effluent of each investigated wetland at the time of sampling using a HACH sensION pH Meter and Dissolved Oxygen Meter (Loveland, CO). The probes were calibrated before each use according to the manufacturer's recommendation. Additionally, the water level in each constructed wetland and air temperature were

recorded at the time of sampling, while the total weekly rainfall for the week preceding each sampling event and the average weekly air temperature were obtained from the Indiana State Climate Archive Data (Indiana State Climate Office, Purdue University, Station ID 124837, NEPAC, 124730). All stations were within ten miles of the study sites.

For the wetland design comparison study, additional historic water quality data for sites A, B, D, E and F were obtained from the LaGrange County Health Department (Lagrange CHD) for the period from 2004 through the time of this study. The analysis of the water samples was from a certified US Environmental Protection Agency approved testing laboratory. Specifically, data included the concentrations of dissolved oxygen, oxidation reduction potential, pH, ammonia nitrogen, nitrates, biochemical oxygen demand, total suspended solids, TKN, *E. coli*, total nitrogen and total phosphorus, which were used to provide an insight into the basic conditions and functioning of these wetlands. The data was collected from the influent and effluent pipes in the wetlands. The data from sites A and B were sampled from the water before separation into the two wetlands and then after the water effluent from two wetlands were combined; therefore, only one value is given for the two sites. Water quality data for Site C were collected in 2007 and 2008 (See Chapter 5 for method details).

### **Analysis of Plant Composition**

At each monitored wetland, the vegetation was assessed for the frequency of occurrence and percent cover using the line-intercept method (Mueller-Dombois and Ellenberg, 1974; Coulloudon, 1999; Madsen, 1999). Ten three-meter transects were established in

each wetland to conduct the analysis. Given that constructed wetlands are planted with a range of different species in zones placed perpendicular to the water flow, the transects were run in the direction of the water flow starting at the influent distribution pipe. For each meter of all transects, every species was noted as either present (1) or absent (0) (Madsen, 1999). The number of meter segments in which a species occurred was recorded as the frequency of occurrence. The relative frequency was calculated as the frequency of occurrence of each species divided by the total number species intercepts recorded for all transects. Species that accounted for less than five percent of the intercepts were excluded from the analysis, as it is unlikely they contribute to the wetland function. Species richness counts included only species that accounted for more than five percent of the transect intercepts.

### **Data Analysis**

All data were recorded in Microsoft Excel (2007) and analyzed with SPSS (SPSS Inc, Chicago, IL). The mean concentration of triclosan in the influent and effluent of the eight constructed wetlands were used to calculate triclosan removal efficiency (the percent removal). For samples that had undetectable concentrations of triclosan,  $0\mu\text{g/L}$  was used for the calculations (Waltman, et al., 2006).

For the wetland design comparison study, a repeated measures ANOVA was used to determine significant differences between triclosan concentrations at the influent and effluent of each wetland. A Games-Howell approach was also employed to determine significant differences among the sites because it is robust to unequal variances. The removal rates for the three Vertical Flow (VF) wetlands were compared to the removal

rates for the three Horizontal Flow (HF) wetlands using a Type III fixed effect model. Precipitation, rainfall, water temperature, and dissolved oxygen concentrations at the effluent were included in the fixed effect model. The level of significance was set at  $p > 0.05$ .

## **Results and Discussion**

This section details the results for three studies: 1) the triclosan injection study at Site C in October and November of 2009, 2) the study of triclosan removal in three HF constructed wetlands in June and July of 2008, and 3) the comparison study of triclosan removal efficiency in HF and VF wetland design types from September through November of 2008.

### **Triclosan Injection Study**

The tracing dye showed the peak dye concentrations in the effluent at 15 days (Run 1) and 17 days (Run 2) after the dye injection (Figure 2.2). The HRT at Site C was then estimated to be 16 days (See Chapter 4 for details on this study). Therefore, it would be expected that in the triclosan injection study at the Site C wetland, the highest concentrations would be found around day 16, depending on the water usage and flow rates.

During the first injection experiment (Run 1), the initial concentration of triclosan ( $C_0$ ) injected into the wetland was 2.2  $\mu\text{g/L}$  at the influent. The effluent concentrations ranged between 0.33 and 1.13  $\mu\text{g/L}$  with the highest concentration detected on day 12 (Appendix D). For Run 2 the initial concentration in the influent pipe was 2.3  $\mu\text{g/L}$  and

the effluent concentration ranged from 0.23-1.35  $\mu\text{g/L}$  with the highest triclosan concentration detected on day 14 (Figure 2.4). The percent removal for Run 1 and 2 was 51.5 % and 58.6%, respectively, achieving the mean removal rate of 55.0% for the Site C wetland.

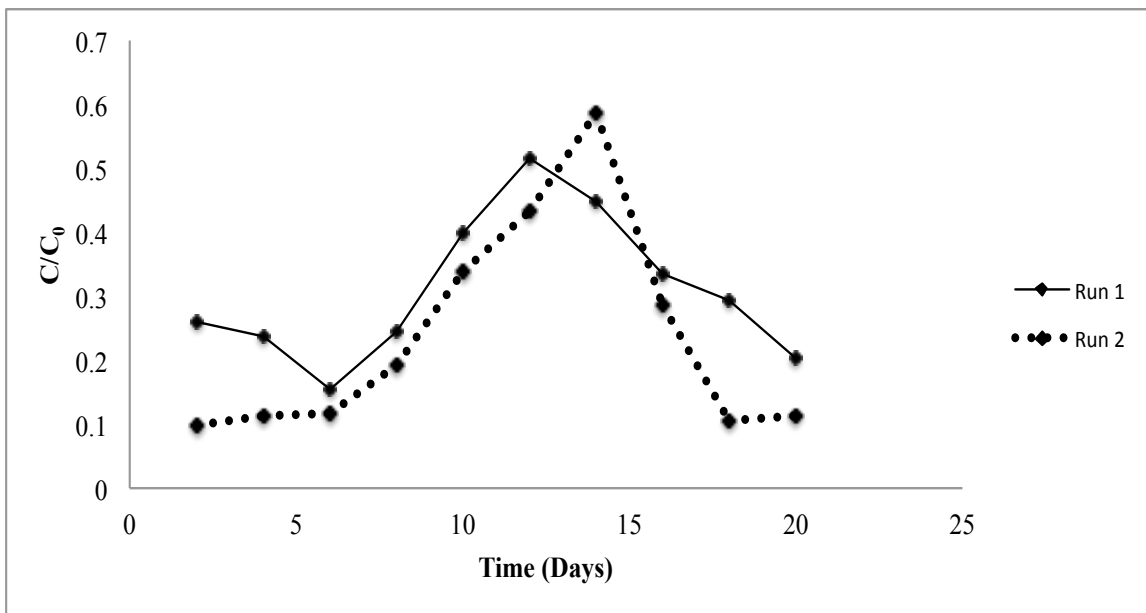


Figure 2.4 – Breakthrough curves for Run 1 and Run 2 of the triclosan injection study. The relative concentration of triclosan in the effluent concentration divided by the initial level of triclosan at the time of injection ( $C_0 = 2.2 \mu\text{g/L}$ ).

### Triclosan Removal in Three HF Wetlands

The DO, water temperature, pH, and depth along with the species richness recorded at sites C, G, and H were similar among the three wetland sites (Table 2.2). The

concentration of DO is within the expected range for subsurface wetland systems that are considered anaerobic (Vymazal, 2005). The DO concentrations were higher in the effluent, which could be a result of wetland plants adding oxygen into the rhizosphere (Brix, 1997; Brisson and Charzarenc, 2009). The DO concentration in the effluent

Table 2.2 –Mean dissolved oxygen, water temperature, pH, and water depth along with species richness at Sites C, G, and H ( $\pm$  SE, N=7).

	Site C	Site G	Site H
<b>DO - Influent (mg/L)</b>	0.200 $\pm$ 0.9	0.212 $\pm$ 0.05	0.234 $\pm$ 0.11
<b>DO - Effluent (mg/L)</b>	0.565 $\pm$ 0.45	0.276 $\pm$ 0.09	0.254 $\pm$ 0.25
<b>Temp. (°C)</b>	20.72 $\pm$ 1.5	17.96 $\pm$ 1.1	18.10 $\pm$ 1.3
<b>Species Richness</b>	4	7	6
<b>pH</b>	7.16 $\pm$ 0.7	7.05 $\pm$ 0.5	7.13 $\pm$ 0.4
<b>Depth (cm)</b>	54 $\pm$ 2	50 $\pm$ 1	52 $\pm$ 4

at Site C was significantly higher than at Sites G and H. There was no correlation between any of these measured environmental parameters and the triclosan removal rates.

A significant amount of triclosan was removed in these three wetlands. The mean influent concentration of triclosan was 0.69  $\mu$ g/L ( $\pm$ 0.1, N=21) with the minimum concentration of 0.38  $\mu$ g/L and a maximum of 0.80  $\mu$ g/L. The mean effluent concentration was 0.46  $\mu$ g/L ( $\pm$ 0.1, N=21) with a minimum of 0.25  $\mu$ g/L and a maximum of 0.70  $\mu$ g/L. The mean percent removal rate for three constructed wetlands (C, G, H) was 32.9% (Table 2.3).

Table 2.3 – Removal of triclosan at Sites C, G, and H constructed wetlands.

	Site C		Site G		Site H	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
<b>Mean (µg/L)</b>	0.629	0.397	0.743	0.501	0.700	0.493
<b>SD</b>	±0.13	±0.14	±0.03	±0.09	±0.01	±0.13
<b>% removal</b>	<b>36.9</b>		<b>32.6</b>		<b>29.6</b>	

SD=Standard Deviation

These three wetlands show a consistent percent removal between 30 and 37%. These wetlands were receiving similar triclosan concentrations in the influent water, they were all designed and built by the same company using similar substrate material and wetland plants. The environmental data that was collected showed the conditions in these wetlands as well as the weather conditions during the sampling time to not differ significantly between sites. This provides good evidence that if these external factors are controlled, a horizontal flow wetland of this design will remove close to the mean removal of 33% as was found in these three wetlands.

Although these three wetlands were of a similar design, Site C had a removal rate that was significantly higher than Site G ( $p=0.05$ ) and Site H ( $p<0.01$ ). Sites G and H were not significantly different ( $p=0.29$ ). One potential reason for this difference may be in the HRTs of wastewater at the sites. Sites G and H were receiving wastewater from fully occupied residences, therefore, the HRT at these sites is likely to be close to the eight days as estimated by the site design. At Site C, however, the wetland design capacity is 2300 GPD but the estimated GPD at the time of testing was 500 GPD based on the building usage at the time of sampling, which would result in a much longer HRT. It is also possible that the higher DO levels in the effluent of this wetland are resulting in

more microbial biodegradation in this wetland. Even though these systems are considered anaerobic, it may be that the higher DO levels in this wetland are allowing for some aerobic degradation to occur. More study would be helpful to examine the correlation between DO levels in HF wetlands and triclosan removal. Another factor could be the age of these wetlands; Sites G and H were constructed in 2000 and Site C was not constructed until 2004. There is the potential, then, that the newer substrate in Site C provided more unsaturated adsorption sites for triclosan.

Although the results of this study demonstrate that HF subsurface constructed wetlands do remove from 30 to 37% of triclosan from wastewater, the rates of removal are much lower than those seen in previous studies at WWTPs where removal rates ranged from 58 to 90% (McAvoy et al., 2002; Singer et al., 2002; Bester, 2003). These removal rates are also lower than those reported by Park et al. (2009) who found that 60-100% of triclosan was removed in subsurface wetlands used as a finishing treatment at a centralized wastewater treatment plant (WWTP). The Park study highlights a problem when comparing percent removals in studies that have dramatically different influent concentrations. Systems with very low influent concentrations can remove a small amount of the pollutant and still show a high percent removal. In the Park et al. study, the concentrations of triclosan entering the wetland ( $< 0.01 \mu\text{g/L}$ ) from the WWTP effluent were much lower than those entering Sites C, G, and H ( $0.6 - 0.7 \mu\text{g/L}$ ), so a 60-100% removal rate in the Park et al. study may only reflect a total removal of  $0.01 \mu\text{g/L}$  of triclosan, whereas Sites C, G, and H removed more triclosan ( $0.2-0.4 \mu\text{g/L}$ ) but resulted in lower percent removal.

The presence of triclosan and other PPCPs in the discharge from constructed wetlands may have a different impact on aquatic ecosystem than when these pollutants are discharged in WWTP effluent. Constructed wetlands release water into drainfields and ultimately into the soil where triclosan sorbs to soil particles while its dispersal in the subsurface is minimal; Carrara et al. (2008) detected triclosan only within 20 meters of the discharge site and not in the surrounding groundwater plumes. In contrast, centralized WWTPs discharge their effluent to surface water bodies where triclosan is highly mobile and often found downstream in lakes where it impacts aquatic plant communities and fish populations (Wilson et al., 2003; Balmer et al., 2004).

### **Plant Composition in Constructed Wetlands**

The plant composition data collected from all eight constructed wetlands showed the dominant plant species to be highly variable among these operational constructed wetlands (Table 2.4). Even though most wetland cells are initially planted with five or six wetland plants, other plants slowly invade the beds causing a change in the plant composition over time. Many non-wetland plant species have migrated into these areas and some of the VF systems (Sites D, E, and F) were even planted with non-wetland plants because the water table is so far below the surface that wetland plants often can't survive. The corn plants observed in Site F were planted in this wetland by the LaGrange County Health Department for experimental purposes to study their efficiency in pollutant removal (Garcia-Perez, 2011). *Scirpus fluviatilis* (River Bulrush) was present in six out of eight wetlands, making it the most commonly observed species. Species

richness was positively correlated to triclosan removal rates ( $r=0.77$ ,  $N=8$ ,  $p=0.02$ ). This sample size is small but it supports the findings of Karathanasis et al. (2003) who found that constructed wetlands with high plant diversity performed better for the removal of fecal bacteria, BOD and TSS. Eight wetland sites did not provide a large enough sample

Table 2.4 – Species found in Sites A-H with relative frequencies of  $\geq 0.5$ .

<b>Species</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>
<i>Amaranthus hybridus</i> (Pigweed)	-	-	-	-	-	-	0.11	-
<i>Aster sp.</i> (Aster species)	-	-	-	0.40	0.27	0.28	0.17	0.06
<i>Cichorium intybus</i> (Chicory)	-	-	-	-	-	0.33	-	-
<i>Cirsium vulgare</i> (Common Thistle)	-	-	-	-	-	0.22	-	-
<i>Cosmos bipinnatus</i> (Garden Cosmos)	-	-	-	-	-	0.28	-	-
<i>Helianthus annuus</i> (Sunflower)	-	-	0.09	-	-	0.17	-	-
<i>Helianthus tuberosus</i> (Jerusalem Artichoke)	-	-	-	0.40	-	-	-	-
<i>Ipomoea tricolor</i> (Morning Glory)	-	-	-	-	-	0.22	-	-
<i>Iris versicolor</i> (Blue Flag Iris)	-	-	-	0.27	0.33	0.28	-	-
<i>Oxalis corniculata</i> (Yellow Woodsorrel)	-	-	-	0.09	-	-	-	-
<i>Phalaris arundinacea</i> (Reed Canary Grass)	0.13	0.08	-	0.33	-	-	-	-
<i>Phragmites australis</i>	0.42	0.33	-	0.27	-	-	0.29	-
<i>Polygonum lapathifolium</i> (Pale Smartweed)	-	-	-	-	-	-	0.05	0.30
<i>Scirpus acutus</i> (Hard-stemmed Bulrush)	-	-	-	-	0.33	-	-	-
<i>Scirpus cyperinus</i> (Wool Grass)	-	-	0.12	-	-	-	0.05	0.08
<i>Scirpus fluviatilis</i> (River Bulrush)	0.28	0.35	0.16	-	0.20	-	0.15	0.19
<i>Schoenoplectus tabernaemontani</i> (Softstem Bulrush)	0.35	0.22	0.17	-	0.27	-	-	0.05
<i>Solidago sp.</i> (Goldenrod species)	-	-	-	-	-	-	-	0.10
<i>Sonchus asper</i> (Spiny Sowthistle)	-	-	-	-	-	-	0.10	-
<i>Typha latifolia</i> (Common Cattail)	0.33	0.22	-	-	-	-	-	-
<i>Zea mays</i> (Corn)	-	-	-	-	-	0.11	-	-
Open Gravel	0.07	0.28	0.40	-	-	0.11	-	-
<b>Species Richness</b>	<b>5</b>	<b>5</b>	<b>4</b>	<b>6</b>	<b>5</b>	<b>8</b>	<b>7</b>	<b>6</b>

size to determine correlations between individual plant species and triclosan removal rates because no individual species were found at all the sites. Future examination of wetland plants and triclosan removal would require either a larger number of wetlands or a controlled laboratory study.

## **Horizontal Flow and Vertical Flow Wetland Design Comparison**

### *Historical Wetland Performance for Physico-chemical Parameters*

The 2004-2008 data (Table 2.5) from the Lagrange County Health Department demonstrate that the wetlands investigated in this study have been functioning effectively as a secondary wastewater treatment for the removal of BOD, TSS, and *E. coli*, and they show Dissolved Oxygen (DO), pH, NH<sub>3</sub>, NO<sub>3</sub>, TN, and TP ranges that are comparable to other studied constructed wetlands (Garcia-Perez et al., 2008; Garcia-Perez et al., 2009; Vymazal, 2009). These data also demonstrate that the HF wetlands (Sites A, B, and C) are performing differently than the VF wetlands (Sites D, E, and F) in that the completely saturated HF wetlands show DO concentrations lower than the DO concentrations in the VF wetland where the upper level of gravel is not saturated.

Data taken at the time of sampling in this study confirm higher DO concentrations at the VF sites (Table 2.6). The mean influent and effluent DO concentrations were the same for the three HF wetlands (0.90 mg/L), whereas in the three VF wetlands the mean influent DO concentration was 0.61 mg/L and the effluent mean was 2.43 mg/L. The lower DO in the HF wetlands may account for the higher concentrations of ammonia nitrogen (NH<sub>3</sub>-N) and TN at Sites A and B because the anaerobic conditions do not allow for ammonia nitrification (Vymazal, 2005). The reduction in TN in the VF wetlands indicates that nitrogen is likely being taken up by the plants. Since these systems are aerobic, denitrification is limited because this process only occurs under anaerobic or anoxic conditions (Vymazal, 2007).

Typically, HF constructed wetlands are considered anaerobic treatment systems (D.O.  $\sim$  1.5 mg/L) while the VF wetlands are considered more aerobic (D.O.  $\sim$  1.8-4.0 mg/L); this may possibly affect the removal of triclosan. In WWTPs the highest triclosan removal is found in aerobic treatments (McAvoy et al., 2002; Singer et al., 2002), which would suggest that VF wetlands have the potential for a higher triclosan removal because of higher DO concentrations. Thompson et al. (2005) found that where DO levels were high (1.5-2.0 mg/L), biodegradation of triclosan was the main removal mechanism and, in contrast, where DO concentrations were below 0.3mg/L, the main removal mechanism shifted to substrate sorption.

Table 2.5 - Mean values for water quality parameters in studied horizontal flow (HF) and vertical flow (VF) constructed wetlands (2004-2008) ± SE.

Sites	Design Type	N	DO (mg/L)	pH (SU)	NH3-N (mg/L)	NO3-N (mg/L)	BOD (mg/L)	TSS (mg/L)	E.coli (org/100ml)	TN (mg/L)	TP (mg/L)	
<b>A &amp; B</b>	<b>inf</b>	HF	9	1.6	7.6	51.5	0.0	96.0	23.1	2.E+06	65.4	NA
				± 1.5	± 0.3	± 23.6	± 0.0	± 51.7	± 8.4	± 0.9E+06	± 27.5	
<b>A &amp; B</b>	<b>eff</b>		9	1.5	7.5	25.5	0.6	8.1	3.8	6.E+03	30.6	NA
				± 1.5	± 0.2	± 14.5	± 0.7	± 4.7	± 2.4	± 7959.0	± 16.8	
<b>C</b>	<b>inf</b>	HF	8	0.2	7.2	16.4	0.06	NA	NA	3.E+04	NA	NA
				± 0.1 (5)	± 0.1 (5)	± 5.7	± 0	± 2.E+04 (3)				
<b>C</b>	<b>eff</b>		8	0.3	7.20	5.1	0.1	NA	NA	533	NA	NA
				± 0.2 (5)	± 0.1 (5)	± 3.9	± 0.2			± 306		
<b>D</b>	<b>inf</b>	VF	1	NA	NA	41.0	NA	185.0	94.0	NA	80.0	NA
				-	-	-	-	-	-	-		
<b>D</b>	<b>eff</b>		18	4.5	7.4	3.2	9.8	5.2	3.4	7.E+03	13.8	NA
				± 0.2	± 0.3	± 1.6	± 8.9	± 3.5	± 3.5	± 13981.0	± 8.4	
<b>E</b>	<b>inf</b>	VF	7	1.8	7.1	42.4	0.0	145.9	87.3	1.E+06	52.8	8.7
				± 1.0	± 0.2	± 17.3	± 0.0	± 75.9	± 23.8	± 0.9E+06	± 12.2	± 1.5 (6)
<b>E</b>	<b>eff</b>		27	1.7	7.2	15.2	3.1	15.0	4.6	4.E+05	21.6	6.4
				± 1.1	± 0.2	± 5.8	± 3.1	± 8.0	± 2.5	± 4.0E+05	± 9.7	± 1.3 (6)
<b>F</b>	<b>inf</b>	VF	11	1.8	7.3	49.3	0.0	195.2	48.7	3.E+06	60.4	13.6
				± 0.8 (10)	0.3 (10)	± 28.6	± 0.0	± 160.9	± 18.2	± 5.0E+06	± 27.0	± 5.9 (7)
<b>F</b>	<b>eff</b>		11	4.0	7.3	2.0	6.2	1.7	0.6	1.E+05	9.7	7.8
				± 1.7 (10)	0.2 (10)	± 2.7	± 6.3	± 2.3	± 1.1	± 2.1E+05	± 6.0	± 3.1 (7)

Data for Sites A, B, D, E, and F tested by Lagrange County Health Department. (Some of this data has been published in Garcia-Perez, 2008) Data for site C conducted by author in 2006 and 2007. Mean values are provided with the standard deviation noted below. Number in paranthesis are N values where they differ from the stated N value. NA designates data that is unavailable.

Table 2.6 - Mean and standard deviations for pH and dissolved oxygen concentrations measured at the time of triclosan sampling at Sites A-F (N=20).

Wetland	Design Type	DO Influent (mg/L)	DO Effluent (mg/L)	pH Influent	pH Effluent
Site A	HF	0.62±0.27	0.36±0.10	7.53±0.29	7.48±0.24
Site B	HF	0.62±0.27	0.33±0.09	7.53±0.29	7.47±0.26
Site C	HF	0.47±0.14	0.53±0.30	7.17±0.07	7.11±0.07
Site D	VF	0.73±0.46	3.68±0.77	7.61±0.20	7.39±0.25
Site E	VF	0.57±0.50	1.65±0.61	7.21±0.21	7.14±0.14
Site F	VF	0.51±0.16	1.97±0.44	7.33±0.32	7.38±0.19

Triclosan has been shown to be affected by cold winter temperatures in surface flow constructed wetlands (Matamoros and Salvadó, 2012). In this study, weekly mean air temperatures ranged from 10-25 °C with a mean of 22 °C (Table 2.7). The ANOVA test for subject effects did not show any significant difference between triclosan removal and air temperature (p=0.44). Similarly, there was no significant difference in weekly rainfall and triclosan removal (p=0.17). Not surprisingly, water temperatures in

Table 2.7 – Statistical data for air and water temperature, and precipitation during the time of sampling (N=20).

	Air Temp. °C	Weekly Air Temp. °C	Water Temp. Influent °C	Water Temp. Effluent °C	Weekly Rainfall
<b>Mean</b>	22.0	18.0	19.3	18.7	16.2
<b>SD</b>	±5.6	±3.5	±1.7	±19.2	±28.3
<b>Max</b>	34.4	25.2	23.0	13.1	116.1
<b>Min</b>	11.1	10.4	15.8	2.5	0.0

the wetland reflected the trends in weekly air temperatures. Temperatures of water were not significantly different between the HF and VF sites despite the differences in water levels recorded for those two designs. The mean depth from the wetland surface to the water table was 3 cm in HF wetlands and 32 cm in the VF wetlands.

### *Triclosan Removal*

Because the investigated wetlands are fully functioning constructed wetlands that continuously receive wastewater for treatment, the influent concentration of triclosan varied at each sampling time and among studied wetlands reflecting the different triclosan use. The mean concentrations of triclosan in the influent water were significantly higher than in the effluent ( $p < 0.00$ ) (Table 2.8). These influent concentrations are within the range of triclosan concentrations measured at WWTP influent in previous studies conducted in the U.S. and Europe (Bester, 2005; Thompson, et al. 2005; Waltman, et al. 2006; Winkler, et al.

Table 2.8 - Concentrations of triclosan in the influent and effluent of six constructed wetlands.

	<b>N</b>	<b>Mean*</b>	<b>SD</b>	<b>Median</b>	<b>Minimum</b>	<b>Maximum</b>
		( $\mu\text{g/L}$ )	( $\mu\text{g/L}$ )	( $\mu\text{g/L}$ )	( $\mu\text{g/L}$ )	( $\mu\text{g/L}$ )
<b>Influent</b>	120	1.14	$\pm 0.32$	1.13	0.28	2.05
<b>Effluent</b>	120	0.76	$\pm 0.38$	0.83	BDL**	1.72

\*dependent T-Test comparison of the means yields a  $p < 0.000$ , \*\*BDL=below detection limit

2007). The lower triclosan influent concentrations found in this study may be due to the fact that constructed wetlands provide secondary treatment and are, therefore, preceded by a septic tank where some triclosan might have been removed by sorption to the retained sludge whereas the levels measured in WWTP influent correspond to the levels in raw wastewater before any treatment occurred. The removal of triclosan in septic tanks was shown by Carrara et al. (2008) and Conn et al. (2010). Conn et al. found a median triclosan concentration in the influent of septic tanks from six different households to be 19.0 µg/L while the median effluent was 5.7 µg/L. They found that the household input of triclosan was highly variable (0.4-230 µg/L) in raw sewage depending on differences in domestic uses of triclosan products. It is reasonable to expect that concentrations of triclosan in onsite systems would be more variable than at centralized WWTP influent from a large population due to differences in hydraulic loading and the dilution of wastewater (Conn et al., 2010). In this study the mean concentration of triclosan in the effluents of the constructed wetlands was lower than in the influent at all sites (Figure 2.5). The repeated measures ANOVA showed that there were significant differences ( $p < .005$ ) between the mean influent and effluent concentrations of triclosan at all sites except site E (Figure 2.3). The removal rates for these six constructed wetlands ranged from 15.6% to 56.2% (Figure 2.5, Table 2.9). The highest triclosan removal rates were recorded in the three gravity-fed HF wetlands (Sites A, B, and C) and the lowest in the three VF wetlands, however, the difference in the triclosan removal between the two design types was not shown to be statistically different. The removal rate of

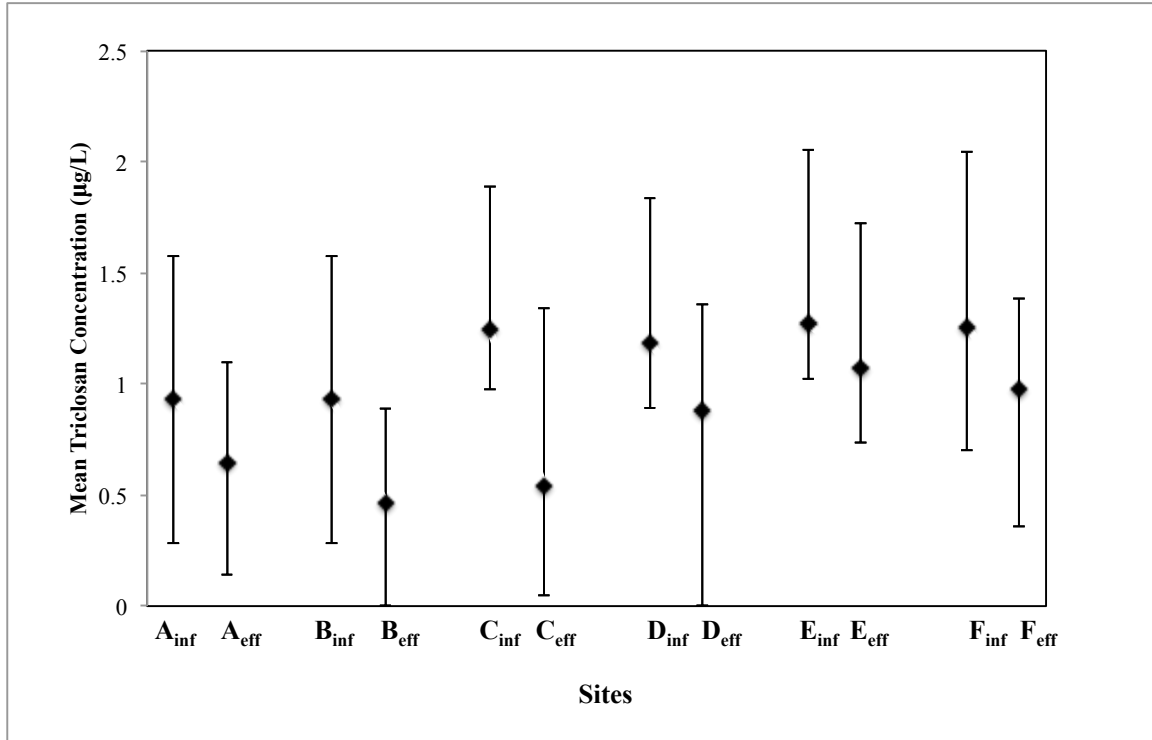


Figure 2.5 - The mean triclosan concentrations found in the influent and effluent water at six constructed wetland sites. Bars indicate the maximum and minimum values. (N=20).

56.2% at Site C is comparable to the 55% removal found in this same wetland in the triclosan injection study supporting the removal rates determined in this investigation / project. However, this removal rate is higher than the removal percentage of 36.9% found in the same wetland during the monitoring period of June and July 2008. This suggests that triclosan removal is related to HRT, as the flow rate in the summer months was considerably higher for this site while students were housed on site as mentioned in the HF wetland study.

Table 2.9 - Mean percent removal of triclosan in six constructed wetlands of two different designs (N=20).

Horizontal Flow (HF)		Recirculating Vertical Flow (VF)	
Site	Percent Removal	Site	Percent Removal
Site A	30.8	Site D	25.4
Site B	49.8	Site E	15.6
Site C	56.2	Site F	22

### *Wetland Design Comparison*

The three HF wetlands had the highest removal rates (Table 2.9). A Type III test for Fixed Effects model was used to determine if the difference in the triclosan concentrations found in the influent and effluents of HF versus VF wetlands was significant. This model compares the triclosan concentrations for the different wetland types (HF and VF) along with other measured variables that may contribute to the variation between the six sites and the two design types (i.e. DO, precipitation, air temperature and water temperature). The model run for Site nested in Wetland Design Type (HF and VF) tested whether the three HF sites are performing significantly different from one another and, also, if the three VF sites are performing differently from one another. The results of this analysis of the “Site nested in Type” was not significant ( $p = 0.216$ ), indicating that the wetlands of the same design are performing similarly. The model also showed no significant difference in triclosan removal rates between the VF and HF design types when all the measured variables were included in the model. This result is surprising, as the percent removal for the three VF sites were clearly much lower than the HF sites (Table 2.8). This is most likely a consequence of the high variability in

the triclosan concentrations in the influent and effluent in these operational wetlands that are receiving different levels of triclosan every day. However, this supports the findings of Matamoros et al. (2009) who compared removal rates for 13 PPCPs (not triclosan) between HF and VF wetlands and found no statistical difference in the removal rates between the designs for any of the compounds, though the VF wetlands did appear to perform more consistently and were more tolerant of varying organic loads. To improve an understanding of this issue, it would be valuable to expand the triclosan injection study conducted by this investigation by including more operational wetlands and compare these two design types in a controlled environment where the triclosan concentrations in the influent could be kept constant.

When all of the measured environmental variables, including wetland design and environmental parameters, were included in the analysis, the only significant effect in the model was the interaction between the wetland design type and dissolved oxygen concentrations in the effluent ( $p = 0.009$ ). This indicates that DO interacts with triclosan differently between the two types of wetlands affecting its removal rates. The results show that with higher concentrations of DO in the effluent of VF wetlands the triclosan removal was lower than in HF wetlands. This relationship was confirmed by the estimate of fixed effects for design type with dissolved oxygen being -0.392, suggesting that the relationship between dissolved oxygen and triclosan removal rates is more strongly negative in VF wetlands than in HF wetlands. This does not necessarily mean, however, that high effluent DO is the direct cause of the lower removal rates in the VF systems. For example, DO could be responding to lower soil saturation in VF systems, while

triclosan removal could be responding to a difference in HRT between VF and HF systems.

### **The Role of HRT in Triclosan Removal**

The importance of HRT in triclosan removal, as demonstrated by previous research (Stottmeister et al., 2003; Matamoros and Salvadó, 2012; Zhang et al., 2012), is supported by the study results from Site C (Table 2.10). The percent removal rates calculated during the wetland design comparison study and the triclosan injection study

Table 2.10 – Comparison of triclosan removal rates from three different studies conducted at Site C constructed wetland; GPD is estimated based on building occupancy at the time of data collection.

Study	Date	Percent Removal	Estimated Hydraulic Load (GPD)
HF Study	June 16 - July 25, 2008	36.9%	500
HF and VF Comparison	August 15 - October 21, 2008	56.2%	150
Injection Study	September 12 - October 15, 2009	55.0%	150

show a higher removal percentage at lower hydraulic loads implying a much higher HRT during those time periods. If HRT is a significant factor in either sorption or biodegradation, as suggested here, this may explain the reasons for lower percent removals in VF wetlands (though not statistically different) compared to HF wetlands. It was expected that triclosan removal would be higher in the VF wetlands, since triclosan has been shown to only biodegrade in the presence of oxygen (McAvoy, 2002). However, the estimated HRT in the VF wetlands is two to four days compared to an eight day or longer HRT in HF wetlands, which may not allow a sufficient time for the aerobic

microbes to break down the triclosan. In this study, Site D had the highest removal rate among the VF wetlands; this wetland treats wastewater from a home with only two people in residence. Therefore, lower flow rate and greater HRT allow more time for biodegradation. On the contrary, Sites E and F were functioning at or above the peak flow, so the shorter HRT most likely affects the treatment efficacy. These results are supported by previous research that has demonstrated the importance of HRT in triclosan removal. Matamoros and Salvadó (2012) found a surface flow constructed wetland to remove more triclosan than a reclamation pond due to a much longer HRT for the wetland. A study of centralized WWTP by Winkler et al. (2007) found that oxidation ditches with a HRT of 30 hours removed 92.1% of triclosan, while a trickling filter and humus tank with a 1.5 hour HRT only removed 39.2%; this difference was attributed, at least in part, to a longer retention time. Zhang et al. (2012) concluded that, in general, the longer the HRT the better the removal of PPCPs in constructed wetlands. An increase in HRT can provide more time for the wastewater to be in contact with plant roots, which has been shown to influence the extent to which plants contribute to the breakdown or removal of pollutants (Stottmeister et al., 2003).

### **Sorption as a Removal Mechanism in Constructed Wetlands**

In both types of wetlands it was expected that some sorption of triclosan to the gravel bed would occur. Avila et al. (2010) found that the behavior of PPCPs in a constructed wetland was strongly dependent on the sorption and biodegradation characteristics of the compound. This is most likely the primary mechanism for removal in the HF system and an important mechanism in the VF systems as well. Because triclosan is a hydrophobic

molecule, though, it would be expected to sorb better to a matter containing organic carbon than to inorganic sediment such as gravel (Jones et al., 2006). Reinhold et al. (2010) conducted a laboratory experiment examining the removal of triclosan in flasks with duckweed and found that sorption to organic material was the dominant removal mechanism. This may explain why the highest removal rates measured in centralized WWTPs occur in stage one of activated sludge systems where the organic load is high; it may also be the mechanism responsible for a reduction of triclosan in septic tanks (Conn, 2010). It has been shown that in onsite treatment systems the filtration of particulate matter to which PPCPs compounds are bound is a more important removal mechanism than microbial degradation (Wilcox et al., 2009). Nakada et al. (2007) found triclosan removal in a sand filter to range from 47 to 50%, similar to levels of HF wetlands found in this study. Considering the low DO levels in the HF wetlands and the low HRT in the VF wetlands, adsorption of triclosan to the substrate may be the dominant removal mechanism in these systems, and, for a hydrophobic compound like triclosan, this will limit the efficiency of constructed wetlands for triclosan removal. It is also possible that the sites where triclosan could adhere to the gravel surface could eventually become saturated, as is the case with phosphorus, decreasing the efficiency of the wetlands over time. Site C had the highest triclosan removal rates in both studies and, as it was also the most recently constructed wetland, it may have more adsorption sites available than the older wetlands investigated in this study.

## **Implications**

It is important to take into consideration that these constructed wetlands are secondary treatment systems that provide only a part of a complete onsite treatment of wastewater. Therefore, the total removal efficiency calculations for triclosan from an onsite system should include additional removal occurring in the primary treatment (i.e. septic tank) as well as in the drainage field. For example, given the median influent triclosan concentration of 19 µg/L found by Conn et al. (2010) in septic tank influents, and the mean triclosan concentration found in the effluent from constructed wetlands in this study of 0.76 µg/L, the overall removal rate of onsite systems would be 96%, comparable to the 58-99% removal seen in centralized plants (McAvoy et al., 2002; Singer et al., 2002; Bester, 2003). A future study should examine a total triclosan removal in onsite systems, including constructed wetlands and drainfields, for comparison to the removal efficiencies found at centralized WWTPs.

A potential advantage of constructed wetlands over WWTP treatment systems in the removal of triclosan is that onsite treatment does not include chlorine disinfection. Triclosan has been shown to form toxic chlorophenols when it interacts with free chlorine in water (Canosa et al., 2005), suggesting an increased environmental and health concern for downstream users and communities caused by effluent discharges from centralized treatment plants.

Even though constructed wetlands contribute significantly to triclosan removal in an onsite wastewater treatment system, it seems likely that, as with WWTPs, not all triclosan is being removed. This is of great concern because a study by Murray et al.

(2010) listed triclosan in a category of pollutants of the highest priority due to its frequent occurrence in the freshwater environment and the possibility to pose a human health hazard at environmental concentrations. The 1) potential of triclosan to act as a potential endocrine disruptor in humans, 2) toxicity of some of its derivative compounds, and 3) potential to increase microbial resistance, have important implications. Work should be done to increase the removal of triclosan through wastewater treatment. Because the removal of triclosan from wastewater is incomplete, it is additionally important to reduce the use of this chemical in household products, such as soaps and detergents.

### **Conclusion**

Subsurface constructed wetlands used in onsite wastewater treatment systems removed a significant amount of triclosan from wastewater (15.6-56.2%). The mean influent concentration of triclosan in the constructed wetlands was 0.69 µg/L in the first study of three HF sites and 1.14 µg/L in the second study of six sites; the mean effluent concentration was 0.46 µg/L and 0.76 µg/L, respectively. Site C showed comparable removal rates between a triclosan injection study (55%) conducted in the fall of 2009, and the study of an operating system that removed 56% of triclosan in the Fall of 2008, supporting the accuracy of the study data. However, the removal rate was lower (36.9%) in the study done in the summer of 2008 when the flow rate of wastewater was greater in this wetland, suggesting the HRT may play a significant role in wetland removal rates. Adsorption to the gravel bed may be a significant mechanism for triclosan removal in

constructed wetlands, though that may be limited by the hydrophobic nature of triclosan. The low concentrations of DO found in the HF systems would limit triclosan biodegradation in these systems and the low HRT (2 to 4 days) in VF systems may not allow time for biodegradation, but more study is needed to determine the removal mechanisms for triclosan in both types of constructed wetlands. Gravity-fed horizontal flow designs may remove a higher percentage of triclosan, though the results from this study did not find statistically significant differences in triclosan removal between two wetland designs. These results are surprising in that it was expected that the higher DO levels in the VF systems would increase the level of biodegradation of triclosan in these systems and, Therefore, show a higher removal rate. However, the low HRT may limit the amount of biodegradation that can occur in these systems, making them similar in triclosan removal rates to the HF systems.

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## CHAPTER 3 – THE REMOVAL OF TRICLOSAN FROM LABORATORY SCALE CONSTRUCTED WETLANDS WITH FOUR SPECIES OF WETLAND PLANTS

### Abstract

Triclosan is a common anti-bacterial agent found in a wide range of medical and personal products and it is frequently detected in wastewater after being washed down the household drains. Removal of triclosan is often incomplete in wastewater treatment allowing it to enter the environment where it has the potential to harm aquatic systems and there is growing concern for its effects on human health (Ferrari et al., 2002; US EPA, 2005). Constructed wetlands used for wastewater treatment often employ saturated gravel beds containing wetland plants that aid in the water treatment process. This study investigated the role these plants may play in triclosan removal from wastewater in constructed wetlands. Four species of wetland plants commonly found in constructed wetlands were grown in containers with a saturated pea-gravel substrate along with a control treatment containing no plants. Triclosan (2 µg/L) was added to the containers and its concentrations were monitored for 28 days. The mean reduction of triclosan was 36.8% for *Scirpus atrovirens*, 37.8% for *Scirpus cyperinus*, 39.1% for *Schoenoplectus fluviatilis*, 40.3% for *Carex comosa*, and 39.4% for the control. A repeated-measures ANOVA test showed no significant difference between any of the plant treatments or the

control ( $p=0.225$ ). This supports the findings of previous research that, although some triclosan is removed in constructed wetlands, the role of wetland plants is insignificant and biodegradation and sorption to the substrate remain the primary removal mechanism in these systems (Stottmeister et al., 2003; Chen et al. 2009b; Wu et al., 2010).

**Keywords:** triclosan, wastewater treatment, wetland plants

## Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) is an antimicrobial agent widely used in personal care products such as toothpaste, soaps, deodorants, cosmetics, and skin care lotions as well as other consumer goods (Adolfsson-Erici et al., 2002). Following the use of these products in daily life, triclosan enters the wastewater stream and ultimately the environment through the effluent from wastewater treatment plants (WWTPs) or onsite wastewater treatment systems (Singer et al., 2002).

Triclosan has been detected in WWTP water effluents as well as in the biosolids that are used as agricultural fertilizers due to its incomplete removal during the wastewater treatment process (McAvoy et al., 2002; Bester, 2003; Bester, 2005; Thompson et al., 2005; Heidler and Holden, 2007). In a national survey of pharmaceuticals and personal care products (PPCPs) in 139 wastewater-receiving streams across the U.S. in 2002, Kolpin et al. found triclosan in 57.6% of the samples making it one of the ten most frequently detected chemicals in the study.

Triclosan has been noted as a chemical of concern by the U. S. Environmental Protection Agency because the recent research evidence suggests that, even at low

concentrations, it may have detrimental effects on aquatic organisms and high concentrations have the potential to cause serious disruption to the ecological balance of aquatic systems (Ferrari et al., 2002; Tatarazako et al., 2004). Triclosan may function as an endocrine disrupter as it has been shown to cause both abnormal growth and behavior in tadpoles, snails, and bivalves and it was found to disrupt thyroid homeostasis in rats (Veldhoena et al., 2006; Canesia, 2007; Crofton et al., 2007; Brown et al., 2012). Because triclosan is a very polar molecule, it has the potential to bioaccumulate in the food chain (Thompson et al., 2005). Triclosan has been found in fish tissue and concentrations as high as 300 µg/kg lipid weight were found in samples of human breast milk (Adolfsson-Erici et al., 2002; Toms et al., 2011).

Constructed wetlands represent an alternative onsite wastewater treatment method that is becoming an increasingly popular method of wastewater treatment for small communities as well as animal farm operations. Although a variety of designs are used in their construction, one common feature of these systems is that they usually contain wetland plants that aid in the removal of pollutants through the wastewater treatment process.

The role of wetland plants as an essential component of constructed wetlands has been well established. They help stabilize the gravel surface of the wetlands, aid in physical filtration, insulate the wetland surface in cold temperatures, provide shade, decrease the current velocity, inhibit algal growth in warm temperatures, and add surface area for microbial growth due to root systems (Brix, 1997). Wetland plants also add oxygen into the rhizosphere and may increase oxidation and biodegradation of pollutants (Brix, 1997; Stottmeister et al., 2003; Brisson and Charzarenc, 2009). Plants have also

been found to facilitate the removal of nitrogen and phosphorous in constructed wetlands. However, the primary role of plants is often considered to be a passive process rather than a direct uptake and removal of contaminants (Tanner, 2001; Zarate Jr. et al., 2012). Nevertheless, recent research has demonstrated that plant uptake can play an important role in the removal of compounds other than nutrients from wastewater such as PPCPs and heavy metals (Liu et al., 2007)

A direct removal of nitrogen and phosphorous through the process of plant uptake has been a subject of debate, but a study by Liu et al. (2012) on a vertical flow (VF) constructed wetland showed clear evidence of nitrogen and phosphorous uptake by roots and the transfer of these nutrients to shoots and leaves (especially in young plants). This supports the research by Jiang et al. (2011) that examined 15 wetland plant species in China and found that nitrogen uptake ranged from 3.7 to 14.3 mg per day and phosphorous uptake was in the range of 1.71 to 4.61 mg per day. They found a positive correlation between plant biomass and nitrogen and phosphorous uptake, and suggested that plant selection for use in constructed wetlands should be based on biomass. However, with an increasing awareness of the occurrence of emerging contaminants in wastewater, nutrient removal should not be the only consideration of wastewater treatment design.

Brisson and Charzarenc (2009) reviewed 35 published studies that had examined plant removal of a wide range of pollutants in constructed wetlands and, although they were able to conclude that there is an overwhelming evidence that the presence of plants is helpful for the removal of many pollutants, the results were species specific and they were unable to draw generalized conclusions about the type of plants that perform best

for overall contaminant removal. Most of the studies reviewed by Brisson and Charzarenc looked at wastewater removal efficiencies so the mechanisms for removal are still largely unknown. A study by Singhakant et al. (2009) showed that a subsurface wetland planted with vetiver grasses removed significantly more arsenic than unplanted wetland, but the tissue samples showed only 0.5-1.0% of the total arsenic input so, although plants are important for arsenic removal, direct uptake was not the primary mechanism at work. Alternatively, Liu et al. (2007) found that direct plant uptake was responsible for 20% of cadmium removal, 23% of lead removal, and 24% of zinc removal by a combination of 19 wetland plant species, though the results varied widely by species. This species specificity was also seen in the work of Zhang et al. (2012), yet overall, the authors found a significant increase in the removal of six out of eight PPCPs in a planted container-scale constructed wetland compared with unplanted containers. Perhaps this plant-specific interaction with pollutants helps to explain why Karathanasis (2003) found that constructed wetland systems planted with a variety of species performed more consistently over a range of parameters and demonstrated less seasonal variation.

The role of wetland plants in the removal of organic pollutants like PPCPs from wastewater as well as the extent of their contribution to the treatment process is still the subject of current research. Previous studies have indicated that, although plants may take up trace amounts of triclosan, this uptake is not a significant mechanism for the removal of triclosan from soil or water (Chen et al., 2009a; Wu et al., 2010). In a controlled study by Chen et al. (2009b) the researchers found that three different wetland plants did not contribute significantly to the removal of triclosan from containers with

biosolid waste from a wastewater treatment plant. However, Reinhold et al. (2010) found that duckweed played an active role in the removal of triclosan from water. These findings may help validate the results of previous research studies that the role of plants in triclosan removal may be species specific. Because of the limited research available to date, further studies are needed to understand the role of aquatic plants in triclosan removal from wastewater. Consequently, the purpose of this study was to 1) determine if the wetland plants *Scirpus atrovirens*, *Scirpus cyperinus*, *Schoenoplectus fluviatilis*, and *Carex comosa*, contribute to the removal of triclosan from wastewater in laboratory constructed wetlands, and 2) to identify any differences in triclosan removal rates among these four different wetland species.

### **Materials and Methods**

A microcosm-scale study was conducted to simulate the removal of triclosan occurring in constructed wetlands by means of different aquatic plant species in laboratory settings. For this laboratory experiment four plant species were selected from wetland plants commonly used in many constructed wetlands: Dark Green Rush (*Scirpus atrovirens*), Woolgrass (*Scirpus cyperinus*), River Bulrush (*Schoenoplectus fluviatilis*), and Bristly Sedge (*Carex comosa*). These species are native to northern Indiana, occur in natural wetlands throughout the area, and are used by local manufacturers of constructed wetlands. Local plants are recommended in constructed wetland design guidelines because they are well adapted to the local environment (US EPA, 1993). The wetland plants were grown in 18x30x15cm plastic containers inside a greenhouse without climate

control. The containers were filled with limestone pea gravel, the common substrate in constructed wetlands, to a depth of 13cm. Each container was planted with two plants of the same species (Figure 3.1). Three replicate containers of each species were prepared, making a total of 12 containers of plants. In addition to these four treatments, a control group of 3 containers without plants (only the substrate) was also prepared, for a total of 5 treatments in 15 containers (Figure 3.2). Since microbes hosted on plant roots are the most likely factor to result in different removal rates between plants (Brix 1997; Stottmeister et al. 2003), plants were planted in the spring 2010 and allowed a full growing season before commencement of the experiment. They were overwintered in a greenhouse with no climate controls so the plants were allowed to die back as they would in an outdoor wetland. The plants were then used in the experiment in July and August (2011) of their second growing season. This allowed for a better establishment and growth of the rhizosphere, as well as for microbial establishment.

During the experiment, water was added once a day to maintain a constant water volume in the containers, and water levels were kept at 2 cm below the surface of the substrate. The containers were clear so the water level could be accurately monitored from the outside. The amount added to each container was recorded. The water level

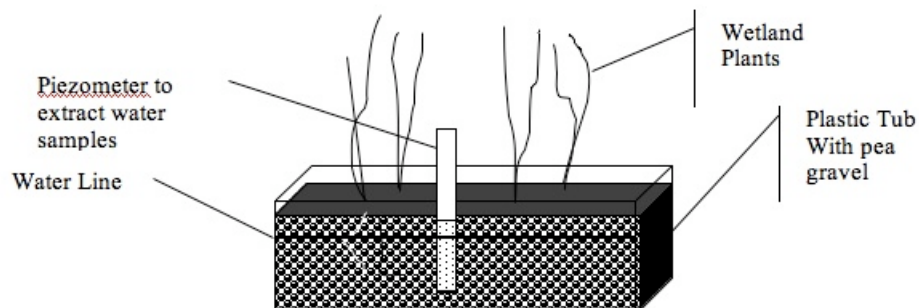


Figure 3.1 - Treatment container filled with limestone pea gravel and planted with wetland plants. Sample water was extracted from the piezometer for testing.

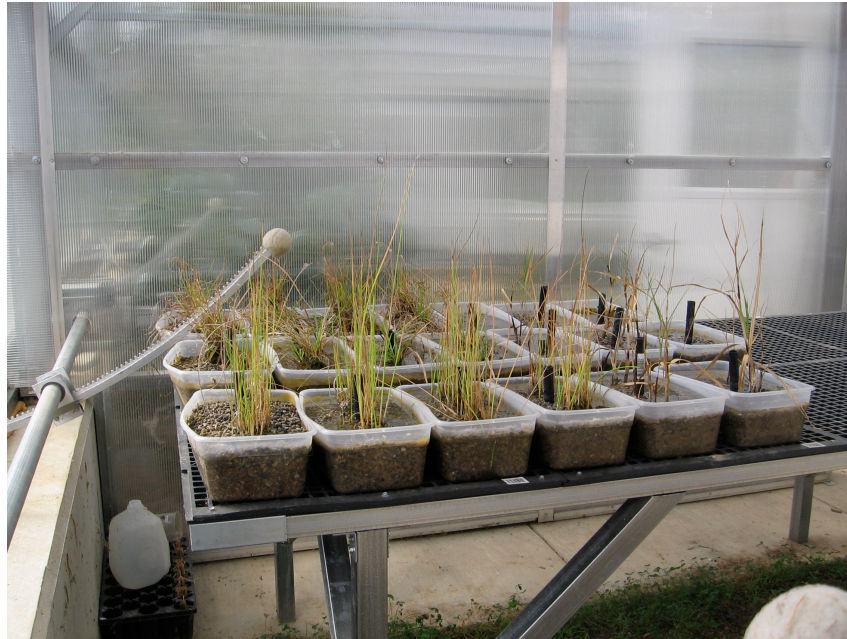


Figure 3.2 – Wetland plants growing in containers with gravel substrate.

decreased in the containers at different rates due to varying levels of transpiration.

Two days before the experiment began, water was removed from the containers and the gravel was allowed to dry out completely for two days, leaving only plants and dry substrate in the containers. Triclosan crystals (5-chloro-2-(2,4-dichlorophenoxy) (Thermo Fisher Scientific, Pittsburg, PA) were dissolved in 15% methanol and 85% distilled water due to its low water solubility (Vandhana et al., 2010). The concentration of triclosan in the stock solution was  $2.0 \mu\text{g/L}$ , which was within the range of concentrations found in the six functioning constructed wetlands in the area and greater than the mean concentration in these wetlands. Triclosan solution was added to each container until the liquid in the containers reached 2cm below the surface.

Water samples were taken from the containers on day 4, 8, 14, and 28 to

determine the residual concentrations of triclosan in the containers. The mean daily air temperatures in the greenhouse was recorded by use of the greenhouse thermometer (Taylor Precision Products, Oak Brook, IL). The pH in each container was also measured daily using a HACH sensION pH Meter. At the end of the testing period the plants were removed from the substrate, air-dried in cloth bags for four months, and then weighed to determine the total plant biomass (including the submerged root system) for each container.

The concentration of triclosan in water was determined by magnetic particle-based immunoassay (ELISA) (Abraxis Testing Solutions, Warminster, PA), which detects combined triclosan and triclosan methyl concentrations in the sample water. Sample handling and testing protocol were performed according to the manufacturer directions. Abraxis-supplied standards of triclosan solutions (0 µg/L, 0.025 µg/L, 0.1 µg/L, and 1.0 µg/L) were used to construct the standard calibration curve and each standard solution was analyzed in triplicates. The mean values from triplicate samples were used for all analyses. The standard curve was constructed by plotting the %B/B<sub>0</sub> (absorbance value for each standard /absorbance value for the zero µg/L standard) against the corresponding known triclosan concentrations. The concentrations of triclosan in water samples extracted from the containers were then determined from this standard curve. All collected samples were analyzed for triclosan in triplicates. The Abraxis Trilosan assay has an estimated minimum detectable concentration in water of 0.1 µg/L based on a 90% B/B<sub>0</sub> (Shelver et al., 2007).

All data were recorded in Microsoft Excel (2007) and analyzed with SPSS (SPSS Inc, Chicago, IL). A repeated-measures ANOVA was used to test the null hypothesis

that the different plant treatments had no effect on the removal of triclosan from the water. The total percent removal in each sample container was calculated as the percent reduction of triclosan from the injected concentration of 2.0µg/L. The mean percent removal was then calculated for each of the four plant treatments as well as the control.

## Results and Discussion

Total plant biomass in each container varied by species (Table 3.1). The minimum biomass weight per container was 44.4g (*Carex comosa*), and the maximum was 81.3g (*Scirpus cyperinus*) while the mean biomass for three replicates of each treatment ranged from 61.4g to 71.8g (Table 1.1). The water replacement volumes varied by species as well as among species' replicates although they did not correlate with the biomass weight (Table 3.1). The mean volume of water replacement for all treatments was 114ml although they were highly variable as is shown by the high standard deviations. The water pH did not differ significantly among the treatments and the values corresponded to the water occurring in the unplanted control of limestone pea gravel substrate (Table 3.1).

The mean air temperature in the greenhouse ranged from 28.4 °C (July 27, 2011)

Table 3.1 – Mean values for plant biomass, daily water replacement and pH in the test containers (N=9).

Species/Treatment	Mean Biomass (g)	Mean Daily Water Replacement (ml)	Mean pH
<i>Carex comosa</i>	51.4 ±9.7	121±56	8.05±0.11
<i>Scirpus cyperinus</i>	71.8 ±9.2	132±85	8.17 ±0.09
<i>Schoenoplectus fluviatilis</i>	66.2 ±5.7	110±61	8.15 ±0.10
<i>Scirpus atrovirens</i>	61.4 ±11.0	103±51	8.29±0.03
Control	0 ±0.0	106±48	8.12±0.17

to 38.6 °C (August 1, 2011). There was no correlation between the air temperature and triclosan removal, which is most likely due to the fact that this study was performed during warm summer months with little temperature fluctuation. Triclosan removal, however, has been shown to be affected by cold winter temperatures in surface flow constructed wetlands (Matamoros and Salvadó 2012).

During the study, the concentration of triclosan decreased in all 15 containers, including the control, with the largest declines occurring between day 1 and day 8 (65.3-83.0% of total removal) (Figure 3.3). Triclosan concentrations continued to decline throughout the 28 days of the experiment but the removal rates decreased after week two.

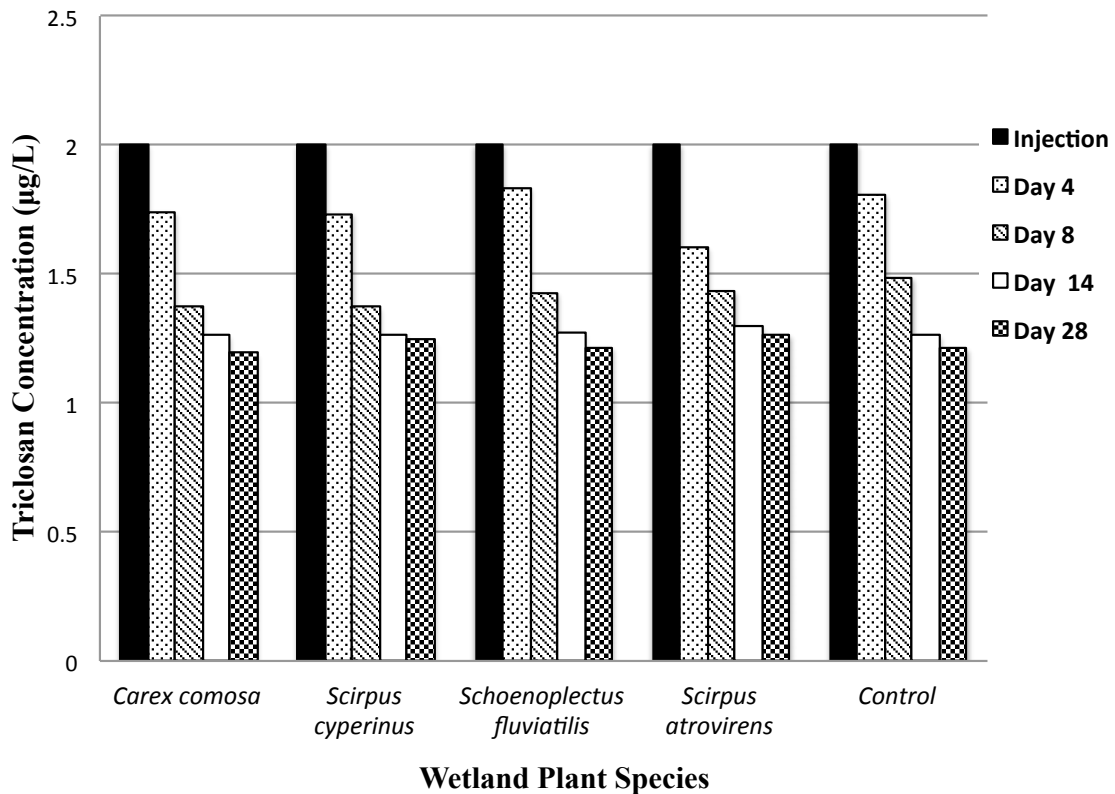


Figure 3.3 - The mean triclosan concentrations (N=3) at the time of injection and on day 4, 8, 14, and day 28 for each of the four macrophyte treatments as well as the control.

After 28 days the recorded mean removal of triclosan was 39.4% (control), 36.8% for *Scirpus atrovirens*, 37.8% (*Scirpus cyperinus*), 39.1% (*Schoenoplectus fluviatilis*), and 40.3% (*Carex comosa*). The final mean concentration of triclosan in water was 1.21, 1.26, 1.24, 1.21, and 1.19 µg/L, respectively.

The repeated measures ANOVA showed no significant differences in the removal of triclosan between the different plant treatments or between any of the planted containers and unplanted control ( $p=0.225$ ). These results support the findings of previous studies that have shown that although plant uptake of triclosan may occur in constructed wetlands, it is insignificant compared to the biodegradation and sorption processes (Stottmeister et al., 2003; Chen et al., 2009a; Wu et al., 2010).

The removal rates for triclosan in this study are comparable to the research of Carr et al. (2011) who showed a reduction of triclosan between 27 and 40% in anaerobic soil conditions after 14 days. In contrast, the removal rates are higher in this study than those found by Matamoros et al. (2012) who tested the removal rates for triclosan in containers with different free floating macrophyte wetland species grown in open water. One of the treatments was covered to remove the effects of photodegradation and with this treatment they found a removal rate of only 19% after 38 days. The authors suggest that a 19% removal rate is low enough that it could be explained by sorption of triclosan to the container. In this study, the gravel substrate provides additional potential sorption sites for triclosan over open water containers, which may explain the higher removal rates. Since there was no significant difference in the triclosan removal rates between the planted containers and the unplanted control container, and, therefore, there was no significant triclosan removal due to plant uptake with the four plant species, the main

removal process is either sorption to the substrate and the container, or the process of microbial degradation . Photodegradation should not play a role in removal in these containers as the water levels were kept below the surface to match the conditions in a subsurface constructed wetland. This is unlike free-surface water constructed wetlands where photodegradation has been shown to be a dominant removal mechanism for triclosan (Matamoros and Salvadó, 2012)

The process of microbial degradation of triclosan as a removal mechanism is supported by the findings of Carr et al., (2011) who found triclosan to have a half-life of 15.3-28.8 days in anaerobic soils due to microbial biodegradation. However, a laboratory study by Reinhold et al. (2010) found that microbial degradation does not significantly contribute to the reduction of triclosan in water and that sorption was the dominant mechanism when organic matter was present. This is consistent with the research of McAvoy et al. (2002) who found that biological degradation of the triclosan compound seems to happen under aerobic conditions and also in the presence of light, but not in anaerobic conditions. Additional study is needed to determine whether the sorption or biodegradation was the main removal mechanism seen in these microcosm container wetlands since the plant removal was not a significant factor.

The fact that no significant difference was observed between the planted and unplanted treatments in this experiment lends evidence to the argument that wetland plants are not likely to be a significant contributor to triclosan removal in constructed wetlands. Some PPCP compounds have been shown to be removed by wetland plants as was seen in the study by Zhang et al. (2012) where six out of eight tested PPCPs had a significant increase in removal rates also in container scale wetland systems. However, a

recent review of published literature by Brisson and Charzarenc (2009) highlighted that removal rates are highly variable depending on the species and the pollutant. Even tests on the same compound have yielded different results when the experimental conditions were different. For example, Brisson and Charzaren highlight five studies comparing removal rates of BOD, TSS, nitrate, and ammonia by use of *Phragmites australis* and *Typha latifolia* in constructed wetlands. The results show two studies where *Phragmites* appears more efficient, one study where *Typha* was more efficient, and two studies where there was no significant difference between the removal rates for the two species. There is little information available regarding the effects of wetland plants on triclosan removal and those that have been performed have shown conflicting results as well (Zarate Jr. et al., 2012). Chen et al. (2009b) found no difference in triclosan removal rates (38-48%) between model sludge reed bed containers planted with bulrush (*Typha latifolia*), reed (*Phragmites australis*) and reed canary grass (*Phalaris arundinacea*) and unplanted containers, and these removal rates are very similar to the 37-40% removal percentages found in this study. Another study by Chen et al. (2009a) also detected no triclosan in samples of the plants in an operational sludge reed bed where the triclosan removal rate was up to 68% in the bottom layer of the reed bed. However, Wu et al. (2010) found triclosan in both the root and leaf tissue of soybean plants harvested from fields where biosolids containing triclosan had been applied and irrigated with triclosan containing water; the highest triclosan concentrations were found in roots (76.8 ng/g), in stems (136.0 ng/g), and, in leaves (120.0 ng/g). Similarly, Zarate Jr. et al. (2012) examined tissue samples from *Typha latifolia*, *Pontederia cordata*, and *Sagittaria graminea* in a free surface water constructed wetland and they found that triclosan concentration in the

roots of *T. latifolia* ranged from 10 to 40 ng/g though the concentration in the shoots were near detection limit as were the levels in both roots and shoots from the other plant species. This led Zarate et al. to conclude that triclosan can readily accumulate in the roots of living wetland plants but the response is species specific. Part of the differences seen in these studies may also be a matter of scale. Though Wu et al. (2010) found triclosan in soybeans, they also analyzed triclosan concentrations in the surrounding soils and determined that triclosan removal from plant uptake is negligible compared to the levels detected in the surrounding soil. Therefore, if experiments are simply measuring the removal of triclosan in a full-scale constructed wetland, even if some triclosan is taken into the plant tissue, it may not be sufficient to significantly reduce the concentration of triclosan in wastewater.

In subsurface constructed wetlands, an eight-day hydraulic retention time (HRT) has been considered effective for the removal of organic matter, TKN, and BOD (Akratos and Tsihrizis, 2007). In this study 65 to 83% of the total triclosan removal was removed after eight days suggesting that constructed wetlands would also remove a significant percentage of triclosan with a standard eight day HRT. There is unlikely to be any significant contribution to triclosan removal in wetlands with the plant species tested in this study, however, the role that species specificity play in the PPCP removal in constructed wetlands suggests that further study on other plant species would be valuable. Though analysis of plant tissue was not performed in this study, the fact that there was no difference between the planted and the control treatments suggest that even if plant uptake is occurring, it is unlikely to remove enough triclosan to make a significant contribution to the removal efficiency of a constructed wetland. Further, this research does

not take into account the role of plant interactions in triclosan removal rates.

Karathanasis et al. (2003) found that constructed wetlands with high plant diversity are more efficient and consistent in removal for many pollutants. Therefore, future research should investigate larger scale systems employing active wastewater and other plant species that were not included in this study, both individually and also in combination.

### **Implications**

Triclosan, along with other pharmaceuticals and personal care products, is entering the environment in the effluent of wastewater treatment systems. This may pose a threat to both ecosystem and human health. It is important to continue investigation into the mechanisms for removal of triclosan and other chemicals in different wastewater treatment systems. The results of this study suggest that sorption is the dominant removal mechanism for constructed wetlands; therefore, additional research could focus on investigation of alternative substrate materials for triclosan sorption potential. A summary of recent research on wetland plants and the removal of PPCPs from wastewater also suggests that wetlands facilitate removal of many of these pollutants, which warrants further study of numerous wetland plants and their role in removal of PPCPs (Brisson and Chazarenc, 2009). Identification of the most efficient species may influence plant selection in future wetland design and wastewater treatment. This study also suggests that subsurface constructed wetlands will not completely remove triclosan given a typical hydraulic residence time. This implies that onsite treatment systems can be an avenue of triclosan entry into the environment, as is the case with centralized WWTPs. This is a concern because triclosan, a potential endocrine disrupter, may

negatively affect human health as well as aquatic ecosystems and their diversity (Tatarazako et al., 2004, Veldhoen et al., 2005).

### **Conclusion**

Triclosan concentrations in water were reduced in saturated gravel bed containers over a 28 day period at the rate of 39.4% (control - unplanted), 36.8% (*Scirpus atrovirens*), 37.8% (*Scirpus cyperinus*), 39.1% (*Schoenoplectus fluviatilis*), and 40.3% (*Carex comosa*). The highest removal rates occurred within the first 8 days. This suggests that subsurface constructed wetlands with similar saturated beds would also remove some triclosan given the recommended eight day hydraulic residence time for subsurface wetlands. However, direct uptake by these four wetland plants appears to play an insignificant role in the triclosan removal process. There was no significant difference between the removal rates of triclosan by any of the four studied plant species and the control without plants. Biodegradation or sorption to the substrate/container might be the primary removal mechanisms but further study is needed to determine the significant removal mechanism in these systems. Previous studies of PPCPs removal by use of aquatic plants suggest that the interactions can be very species and pollutant specific. Therefore, other wetland species used in constructed wetlands should also be investigated together with an analysis of triclosan in plant tissue.

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## CHAPTER 4 – ADDITIONAL RESEARCH: DETERMINATION OF THE HYDRAULIC RETENTION TIME IN AN OPERATIONAL SUBSURFACE CONSTRUCTED WETLAND

### **Abstract**

Hydraulic retention time (HRT) has been shown to be an important factor in the removal of nutrients and organic contaminants during the treatment of wastewater in constructed wetlands (Tanner 2001; Healy and Cawley 2002; Browning, 2003; Matamoros and Salvadó 2012). The estimated HRT for a wetland is calculated using the medium porosity, bed volume and estimated flow; however, the actual HRTs have at times been found to differ from the theoretical HRT due to factors not considered in the equation such as plant root systems (US EPA, 1993; US EPA, 1999). This study used a tracing dye to determine the HRT for a Horizontal Flow subsurface constructed wetland in Noble County, Indiana. Two trials showed the wastewater HRT of 15 days and 17 days from the time of dye injection in the influent until the peak dye concentration in the effluent. The HRT for this wetland was determined as the mean of these two trials or 16 days. The results also suggested that some water short-circuiting could be occurring in this wetland.

## **Introduction**

### **Research Goals**

The goal of this study was to determine the actual HRT for the subsurface constructed wetland at Reith Village in Wolf Lake, IN. The constructed wetland that treats the wastewater at Reith Village Ecological Field Station (Site C) is one of the sites used in the investigation of triclosan removal as well as the triclosan injection study described in Chapter Two. Because the injection study involved dosing of the wetland with triclosan at the influent and monitoring the subsequent concentrations in the effluent, it was important to determine the hydraulic retention time (HRT) of wastewater in the wetland to estimate the length of the monitoring period as well as the frequency of sampling that would be used in the triclosan removal and injection study.

An additional goal of this experiment was to compare the experimentally measured HRT to the HRT estimated by the manufacturer of this wetland (14 days) to observe if there was a significant discrepancy between the two estimates. Experimentally testing the HRT for all eight wetlands investigated for triclosan (discussed in Chapter Two) would have been helpful because tracing dye testing can provide a more reliable determination of HRT than the theoretical estimate in subsurface wetlands (US EPA, 1999) and, in general, longer HRTs have been shown to be more effective at removing BOD (Biochemical oxygen demand) and TSS (total suspended solids) as well as PPCPs (pharmaceuticals and personal care products) in wetlands (Akratos and Tsihrintzis, 2007; Zhang et al., 2012). However, the tracing dye experiment cannot be performed simultaneously with the triclosan experiments because the presence of dye in water samples can interfere with the triclosan analysis. Similar HRT times between the

experimentally derived HRT determined with the tracing dye and the design estimate HRT in this study will provide support for the use of the design estimates in the wetlands investigated for triclosan removal.

### **Hydraulic Retention Time – Background Information**

Hydraulic retention time (HRT) in a constructed wetland is the term given to the length of time that water spends in the wetland treatment bed. HRT has been shown to be an important factor in the removal of nutrients and organic contaminants during wastewater treatment in constructed wetlands (Tanner 2001; Healy and Cawley, 2002; Browning, 2003; Akrotos and Tsihrintzis, 2007; Matamoros and Salvadó 2012). Hydraulic load rates (volumetric flow rate/wetland surface area in gal/ft<sup>2</sup>/d) and HRT are closely related in that a shorter HRT usually requires a lower hydraulic loading rate for the wastewater to attain the same level of treatment (US EPA, 1999; Carrara et al., 2008). This can be especially important in constructed wetlands that have vegetation, as an increase in HRT can provide more time for the water to be in contact with plant roots, which may increase the contribution of plants to the degradation or removal of pollutants (Stottmeister et al., 2003). Horizontal flow (HF) wetlands have longer HRTs than Vertical flow (VF) wetlands suggesting that plants may play a more significant role in treatment of wastewater in HF wetlands (US EPA, 1999).

Design specifications for the HRT in a subsurface constructed wetland is usually based on a simple calculation of the effective porosity of the media times the length, width, and average depth of the bed, divided by the average daily flow (US EPA, 1993). In HF subsurface constructed wetlands, an eight-day HRT has been considered sufficient for effective removal of organic matter, TKN (Total Kjeldahl Nitrogen or sum of

ammonia-nitrogen and organic nitrogen), and BOD (Akratos and Tsihristzis, 2007). The development of plant root systems can influence the HRT in an HF system and tracer dye studies have sometimes shown a difference between the measured HRT and the theoretical HRT (US EPA, 1999).

### Materials and Methods

The HRT was tested in a horizontal flow (HF) subsurface constructed wetland at Rieth Village (Merry Lea Environmental Learning Center of Goshen College), an ecological field station in Wolf Lake, IN that is comprised of one office building and two small student residences (Figure 4.1). This site serves 32 student residents and 5

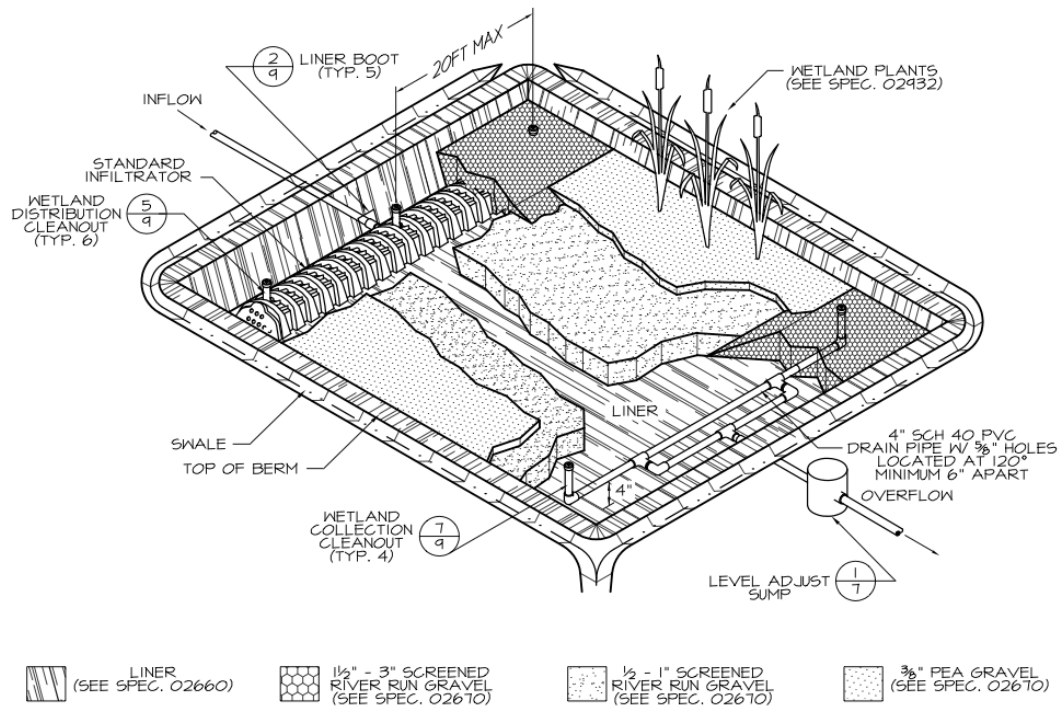


Figure 4.1 – Schematic of the horizontal flow constructed wetland at Reith Village. Image by Natural Systems Inc., provided by Merry Lea Environmental Learning Center of Goshen College.

employees at peak flow, and the wetland was designed to receive 2300 gallons per day (GPD); however, during the time of testing these buildings were not occupied at full capacity. Because these buildings are LEED (Leadership in Energy and Environmental Design) certified (U.S. Green Building Council) they are set up with several monitoring devices including a wastewater flow meter. However, the monitor was not working correctly during the time of data collection and wastewater flow had to be estimated from the occupancy of the buildings during the testing times and the recommended hydraulic load per capita (U.S. EPA manual).

In order to determine the HRT for this wetland, a total volume of 473 mL of fluorescent yellow/green liquid fluorescein tracing dye (Bright Dyes, Miamisburg, OH) was added to the central cleanout pipe at the influent, and subsequently, the effluent water was sampled from one of the effluent clean out pipes every day between 3 and 6 p.m. for 23 days (Figure 4.1). The first dye test (Run 1) was performed from June 2 to June 25, 2009 and the second test (Run 2) was completed between July 1 and July 23, 2009. Temperature and precipitation data was obtained from the Indiana State Climate Office (Purdue, University) from the nearest weather station in Columbia City, IN (Appendix C, Table 3).

The concentration of dye in the daily samples collected from the effluent was analyzed using an HACH DR/890 colorimeter (HACH Company, Loveland, CO) at a 500 nm wavelength according to the specifications of dye manufacturer (Appendix C, Tables 1 and 2). The absorbance levels in water samples were plotted on a graph for each day to establish a residence time curve for each run for a total of 23 readings for each of the two dye runs. The number of days between the dye injection and the curve peak

concentrations (dye breakthrough) was averaged for the two runs and this was considered the actual residence time for the constructed wetland.

### Results and Discussion

Both Run 1 and Run 2 clearly showed a breakthrough peak for the dye concentration in the effluent (Figure 4.2). The breakthrough occurred on day 17 for Run 1 and on day 15 for Run 2 (Daily results in Appendix C). The HRT for this wetland was determined as the mean of 16 days, which is well above the minimum suggested HRT for

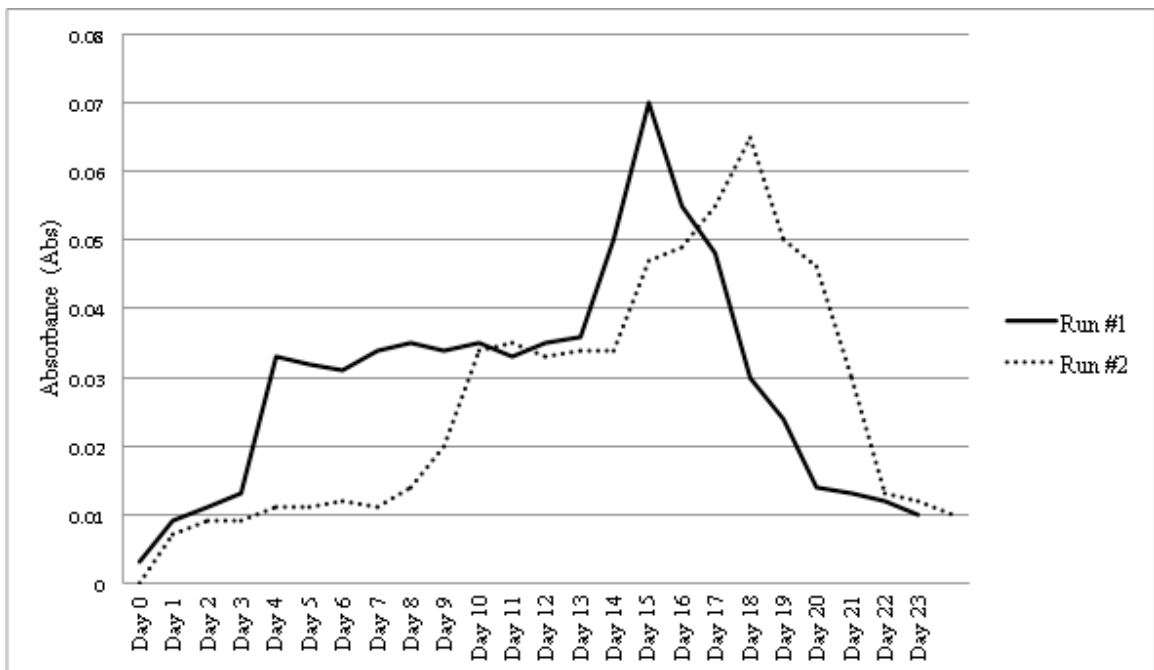


Figure 4.2-Dye absorbance in the effluent of a constructed wetland at Rieth Village following dye injection.

HF wetlands of eight days and above the theoretical estimated HRT of 14 days for this wetland. This is not surprising as the buildings making use of this wetland were not at capacity and, given the housing and office occupancies, the estimated flow rates were

500 gallons per day (GPD) during the first run and 400 GPD during the second run. With lower flow, the hydraulic load per square foot of the wetland surface would decrease and water would move more slowly through the wetland resulting in a higher HRT. The differences in the peak dye breakthrough time for Run 1 and Run 2 are also likely to be caused by the different flow rates between those time periods, however it is apparent that the HRT of this wetland is well above the recommendation for the removal of organic matter, TKN and BOD.

The mean daily temperature and the mean daily precipitation for the two testing period are reported in Table 4.1 along with the maximum daily precipitation. The data shows very little temperature or precipitation variation between the testing periods. The maximum daily precipitation is reported because high levels of precipitation can raise the water level thus increasing the flow rate and decreasing the HRT (US EPA, 1999). A large precipitation event could also raise the water levels enough to allow water to flow over the surface and result in wastewater short-circuiting in the wetland cell. The maximum precipitation recorded during these testing times was not significant enough to cause a surface flow event.

Table 4.1 – Mean temperature and daily precipitation data during the test periods for Run 1 and Run 2.

	Run 1	Run 2
Mean Temperature (°C)	18.6 ±3	18.4 ±2
Mean Precipitation (cm)	0.23 ±0.6	0.23 ±0.7
Max. Precipitation (cm)	2.24	2.41

Data from Indiana State Climate Office

Even though a surface short-circuiting caused by excessive precipitation did not occur, an early breakthrough peak of dye concentration was evident during both Run 1 and Run 2 (Figure 4.2). The presence of some dye before peak breakthrough is to be expected since, given the hydrology of a wetland, water will disperse throughout the bed and flow at slightly different rates depending on the gravel medium and the effective porosity (García et al., 2004). Subsurface wetlands have been shown to have a moderate to high degree of dispersal meaning that some water will travel quickly from influent to effluent pipe by the most direct route (preferential paths) and some will be pushed into other paths and move more slowly to the exit (US EPA, 1999). However, in this case, the amount of dye shown to be reaching the effluent early in these runs may be greater than what would occur through a natural dispersal in a subsurface wetland. During Run 1 about 50% of the maximum dye concentration was detected in the effluent by day four. This same pattern is seen in Run 2 but the time needed to reach 50% detection was 10 days. This suggests that some short-circuiting may be occurring in the wetland that allowed some water to pass through the bed at a much faster rate. Short-circuiting can occur not only on the surface but also at the bottom or sides of the bed liner (US EPA, 1999). Such short-circuiting is a concern because the water reaching the effluent in four days obtains less treatment than would occur during the eight-day recommended minimum HRT, which may result in a decreased efficiency and removal of pollutants from wastewater.

Given the fact that the experimentally determined HRT for this wetland of 16 days was comparable to the HRT estimated by the design manufacturers, the

manufacturer's estimates of HRT were used in the triclosan study for all wetlands discussed in chapter two.

### **Conclusion**

The results from two tracing dye runs through the constructed wetland showed the residence times of 15 and 17 days from the dye injection until the dye peak breakthrough in the wetland effluent. The HRT for the HF subsurface constructed wetland at Rieth Village was determined to be the mean of these results, or 16 days. This is similar to the design estimated HRT of 14 days and more than the recommended eight-day minimum for acceptable wastewater treatment performance. The variability between the two runs is likely due to different water usage and subsequent flow rates during the testing period. The flow rate for Run 1 was estimated to be 500 GPD based on the building usage and occupancy at the time and the flow rate during Run 2 was estimated to be lower at 400 GPD. The results also show that there is a possibility of some short-circuiting of wastewater through the wetland because of early dye breakthrough.

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## CHAPTER 5: ADDITIONAL RESEARCH METHODS - THE EVALUATION OF A SUBSURFACE CONSTRUCTED WETLAND FOR THE TREATMENT OF IRON, PHOSPHORUS, AMMONIA, NITRITE, NITRATE, DISSOLVED OXYGEN, PH, AND *E. COLI*.

### **Purpose and Background**

This section describes the methodology used to collect data in support of the evaluation of the wetland treatment performance. Specifically, both the influent and effluent of the constructed wetland at the Rieth Village Ecological Field Station were monitored for iron, phosphorus, ammonia, nitrite, nitrate, dissolved oxygen, pH and *E. coli*. This constructed wetland is one of the six study sites used in wetland investigation discussed in chapter two. The other five sites, located in LaGrange County, IN, were monitored for a range of parameters by the LaGrange county health department (Table 2.2). The Rieth Village site (Site C) is located in Noble County and has not been monitored by the local health department thus the data collected by the author in 2007 and 2008 were used to provide historical background information on the performance of this wetland in support of the triclosan study in Chapter two.

### **Constructed Wetland Background**

Constructed wetlands are shallow ponds, beds, or trenches that contain floating or emergent wetland vegetation (Matamoros et al., 2005). They utilize natural processes

including biological, chemical, and physical mechanisms for water treatment (Song et al., 2001). In these systems pollutants are removed from wastewater through plant uptake, sorption, chemical transformation, volatilization, and microbial degradation (Stearman et al., 2003).

There are two main kinds of constructed wetlands: free water surface flow wetlands and subsurface flow wetlands (Browning, 2003). Free water surface wetlands contain areas of gravel and vegetation as well as areas of open water, where wastewater flows through the constructed wetland as it would through a natural marsh. Subsurface flow wetlands are sealed basins containing vegetated porous gravel beds through which wastewater percolates (US EPA, 2000) (Chapter 1, Figure 1.2; Chapter 4, Figure 4.1). The gravel in these wetlands is saturated to just below the surface of the gravel substrate so the processes in the wetland are predominantly anaerobic (US EPA, 1999).

Constructed wetlands are effective at removing BOD and total suspended solids (TSS) from wastewater. A study of 13 constructed wetlands by Matamoros et al. (2009) found a greater than 95% removal of both BOD and TSS in all studied wetland systems. One common parameter often studied in constructed wetlands is total nitrogen because of its high concentration in wastewater and its potential harmful effects on the environment and human health. Some studies have shown that subsurface constructed wetlands can remove nitrogen quite effectively from wastewater with reported removal rates often from 70 to 80% (Healy and Cawley, 2002). However, because anaerobic processes predominate in these wetlands, nitrification of ammonia is limited (US EPA, 1999). As a result, even though the anaerobic conditions would facilitate denitrification, a complete

removal of nitrogen is limited or nonexistent because ammonia can not be nitrified (Vymazal, 2011).

## **Materials and Methods**

The study of basic wetland performance for removal of wastewater pollutants at Site A, B, D, E, F was conducted by the LaGrange County Health Department, while the monitoring of wastewater parameters at Site C was performed by the author of this dissertation as described here. The constructed wetland site used in this study was a horizontal flow (HF) subsurface constructed wetland at Rieth Village (Merry Lea Environmental Learning Center of Goshen College), an ecological field station in Wolf Lake, Indiana with one office building and two small student residences. This wetland has a footprint of 714 square feet and it is designed for a hydraulic load of 2300 gallons per day (GPD). This constructed wetland cell is part of a functioning onsite treatment where the wastewater goes first to a primary settling tank, then to the constructed wetland, and finally to a recirculating sand filter. The original design was intended for the final effluent to be used in a drip irrigation system, however, current Indiana regulations mandate that the effluent is to be discharged to the local centralized treatment facility. This system was constructed and planted in May of 2005, and began receiving wastewater in September, 2005.

The constructed wetland was originally planted with five native plant species: Softstem Bulrush (*Schoenoplectus tabernaemontani*), Dark Green Rush (*Scirpus atrovirens*), Woolgrass (*Scirpus cyperinus*), River Bulrush (*Scirpus fluviatilis*), and

Bristly Sedge (*Carex comosa*). These five species still represent the dominant plants in this wetland, although other species have begun to grow in the gravel medium as well (Appendix B, Table B.1). During this study the wetland received wastewater from two cottages that, at the time of this testing, housed approximately six people and one small office building. The estimated daily hydraulic load for the testing period was 400 GPD, considerably lower than the design flow rate of 2300 GPD for this wetland. This means the hydraulic retention time is likely longer than the 14 day design estimate. The dye tracing experiment performed on this wetland described in Chapter 4 found an HRT of 16 days.

There were two water sampling periods for this study. The first set of samples was collected eight times over a period of five weeks in June and July of 2006. Grab samples of influent and effluent water were taken from the clean-out pipes at the wetland using polyethylene bailers and then transferred to glass bottles for transportation to the laboratory (Figure 4.1).

Water temperature was measured at the influent clean-out pipe with a glass laboratory thermometer and the water level in the distribution pipe was measured with a meter stick at the time of sampling. All water analyses were performed within two hours of sample collection; samples were refrigerated until all tests were completed. Samples were analyzed for five different parameters (total iron, reactive orthophosphate-P, nitrite-N, ammonia-N, and nitrate-N) using a Hach water chemical testing kit and a DR/800 colorimeter (HACH Co., Loveland, CO).

Total iron was analyzed using US EPA FerroVer® method, reactive orthophosphate ( $\text{PO}_4^{3-}$ ) was analyzed using the PhosVer 3 (Ascorbic Acid) EPA

compliant method, nitrite-N was tested using Diazotization EPA compliant method, ammonia-N was analyzed using the Salicylate method, and nitrate-N was tested using the Cadmium Reduction method (HACH Co., Loveland, CO). When the measured concentration exceeded the range limits of the method, then analysis was performed again with samples diluted with deionized water. Duplicate samples, laboratory blanks and HACH verification standards were analyzed for quality control of each parameter (Appendix Table F.7 and F.8).

Analysis of *E. coli* (*Escherichia coli*) was performed on three sampling dates (June 7, 14, and 21, 2007) at the Rieth Village wetland using the US EPA approved Coliscan method (Micrology Laboratories, Goshen, IN). A 1 ml sample was taken with a sterile dropper, added to the Coliscan Easygel and immediately poured onto the prepared Coliscan petridish that was incubated at 35 °C for 24 hours. The counting of the colony forming units (CFU) was performed as instructed by the Coliscan manufactureres. Influent and effluent water samples were tested in triplicates. (Appendix Table F.9).

Concentrations of Dissolved Oxygen (DO), water temperature, and pH levels in the influent and effluent water of the constructed wetland were tested once a week for six weeks during a second monitoring period between June 16 and July 25, 2007. All parameters were monitored by use of digital probe sensors that were lowered directly into the influent and effluent pipes. The probes were calibrated before each use according to the manufacturer's recommendation. A YSI DO 200 Dissolved Oxygen Meter Probe (Cole-Parmer, Vernon Hills, IL) was used to measure both temperature and DO, and a HACH SensION1 Portable pH Meter (HACH Co., Loveland, CO) was used to measure water pH.

*All results for this background research are available in Appendix A.* The data is also summarized in Table 2.2 in Chapter two.

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APPENDIX A – CONSTRUCTED WETLAND PERFORMANCE DATA FOR SITE C.

**Results Summary**

The removal of ammonia and phosphorus were both significant at 69% and 96%, respectively). There was no significant change in the concentrations of nitrite and nitrate. The mean *E. coli* concentrations was reduced from 26000 cfu/100ml to 533 cfu/100ml (98% removal). The total iron concentration unexpectedly increased by 98%, and the cause of this increase is unknown. Dissolved oxygen levels were found to be between 0.12-0.56 mg/L and the pH ranged from 7.02 to 7.21 in the influent and from 7.11 to 7.22 in the effluent.

Table A.1 - Concentrations of various water quality indicators or pollutants in the Reith Village wetland.

Day		1-Jun-07	7-Jun-07	8-Jun-07	9-Jun-07	14-Jun-07	17-Jun-07	21-Jun-07	22-Jun-07	Mean	Std.Dev.	
<b>Iron</b> (mg/L)	RV-IN	Run 1	0.058	0.177	0.060	0.043	0.011	0.050	0.118	0.116	0.08	0.06
		Run2	0.065	0.172	0.058	0.052	0.009	0.045	0.114	0.120		
	RV-OUT	Run 1	4.080	2.360	2.040	3.010	4.530	4.050	4.220	4.120	3.56	1.69
		Run2	4.250	2.420	1.960	2.920	4.640	3.980	4.130	4.220		
<b>Phosphorus</b> (mg/L)	RV-IN	Run 1	7.000	8.400	9.100	8.700	10.400	8.600	7.800	7.900	8.49	1.01
		Run2	6.890	8.530	8.950	8.650	10.750	8.490	7.610	8.030		
	RV-OUT	Run 1	0.120	0.110	0.800	0.250	0.200	0.270	0.220	0.610	0.32	0.25
		Run2	0.230	0.180	0.110	0.350	0.280	0.340	0.320	0.780		
<b>Ammonia</b> (mg/L)	RV-IN	Run 1	17.200	16.400	13.600	5.600	20.400	13.600	20.000	24.400	16.40	5.68
		Run2	16.800	17.100	12.900	5.300	21.000	13.400	20.400	25.000		
	RV-OUT	Run 1	7.200	3.600	0.800	2.400	8.400	0.800	5.800	11.800	5.10	3.91
		Run2	7.000	3.200	1.100	2.300	7.900	1.300	6.300	11.500		
<b>Nitrite</b> (mg/L)	RV-IN	Run 1	0.007	0.000	0.077	0.010	0.093	0.027	0.032	0.158	0.05	0.06
		Run2	0.007	0.005	0.083	0.017	0.089	0.025	0.040	0.139		
	RV-OUT	Run 1	0.013	0.076	0.016	0.033	0.041	0.002	0.002	0.000	0.02	0.03
		Run2	0.009	0.077	0.022	0.028	0.044	0.000	0.003	0.002		
<b>Nitrate</b> (mg/L)	RV-IN	Run 1	0.010	0.000	0.330	0.000	0.010	0.040	0.020	0.080	0.06	0.01
		Run2	0.014	0.008	0.290	0.003	0.010	0.041	0.027	0.085		
	RV-OUT	Run 1	0.020	0.180	0.110	0.090	0.550	0.010	0.000	0.000	0.12	0.19
		Run2	0.040	0.160	0.120	0.090	0.560	0.020	0.005	0.005		
<b>Water Temp</b>   RV (°C)		19	20	23	22	20	21.8	19	20	21	1	
<b>Water Level</b>   RV (cm)		22.5	22.5	21.5	21.0	22.0	22.0	21.5	21.0	21.8	0.6	

Table A.2 – Mean concentrations of iron, phosphorus, ammonia, nitrite, and nitrate in the influent and effluent of the Rieth Village constructed wetland.

	Iron (mg/L)**	Phosphorus (mg/L)**	Ammonia (mg/L)**	Nitrite (mg/L)	Nitrate (mg/L)	Water Temp. (°C)	Water Level (cm)
Influent	0.08	8.49	16.40	0.05	0.06	20.60	55.25
SD	±0.06	±1.0	±5.7	±0.05	±0.01	±1.5	±1.5
Effluent	3.56	0.32	5.10	0.02	0.12		
SD	±1.7	±0.2	±3.9	±0.02	±0.19		
Paired T-test	p<0.00	p<0.00	p<0.00	p=0.10	p=0.27		

\*\*indicates parameters that showed a significant difference in their means from a paired T-test.

Table A.3 – *E. coli* density (cfu per 100 ml of sample) at the influent and effluent at Rieth Village constructed wetland.

Date	6/7/07	6/14/07	6/21/07	Mean	SD
<b>Influent</b> cfu/100 ml	6400	46000	25600	26000	19803
<b>Effluent</b> cfu/100 ml	800	600	200	533	306

Table A.4 – Measured concentrations and means for DO, pH, water temperature and water depth in the influent and effluent water of the Rieth Village constructed wetland.

Day	23-Jun-08	30-Jun-08	7-Jul-08	14-Jul-08	21-Jul-08	28-Jul-08	Mean	SD
DO In (mg/L)	0.12	0.38	0.14	0.21	0.20	0.15	0.20	0.09
DO Out (mg/L)	0.14	0.54	0.56	0.35	0.28	0.14	0.34	0.19
pH In	7.02	7.20	7.21	7.13	7.32	7.14	7.17	0.10
pH Out	7.11	7.13	7.22	7.17	7.15	7.14	7.15	0.04
Temp In °C	18.60	18.30	20.60	20.00	21.80	22.40	20.28	1.66
Water Depth	56.00	55.00	53.00	53.80	54.60	56.50	54.82	1.32

APPENDIX B – VEGETATION DATA FROM ALL STUDIED CONSTRUCTED  
WETLANDS

Table B.1 - Relative frequencies for vegetation found in constructed wetlands Sites A-F.

Species	Site A	Site B	Site C	Site D	Site E	Site F
<i>Aster novae-anglia</i> (New England Aster)	0.00	0.00	0.00	0.40	0.27	0.28
<i>Cichorium intybus</i> (Chicory)	0.00	0.00	0.00	0.00	0.00	0.33
<i>Cirsium vulgare</i> (Common Thistle)	0.00	0.00	0.00	0.00	0.00	0.22
<i>Cosmos bipinnatus</i> (Garden Cosmos)	0.00	0.00	0.00	0.00	0.00	0.28
<i>Helianthus annuus</i> (Sunflower)	0.00	0.00	0.00	0.00	0.00	0.17
<i>Helianthus tuberosus</i> (Jerusalem Artichoke)	0.00	0.00	0.00	0.40	0.00	0.00
<i>Ipomoea tricolor</i> (Morning Glory)	0.00	0.00	0.00	0.00	0.00	0.22
<i>Iris versicolor</i> (Blue Flag Iris)	0.00	0.00	0.00	0.27	0.33	0.28
Open Gravel	0.07	0.28	0.40	0.00	0.00	0.11
<i>Phalaris arundinacea</i> (Reed Canary Grass)	0.13	0.08	0.00	0.33	0.00	0.00
<i>Phragmites australis</i>	0.42	0.33	0.00	0.27	0.00	0.00
<i>Scirpus acutus</i> (Hard-stemmed Bulrush)	0.00	0.00	0.00	0.00	0.33	0.00
<i>Scirpus cyperinus</i> (wool grass)	0.00	0.00	0.12	0.00	0.00	0.00
<i>Scirpus fluviatilis</i> (River Bulrush)	0.28	0.35	0.16	0.00	0.20	0.00
<i>Scirpus validus creber</i> (Soft-stemmed Bulrush)	0.35	0.22	0.17	0.00	0.27	0.00
<i>Typha latifolia</i> (Common Cattail)	0.33	0.22	0.00	0.00	0.00	0.00
<i>Zea mays</i> (Corn)	0.00	0.00	0.00	0.00	0.00	0.11

Table B.2 - Relative frequencies for vegetation found in constructed wetland Sites G and H.

Site G			Site H		
Species	Int.	Freq.	Species	Int.	Freq.
<b>Aster sp</b>	<b>27</b>	<b>0.17</b>	<b>Aster sp.</b>	<b>13</b>	<b>0.06</b>
Curly Doc	6	0.04	Bone set	5	0.02
Orchard Grass	2	0.01	Carex Scoparia	9	0.04
<b>Pale Smartweed</b>	<b>8</b>	<b>0.05</b>	Cinque foil	4	0.02
<b>Phragmites</b>	<b>46</b>	<b>0.29</b>	Phragmites	4	0.02
<b>Pigweed</b>	<b>17</b>	<b>0.11</b>	<b>Goldenrod sp.</b>	<b>21</b>	<b>0.10</b>
<b>River bulrush</b>	<b>24</b>	<b>0.15</b>	<b>Pale Smartweed</b>	<b>61</b>	<b>0.30</b>
Smart Weed	1	0.01	Pigweed	4	0.02
Soft Stem Bulrush	1	0.01	<b>River Bulrush</b>	<b>38</b>	<b>0.19</b>
<b>Spiny Snow Thistle</b>	<b>15</b>	<b>0.10</b>	Smart weed	7	0.03
<b>Wool grass</b>	<b>8</b>	<b>0.05</b>	<b>Softstem bulrush</b>	<b>11</b>	<b>0.05</b>
Yellow Clover	1	0.01	Spiny Snowthistle	8	0.04
			Wild Carrot	2	0.01
			<b>Wool grass</b>	<b>17</b>	<b>0.08</b>
<b>total</b>	<b>156</b>			<b>204</b>	

APPENDIX C – RESULTS FOR DYE TRACING STUDY IN THE RIETH VILLAGE  
CONSTRUCTED WETLAND

Table C.1 - Run #1: Absorbance of dye in the wetland effluent.

<b>Date</b>	<b>Day</b>	<b>Time of Reading</b>	<b>Absorbance</b>	<b>Visual description</b>
6/2/09	Day 0	4:00 PM		No apparent Color
6/3/09	Day 1	3:00 PM	0.009	No apparent Color
6/4/09	Day 2	4:00 PM	0.009	No apparent Color
6/5/09	Day 3	4:00 PM	0.011	No apparent Color
6/6/09	Day 4	4:00 PM	0.011	No apparent Color
6/7/09	Day 5	4:00 PM	0.012	No apparent Color
6/8/09	Day 6	4:00 PM	0.011	No apparent Color
6/9/09	Day 7	4:00 PM	0.014	No apparent Color
6/10/09	Day 8	4:00 PM	0.036	No apparent Color
6/11/09	Day 9	4:00 PM	0.036	No apparent Color
6/12/09	Day 10	4:00 PM	0.036	No apparent Color
6/13/09	Day 11	5:00 PM	0.038	No apparent Color
6/14/09	Day 12	4:00 PM	0.047	No apparent Color
6/15/09	Day 13	4:00 PM	0.049	Very Light Green
6/16/09	Day 14	4:00 PM	0.055	Light Green
6/17/09	Day 15	4:00 PM	0.065	Green
6/18/09	Day 16	4:00 PM	0.05	Green
6/19/09	Day 17	3:00 PM	0.046	Green
6/20/09	Day 18	4:00 PM	0.0365	Light Green
6/21/09	Day 19	4:00 PM	0.026	Light Green
6/22/09	Day 20	5:00 PM	0.025	No apparent Color
6/23/09	Day 21	5:00 PM	0.02	No apparent Color
6/24/09	Day 22	4:00 PM	0.01	No apparent Color
6/25/09	Day 23	4:00 PM	0.007	No apparent Color

Table C.2 - Run #2: Absorbance of dye in the wetland effluent.

<b>Date</b>	<b>Day</b>	<b>Time of Reading</b>	<b>Absorbance</b>	<b>Visual description</b>
7/1/09	Day 0	10:00 AM		No apparent Color
7/2/09	Day 1	3:00 PM	0.009	No apparent Color
7/3/09	Day 2	2:00 PM	0.011	No apparent Color
7/4/09	Day 3	3:00 PM	0.013	No apparent Color
7/5/09	Day 4	4:00 PM	0.033	No apparent Color
7/6/09	Day 5	4:00 PM	0.032	No apparent Color
7/7/09	Day 6	3:00 PM	0.031	No apparent Color
7/8/09	Day 7	3:30 PM	0.034	No apparent Color
7/9/09	Day 8	3:00 PM	0.035	No apparent Color
7/10/09	Day 9	3:30 PM	0.034	No apparent Color
7/11/09	Day 10	4:00 PM	0.035	No apparent Color
7/12/09	Day 11	5:00 PM	0.033	No apparent Color
7/13/09	Day 12	4:00 PM	0.036	No apparent Color
7/14/09	Day 13	5:00 PM	0.05	Very Light Green
7/15/09	Day 14	4:00 PM	0.07	Light Green
7/16/09	Day 15	3:00 PM	0.055	Green
7/17/09	Day 16	3:00 PM	0.048	Green
7/18/09	Day 17	3:00 PM	0.03	Yellow/green
7/19/09	Day 18	3:30 PM	0.024	Light Green
7/20/09	Day 19	3:30 PM	0.014	No apparent Color
7/21/09	Day 20	5:30 PM	0.014	No apparent Color
7/22/09	Day 21	5:30 PM	0.013	No apparent Color
7/23/09	Day 22	4:30 PM	0.012	No apparent Color
7/24/09	Day 23	4:30 PM	0.01	No apparent Color

Table C.3 – Mean precipitation for the periods of June 2-25, 2009 (Run 1) and July 1-24, 2009 (Run 2).

	Run 1	Run 2
Mean Temperature (°C)	18.6 ±3	18.4 ±2
Mean Precipitation (cm)	0.23 ±0.6	0.23 ±0.7

APPENDIX D – RESULTS OF THE TRICLOSAN INJECTION STUDY AT SITE C  
(RIETH VILLAGE) CONSTRUCTED WETLAND

Table D.1- Triclosan concentrations in the influent and effluent after injection in Rieth Village constructed wetland (Site C). Methods detailed in Chapter 2.

TRICLOSAN INJECTION IN REITH VILLAGE CONSTRUCTED WETLAND (µg/L)												
	Injected	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18	DAY 20	
<b>IN - R1</b>	2.2	1.743	1.669	0.370	0.591	0.593	0.477	0.432	0.344	0.358	0.341	
<b>OUT - R1</b>		0.570	0.520	0.339	0.535	0.875	1.134	0.983	0.733	0.650	0.446	
<b>IN - R2</b>	2.3	1.843	1.778	0.467	0.448	0.367	0.465	0.513	0.366	0.445	0.289	
<b>OUT - R2</b>		0.226	0.256	0.267	0.446	0.777	0.993	1.347	0.659	0.246	0.257	
<b>Time (Days)</b>		2	4	6	8	10	12	14	16	18	20	
<b>C/C<sub>0</sub></b>	<b>Run 1</b>	0.259	0.236	0.154	0.245	0.398	0.515	0.447	0.333	0.295	0.203	
<b>C/C<sub>0</sub></b>	<b>Run 2</b>	0.098	0.111	0.116	0.194	0.338	0.432	0.586	0.287	0.107	0.112	
<b>Min</b>	Run 1	0.34	0.23									
<b>Max</b>	Run 2	1.13	1.35									

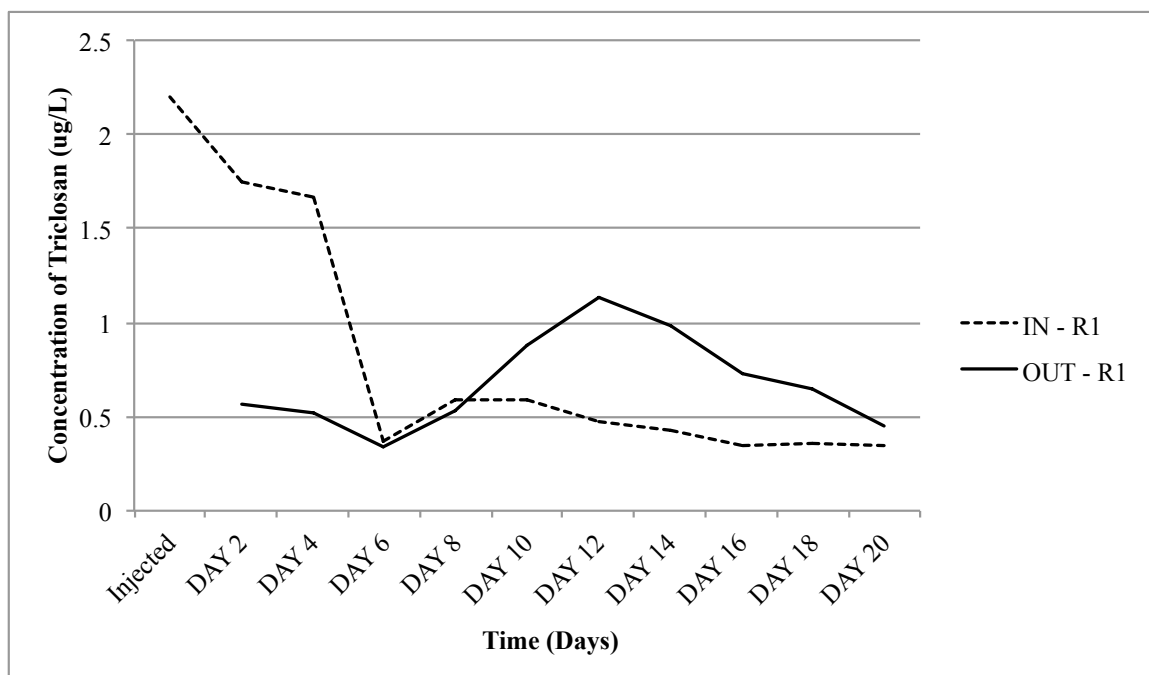


Figure D.1 – Graph of triclosan levels in the influent and effluent water of the Rieth Village constructed (Site C) wetland after the injection of triclosan for Run 1. Methods detailed in Chapter 2 (Run 1).

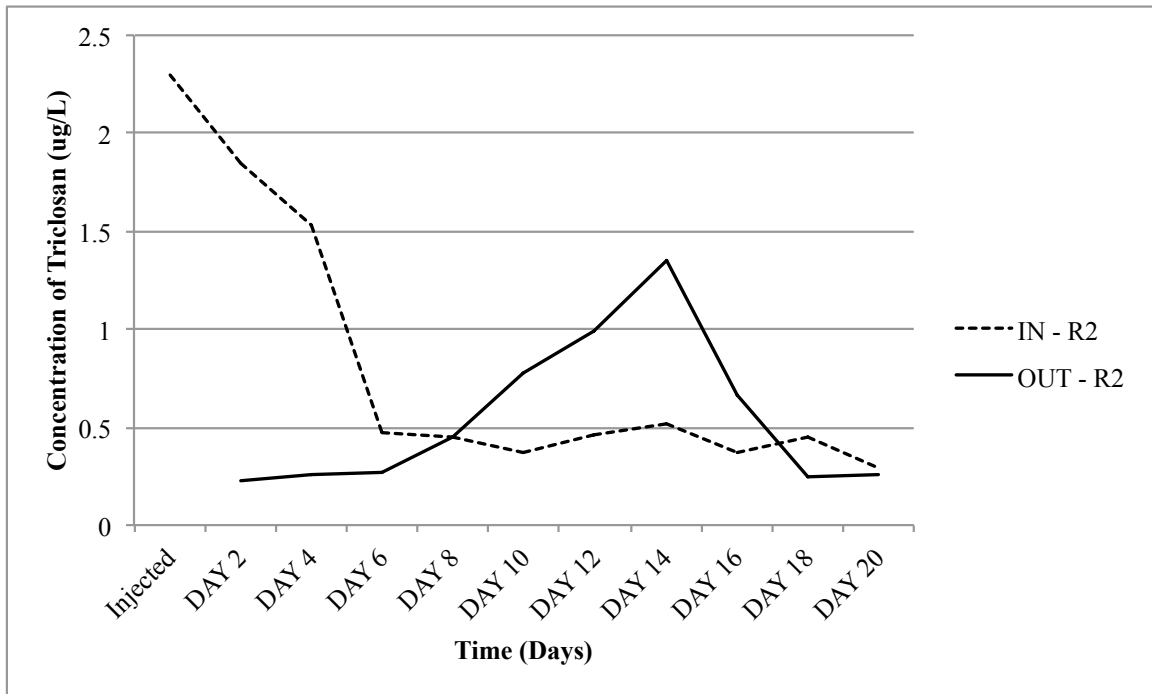


Figure D.2 – Graph of triclosan levels in the influent and effluent water of the Rieth Village constructed (Site C) wetland after the injection of triclosan for Run 2. Methods detailed in Chapter 2 (Run 2).

APPENDIX E – RESULTS OF TRICLOSAN TESTING AT SIX CONSTRUCTED WETLANDS AND ENVIRONMENTAL PARAMETERS TESTED AT THE TIME OF TRICLOSAN TESTING

Table E.1 - Triclosan concentrations in the influent and effluent constructed wetland Sites A-F for each sample day (µg/L). Methods detailed in Chapter 2.

Date	Site A IN	Site A OUT	Site B IN	Site B OUT	Site C IN	Site C OUT	Site D IN	Site D OUT	Site E IN	Site E OUT	Site F IN	Site F OUT
8/12/08	0.44	0.58	0.44	0	1.18	1.05	1.13	0	1.04	0.92	0.84	0.36
8/15/08	0.28	0.14	0.28	0.82	1.22	0.13	0.95	1.26	1.03	1.22	1.29	1.26
8/19/08	0.91	0.43	0.91	0.78	1.01	0.76	1.21	1.05	1.02	0.86	1.15	1.09
8/22/08	0.7	0.95	0.7	0.02	1.18	1.34	1.44	0.75	1.24	1.06	1.01	0.94
8/29/08	0.75	0.64	0.75	0.84	1.15	1.13	1.14	0.79	1.26	0.81	1.01	0.94
9/2/08	0.91	0.15	0.91	0.49	1.21	0.61	1.07	1.1	1.32	1	1.12	0.51
9/5/08	0.79	0.32	0.79	0.23	1.36	0.1	1.58	0.83	1.09	1.22	1.21	0.75
9/9/08	0.8	0.41	0.8	0.73	1.19	0.51	1.12	0.9	1.22	0.94	0.7	1.09
9/12/08	1.23	0.36	1.23	0.42	1.03	0.58	1.06	0.96	1.14	1	1.11	1.2
9/16/08	1.19	1.09	1.19	0.89	1.22	1.02	1.14	1.05	1.22	1.2	1.23	1.17
9/20/08	1.06	0.44	1.06	0	1.61	0.11	1.84	1.11	2.05	1.48	1.98	1.39
9/24/08	1.28	0.36	1.28	0	1.89	0.11	1.52	0.95	1.59	1.59	2.05	1.39
9/27/08	1.58	1.09	1.58	0.74	1.88	0.22	1.47	1.35	1.9	1.72	1.98	1.13
9/30/08	1.01	0.97	1.01	0.58	1.22	0.37	1.07	0.98	1.25	1.11	1.25	0.89
10/3/08	1.1	1.01	1.1	0.29	1.19	0.34	1.07	0.62	1.22	1.01	1.2	0.92
10/7/08	0.77	0.84	0.77	0.41	1.06	0.25	0.96	0.62	1.12	0.74	1.24	0.84
10/10/08	0.85	0.49	0.85	0.1	1.08	0.05	0.93	0.79	1.18	0.88	1.3	0.87
10/14/08	1.13	0.91	1.13	0.5	1.02	0.83	0.89	0.77	1.17	0.96	1.19	0.82
10/17/08	0.96	0.86	0.96	0.81	0.97	0.76	1.03	0.92	1.13	1.01	1.1	1.03
10/21/08	0.87	0.82	0.87	0.68	1.15	0.6	1.02	0.84	1.22	0.75	1.18	1.03

Table E.2 – Triclosan concentrations in the influent and effluent water for constructed wetland Sites C, G and H (µg/L). Methods detailed in Chapter 2.

Date	Site G		Site H		Site C		
	Influent	Effluent	Influent	Effluent	Influent	Effluent	
6/16/07		0.789	0.576	0.797	0.673	0.712	0.351
6/23/07		0.773	0.562	0.731	0.523	0.750	0.444
6/30/07		0.727	0.411	0.719	0.443	0.605	0.346
7/7/07		0.733	0.561	0.739	0.469	0.381	0.279
7/14/07		0.726	0.380	0.484	0.248	0.611	0.329
7/21/07		0.719	0.432	0.685	0.516	0.613	0.339
7/27/07		0.736	0.581	0.747	0.579	0.734	0.692

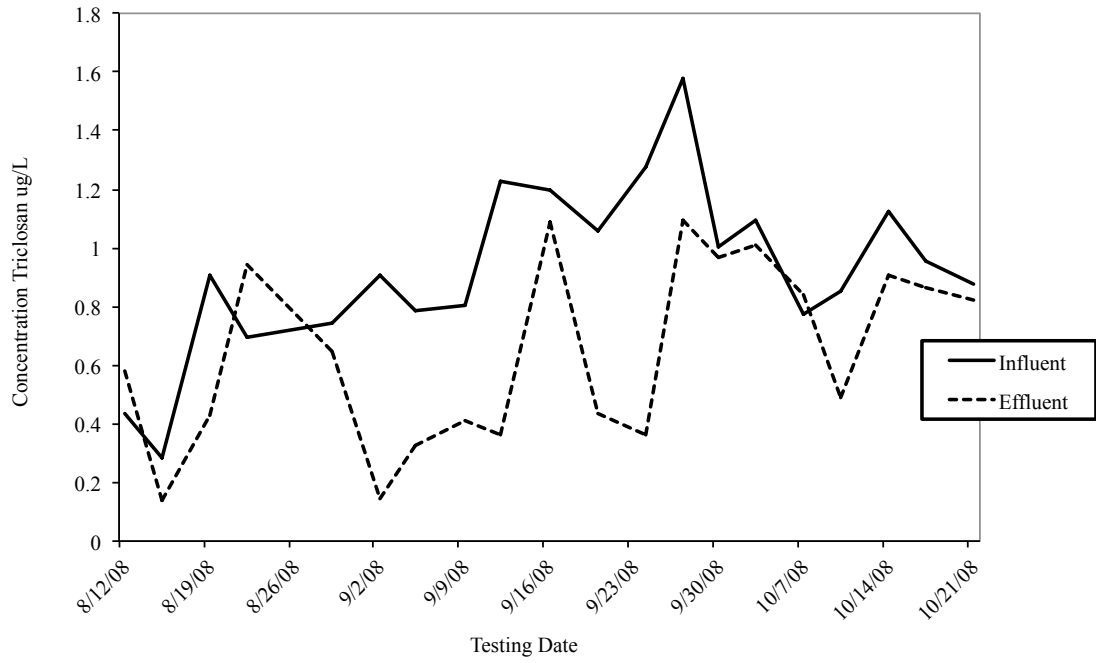


Figure E.1 - Measured triclosan concentrations for Site A constructed wetland.

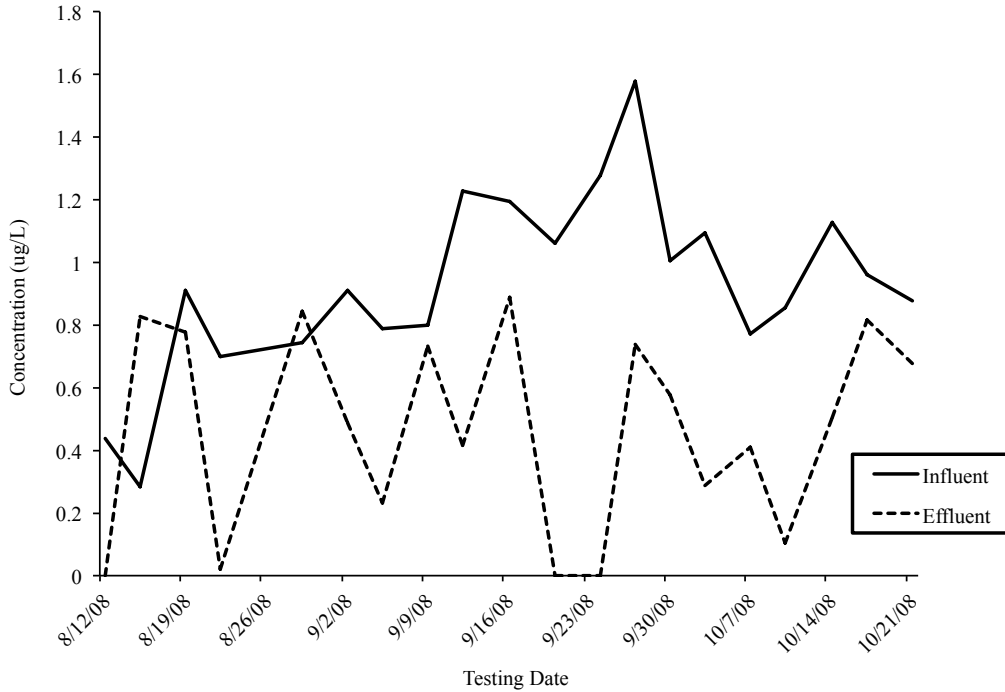


Figure E.2- Measured triclosan concentrations for Site B constructed wetland.

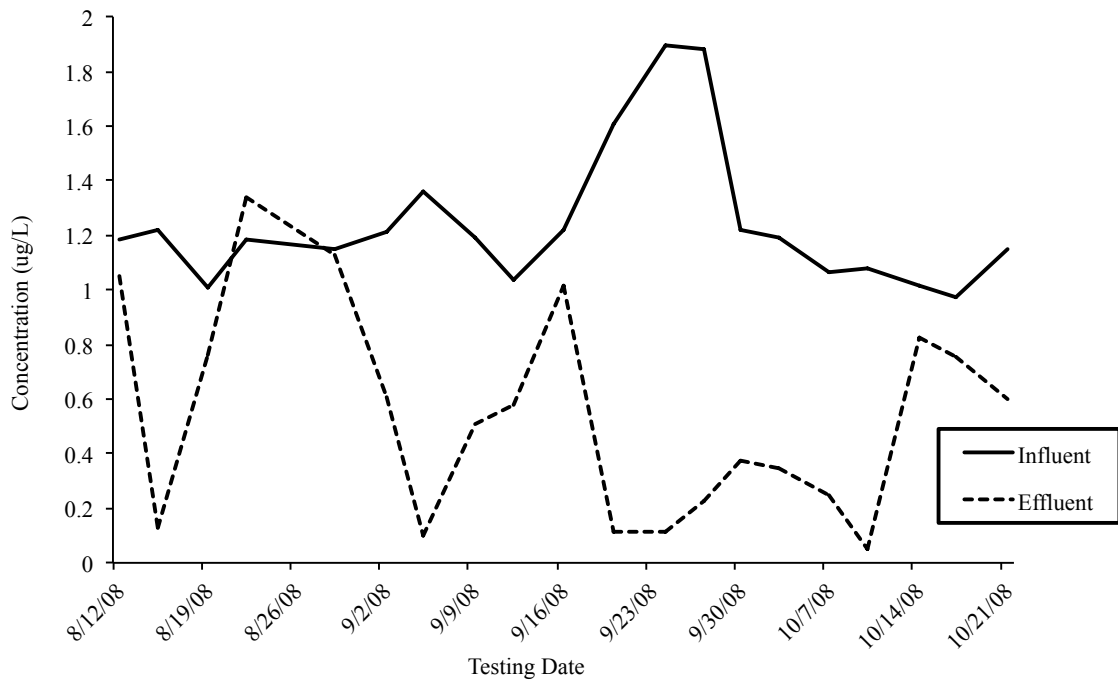


Figure E.3- Measured triclosan concentrations for Site C constructed wetland.

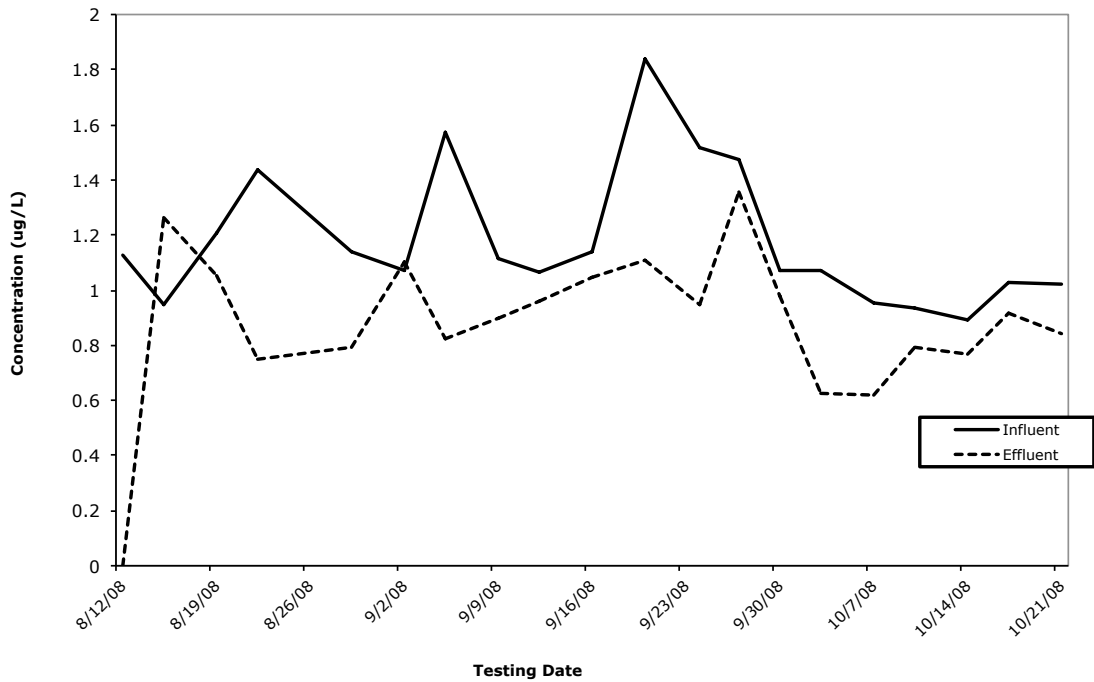


Figure E.4- Measured triclosan concentrations for Site D constructed wetland.

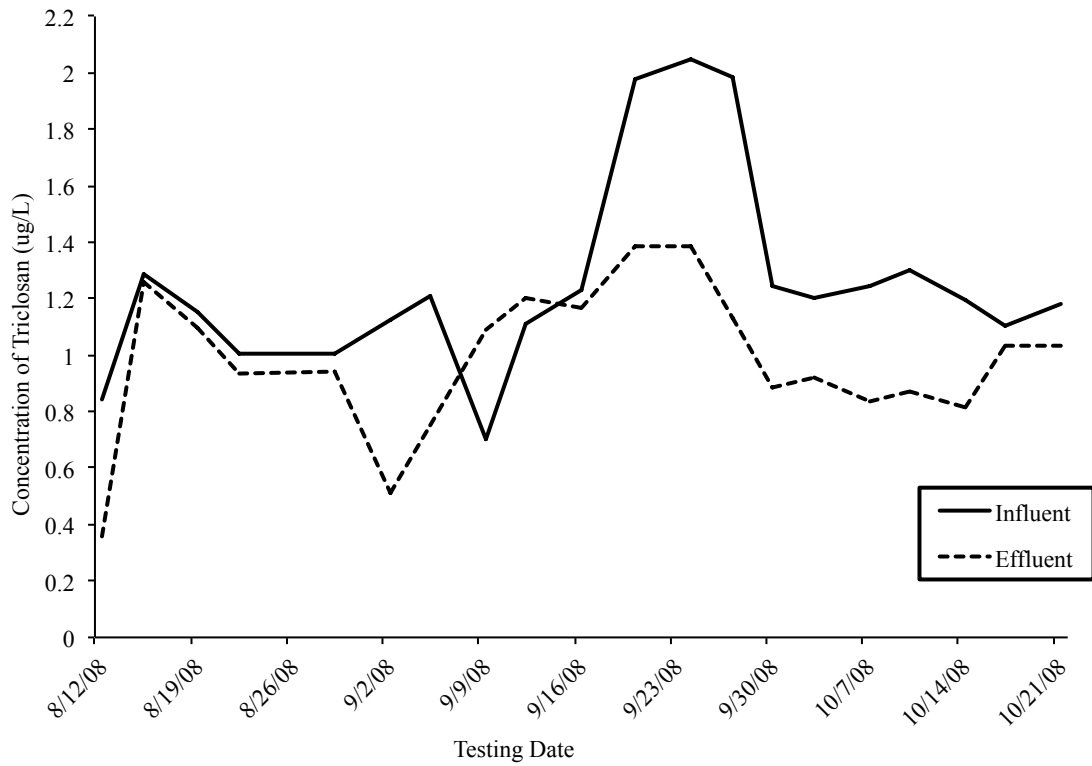


Figure E.5- Measured triclosan concentrations for Site E constructed wetland.

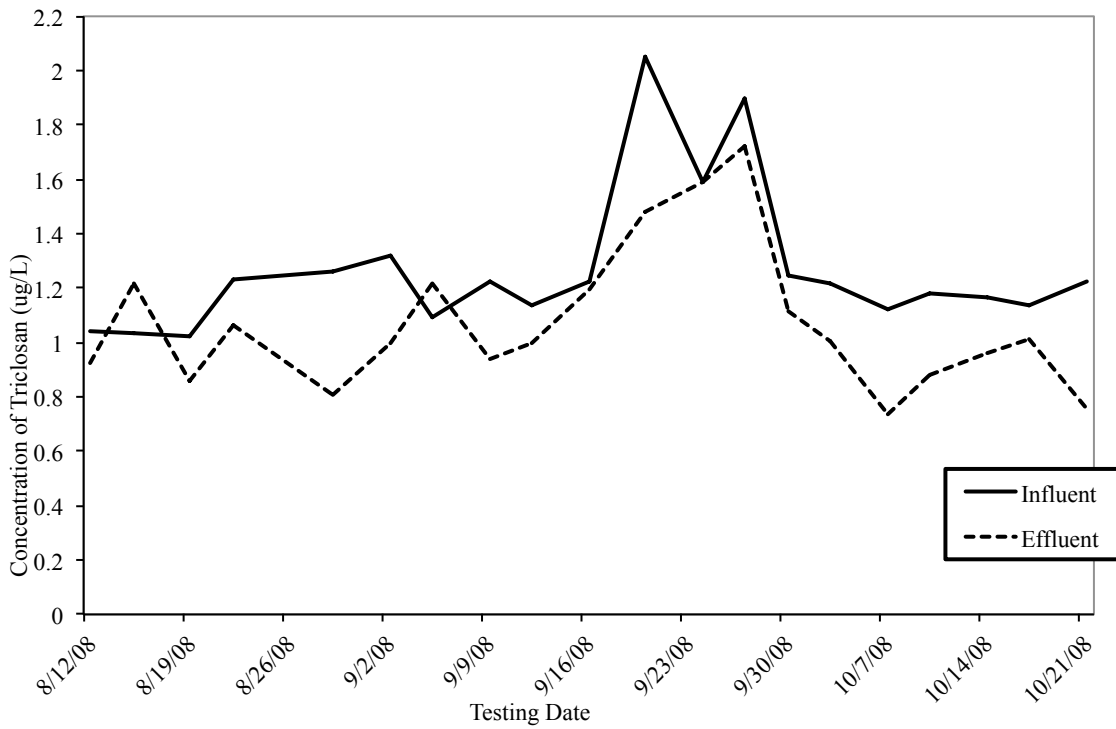


Figure E.6- Measured triclosan concentrations for Site F constructed wetland.

Table E.3—Air temperature at the time of water sample collection at the six constructed wetlands.

Date	Air Temperature at Time of Sample Collection					
	°C	°C	°C	°C	°C	°C
	Site A SA Air Temp	Site B SB Air Temp	Site C SC Air Temp	Site D SD Air Temp	Site E SE Air Temp	Site F SF Air Temp
8/12/08	25.6	25.6	26.7	26.1	26.1	26.7
8/15/08	30.5	30.5	29.2	29.4	29.4	29.4
8/19/08	26.1	26.1	25.0	25.6	25.6	25.6
8/22/08	25.6	25.6	23.9	23.9	23.9	23.9
8/29/08	25.0	25.0	29.4	25.0	26.1	26.1
9/2/08	31.7	31.7	34.4	32.2	32.2	32.2
9/5/08	23.9	23.9	21.1	23.9	23.9	23.9
9/9/08	20.0	20.0	19.4	20.0	20.0	19.4
9/12/08	21.7	21.7	21.7	21.7	21.7	21.7
9/16/08	18.3	18.3	20.0	18.9	18.9	19.4
9/20/08	25.6	25.6	23.9	24.4	24.4	23.9
9/24/08	28.9	28.9	26.1	28.3	28.3	28.3
9/27/08	25.6	25.6	23.9	25.0	25.0	25.0
9/30/08	16.1	16.1	12.8	15.6	15.0	14.4
10/3/08	15.0	15.0	12.2	13.3	13.3	13.3
10/7/08	20.0	20.0	18.3	18.9	18.9	18.9
10/10/08	22.8	22.8	22.8	22.8	22.8	22.2
10/14/08	19.4	19.4	18.3	18.3	18.3	18.3
10/17/08	13.9	13.9	12.8	13.9	13.9	13.3
10/21/08	11.7	11.7	11.1	11.7	11.7	11.1
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	23.33	23.33	22.22	23.33	23.33	23.06
<b>Mean</b>	22.36	22.36	21.65	21.94	21.97	21.86
<b>Stan Dev</b>	5.50	5.50	6.24	5.56	5.63	5.77
<b>Max</b>	31.7	31.7	34.4	32.2	32.2	32.2
<b>Min</b>	11.7	11.7	11.1	11.7	11.7	11.1

Table E.4 - Water temperature of influent water in the constructed wetlands at the time of water sample collection.

Date	Water Temp of Influent at Time of Sample Collection					
	°C Site A	°C Site B	°C Site C	°C Site D	°C Site E	°C Site F
8/12/08	19.1	19.1	19.4	19.1	22.1	20
8/15/08	21	21	18.4	19.1	20	20.7
8/19/08	21.4	21.4	23	19	22.8	21.2
8/22/08	21.5	21.5	21.2	19	21	21.3
8/29/08	20.8	20.8	22	19.6	22.1	21.3
9/2/08	20.3	20.3	21.4	19.6	22.6	21.5
9/5/08	19.2	19.2	19.4	19.4	22.3	21.1
9/9/08	20	20	19.8	18.9	20.4	19.7
9/12/08	19	19	19.9	18.5	20.8	19.6
9/16/08	19.8	19.8	20.4	18.8	20.8	20.1
9/20/08	20	20	20.4	18.4	20.5	19.5
9/24/08	20	20	20.2	18.8	20.9	19.9
9/27/08	19.3	19.3	20.1	18.6	20.3	19.8
9/30/08	19.1	19.1	17.5	17.8	19.5	18.8
10/3/08	20.8	20.8	16.5	17	18.4	17.6
10/7/08	17.9	17.9	15.9	16.2	18.5	17.7
10/10/08	17.2	17.2	15.8	16.1	18.5	17.6
10/14/08	17.2	17.2	17.8	16	18.3	16.9
10/17/08	17.6	17.2	16.5	16.2	18.2	17
10/21/08	16.8	16.8	16.2	16	17.2	16.8
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	19.55	19.55	19.60	18.70	20.45	19.75
<b>Mean</b>	19.40	19.38	19.09	18.11	20.26	19.41
<b>Stan Dev</b>	1.44	1.47	2.15	1.32	1.66	1.61
<b>Max</b>	21.50	21.50	23.00	19.60	22.80	21.50
<b>Min</b>	16.80	16.80	15.80	16.00	17.20	16.80

Table E.5—Water temperature in the effluent water at constructed wetlands at the time of water sample collection.

Date	Water Temp of Effluent at Time of Sample Collection					
	°C Site A	°C Site B	°C Site C	°C Site D	°C Site E	°C Site F
8/12/08	19	19.1	20.2	19.6	20	20
8/15/08	21.1	21.1	18.7	19	20.1	20.5
8/19/08	21.8	21.8	22.5	19.2	22.2	20.7
8/22/08	22.5	22.6	21.2	19.6	22.6	21
8/29/08	21.7	21.7	21.8	19.5	22.2	21.2
9/2/08	23.1	22.9	21.7	20.5	21.7	21.3
9/5/08	21.5	21.4	19.5	19.5	22.5	21.4
9/9/08	20.1	20.1	20	19.4	20.5	19.5
9/12/08	17.4	18.3	20.2	18.1	20.2	19.9
9/16/08	17.9	18.2	18.3	19.1	20.2	19.8
9/20/08	19.8	19.4	19.1	19	19.8	19.5
9/24/08	21.5	20.9	19.2	18.4	20.2	19.8
9/27/08	18.7	18.4	18.7	18.4	20.2	19.7
9/30/08	16.5	17.6	16.7	17.8	18.5	19.1
10/3/08	16.4	16.8	14.88	17.2	17.5	17.8
10/7/08	14.9	14.2	14.4	16.4	16.3	17
10/10/08	15.1	15.7	13.6	16.3	16.9	17
10/14/08	14.2	15.3	15.9	16.3	17.5	17.4
10/17/08	14.9	13.8	14	15.7	16	16.8
10/21/08	13.4	13.8	13.1	14.2	15.4	16.5
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	18.85	18.75	18.90	18.70	20.15	19.75
<b>Mean</b>	18.58	18.66	18.18	18.16	19.53	19.30
<b>Stan Dev</b>	3.07	2.96	2.95	1.64	2.26	1.63
<b>Max</b>	23.10	22.90	22.50	20.50	22.60	21.40
<b>Min</b>	13.40	13.80	13.10	14.20	15.40	16.50

Table E.6 - Dissolved oxygen concentrations in the constructed wetland influent at the time of water sample collection.

<b>D.O. Concentrations in Influent at Time of Sample Collection</b>							
<b>Date</b>	(mg/l) <b>Site A</b>	(mg/l) <b>Site B</b>	(mg/l) <b>Site C</b>	(mg/l) <b>Site D</b>	(mg/l) <b>Site E</b>	(mg/l) <b>Site F</b>	
8/12/08	0.5	0.5	0.4	0.43	0.25	0.53	
8/15/08	0.47	0.47	0.38	0.6	2.27	0.35	
8/19/08	0.32	0.32	0.28	0.58	0.3	0.19	
8/22/08	0.28	0.28	0.26	0.36	0.36	0.27	
8/29/08	0.32	0.32	0.31	0.27	0.13	0.28	
9/2/08	0.75	0.75	0.54	0.25	0.18	0.4	
9/5/08	0.52	0.52	0.52	0.24	0.19	0.48	
9/9/08	0.75	0.75	0.46	1.25	0.24	0.65	
9/12/08	0.65	0.65	0.44	0.85	0.23	0.38	
9/16/08	0.94	0.94	0.4	0.51	0.26	0.51	
9/20/08	0.46	0.46	0.53	0.37	0.31	0.49	
9/24/08	0.47	0.47	0.47	0.8	0.41	0.67	
9/27/08	0.51	0.51	0.53	1.12	0.52	0.54	
9/30/08	0.52	0.52	0.69	2.16	0.78	0.76	
10/3/08	0.66	0.66	0.69	0.75	0.84	0.62	
10/7/08	0.69	0.69	0.64	0.51	1.27	0.63	
10/10/08	0.73	0.73	0.31	1.11	0.78	0.6	
10/14/08	0.43	0.43	0.48	0.43	0.52	0.57	
10/17/08	1.41	1.41	0.3	0.95	0.82	0.72	
10/21/08	1.02	1.02	0.68	1.11	0.75	0.6	
	Site A	Site B	Site C	Site D	Site E	Site F	
<b>Median</b>	0.52	0.52	0.47	0.59	0.39	0.54	
<b>Mean</b>	0.62	0.62	0.47	0.73	0.57	0.51	
<b>Stan Dev</b>	0.27	0.27	0.14	0.46	0.50	0.16	
<b>Max</b>	1.41	1.41	0.69	2.16	2.27	0.76	
<b>Min</b>	0.28	0.28	0.26	0.24	0.13	0.19	

Table E.7 - Dissolved oxygen concentrations in the constructed wetland effluent at the time of water sample collection.

<b>D.O. Concentrations in Effluent at Time of Sample Collection</b>							
<b>Date</b>	(mg/l) <b>Site A</b>	(mg/l) <b>Site B</b>	(mg/l) <b>Site C</b>	(mg/l) <b>Site D</b>	(mg/l) <b>Site E</b>	(mg/l) <b>Site F</b>	
8/12/08	0.24	0.24	0.33	6.15	0.25	2.16	
8/15/08	0.23	0.24	0.32	4	0.27	2.17	
8/19/08	0.35	0.37	1.15	2.48	1.75	1.9	
8/22/08	0.36	0.22	0.97	3.65	1.65	1.42	
8/29/08	0.27	0.25	1.23	3.05	1.61	2.3	
9/2/08	0.54	0.33	0.25	2.75	1.34	2.5	
9/5/08	0.19	0.28	0.47	3.5	1.6	1.93	
9/9/08	0.41	0.28	0.46	3.5	2.2	1.71	
9/12/08	0.34	0.46	1	3.43	1.89	2.7	
9/16/08	0.3	0.23	0.62	4.2	1.39	1.89	
9/20/08	0.28	0.29	0.36	3.92	1.26	1.58	
9/24/08	0.55	0.28	0.44	4.52	1.23	2.27	
9/27/08	0.33	0.29	0.27	3.55	1.51	1.82	
9/30/08	0.35	0.34	0.42	3.71	1.9	2.42	
10/3/08	0.47	0.4	0.47	4.09	2.5	2.81	
10/7/08	0.42	0.5	0.4	3.91	2.21	1.17	
10/10/08	0.3	0.38	0.42	3.57	2.11	1.69	
10/14/08	0.36	0.37	0.32	3.39	1.72	1.62	
10/17/08	0.43	0.42	0.31	3.12	2.54	1.76	
10/21/08	0.54	0.48	0.42	3.02	2	1.57	
	Site A	Site B	Site C	Site D	Site E	Site F	
<b>Median</b>	0.35	0.31	0.42	3.56	1.69	1.90	
<b>Mean</b>	0.36	0.33	0.53	3.68	1.65	1.97	
<b>Stan Dev</b>	0.10	0.09	0.30	0.77	0.61	0.44	
<b>Max</b>	0.55	0.50	1.23	6.15	2.54	2.81	
<b>Min</b>	0.19	0.22	0.25	2.48	0.25	1.17	

Table E.8 – Depth of water table at the time of water sample collection. (Depth = the distance the top of the water table from the constructed wetland surface.)

Date	Distance from gravel surface to water table at Time of Sample Collection					
	cm Site A	cm Site B	cm Site C	cm Site D	cm Site E	cm Site F
8/12/08	4	4	5	40	25	20
8/15/08	3	3	5	40	25	20
8/19/08	2	2	3	45	25	25
8/22/08	7	7	5	40	20	15
8/29/08	4	4	5	40	33	30
9/2/08	10	10	10	60	45	40
9/5/08	10	10	5	40	35	30
9/9/08	2	2	3	35	15	20
9/12/08	2	2	4	40	30	25
9/16/08	2	2	2	45	27	37
9/20/08	3	3	4	30	30	20
9/24/08	2	2	2	40	33	30
9/27/08	2	2	4	44	30	30
9/30/08	2	2	2	46	35	25
10/3/08	2	2	2	40	33	25
10/7/08	2	2	2	40	30	25
10/10/08	2	2	2	40	35	25
10/14/08	2	2	2	40	30	25
10/17/08	2	2	2	38	30	30
10/21/08	2	2	2	38	34	25
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	2.00	2.00	3.00	40.00	30.00	25.00
<b>Mean</b>	3.35	3.35	3.55	41.05	30.00	26.10
<b>Stan Dev</b>	2.58	2.58	1.99	5.68	6.29	5.94
<b>Max</b>	10.00	10.00	10.00	60.00	45.00	40.00
<b>Min</b>	2.00	2.00	2.00	30.00	15.00	15.00

Table E.9 - Total rainfall amounts for the week proceeding the time of water sample collection.

Date	Rainfall for Week Proceeding Time of Sample collection					
	(mm) Site A	(mm) Site B	(mm) Site C	(mm) Site D	(mm) Site E	(mm) Site F
8/12/08	5.58	5.58	24.64	5.58	5.58	5.58
8/15/08	5.08	5.08	32.25	5.08	5.08	5.08
8/19/08	0	0	20.31	0	0	0
8/22/08	0.76	0.76	0.25	0.76	0.76	0.76
8/29/08	16.76	16.76	0.25	16.76	16.76	16.76
9/2/08	10.41	10.41	0.25	10.41	10.41	10.41
9/5/08	8.63	8.63	5.59	8.63	8.63	8.63
9/9/08	12.7	12.7	10.41	12.7	12.7	12.7
9/12/08	6.86	6.86	9.65	6.86	6.86	6.86
9/16/08	116.08	116.08	33.53	116.08	116.08	116.08
9/20/08	84.58	84.58	108.2	84.58	84.58	84.58
9/24/08	0.25	0.25	0	0.25	0.25	0.25
9/27/08	0	0	0	0	0	0
9/30/08	2.54	2.54	0.25	2.54	2.54	2.54
10/3/08	10.16	10.16	1.26	10.16	10.16	10.16
10/7/08	7.62	7.62	2.28	7.62	7.62	7.62
10/10/08	3.56	3.56	6.61	3.56	3.56	3.56
10/14/08	3.56	3.56	4.83	3.56	3.56	3.56
10/17/08	18.29	18.29	10.67	18.29	18.29	18.29
10/21/08	18.03	18.03	11.18	18.03	18.03	18.03
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	7.24	7.24	6.10	7.24	7.24	7.24
<b>Mean</b>	16.57	16.57	14.12	16.57	16.57	16.57
<b>Stan Dev</b>	29.66	29.66	24.55	29.66	29.66	29.66
<b>Max</b>	116.08	116.08	108.20	116.08	116.08	116.08
<b>Min</b>	0.00	0.00	0.00	0.00	0.00	0.00

Table E.10 - The mean air temperature for the week proceeding each water sample collection time.

Date	Mean Air Temp for Week Proceeding Time of Sample Collection					
	°C Site A	°C Site B	°C Site C	°C Site D	°C Site E	°C Site F
8/12/08	20.2	20.2	21.6	20.2	20.2	20.2
8/15/08	19	19	20.7	19	19	19
8/19/08	20.5	20.5	22.3	20.5	20.5	20.5
8/22/08	21.4	21.4	23	21.4	21.4	21.4
8/29/08	21.4	21.4	24.2	21.4	21.4	21.4
9/2/08	21.6	21.6	25.2	21.6	21.6	21.6
9/5/08	22.6	22.6	22.3	22.6	22.6	22.6
9/9/08	20	20	19	20	20	20
9/12/08	19	19	18.9	19	19	19
9/16/08	16.7	16.7	20.2	16.7	16.7	16.7
9/20/08	17.5	17.5	20.1	17.5	17.5	17.5
9/24/08	17.4	17.4	19.9	17.4	17.4	17.4
9/27/08	18.7	18.7	20.7	18.7	18.7	18.7
9/30/08	18.3	18.3	19.9	18.3	18.3	18.3
10/3/08	14.7	14.7	15.9	14.7	14.7	14.7
10/7/08	10.4	10.4	12.4	10.4	10.4	10.4
10/10/08	11.5	11.5	11.9	11.5	11.5	11.5
10/14/08	15.2	15.2	16.9	15.2	15.2	15.2
10/17/08	14.7	14.7	17.2	14.7	14.7	14.7
10/21/08	13.4	13.4	13.2	13.4	13.4	13.4
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	18.50	18.50	20.00	18.50	18.50	18.50
<b>Mean</b>	17.71	17.71	19.28	17.71	17.71	17.71
<b>Stan Dev</b>	3.45	3.45	3.72	3.45	3.45	3.45
<b>Max</b>	22.60	22.60	25.20	22.60	22.60	22.60
<b>Min</b>	10.40	10.40	11.90	10.40	10.40	10.40

Table E.11 – Water pH in the influent water in the constructed wetland at the time of sample collection.

<b>pH Concentrations in Influent Water at Time of Sample Collection</b>							
<b>Date</b>	SU <b>Site A</b>	SU <b>Site B</b>	SU <b>Site C</b>	SU <b>Site D</b>	SU <b>Site E</b>	SU <b>Site F</b>	
8/12/08	7.06	7.06	7.14	7.30	7.09	7.35	
8/15/08	7.30	7.30	7.22	7.45	7.29	7.33	
8/19/08	7.77	7.77	7.14	7.80	7.55	7.45	
8/22/08	7.67	7.67	7.14	7.41	7.00	7.11	
8/29/08	8.00	8.00	7.21	7.45	7.25	7.00	
9/2/08	7.19	7.19	7.00	7.65	7.25	8.00	
9/5/08	7.18	7.18	7.15	7.77	7.16	7.40	
9/9/08	7.50	7.50	7.22	7.48	6.89	7.01	
9/12/08	7.41	7.41	7.24	7.80	7.21	7.14	
9/16/08	7.66	7.66	7.30	7.91	7.63	7.00	
9/20/08	7.40	7.40	7.16	7.83	6.94	7.17	
9/24/08	7.86	7.86	7.24	7.50	7.30	7.14	
9/27/08	7.75	7.75	7.19	7.65	7.15	7.15	
9/30/08	8.10	8.10	7.17	7.74	7.16	7.23	
10/3/08	7.21	7.21	7.09	7.74	7.12	8.01	
10/7/08	7.27	7.27	7.06	7.22	7.06	7.42	
10/10/08	7.50	7.50	7.22	7.35	6.88	7.12	
10/14/08	7.43	7.43	7.24	7.72	7.30	7.23	
10/17/08	7.66	7.66	7.17	7.81	7.53	7.98	
10/21/08	7.60	7.60	7.17	7.68	7.45	7.42	
	Site A	Site B	Site C	Site D	Site E	Site F	
<b>Median</b>	7.50	7.50	7.17	7.67	7.19	7.23	
<b>Mean</b>	7.53	7.53	7.17	7.61	7.21	7.33	
<b>Stan Dev</b>	0.29	0.29	0.07	0.20	0.21	0.32	
<b>Max</b>	8.10	8.10	7.30	7.91	7.63	8.01	
<b>Min</b>	7.06	7.06	7.00	7.22	6.88	7.00	

Table E.12 – Water pH in the constructed wetland effluent water at the time of sample collection.

Date	pH Levels in Effluent at Time of Sample Collection					
	SU Site A	SU Site B	SU Site C	SU Site D	SU Site E	SU Site F
8/12/08	7.46	7.37	7.14	7.30	7.09	7.35
8/15/08	7.24	7.14	7.01	6.50	7.20	7.28
8/19/08	7.42	7.48	7.04	7.80	7.21	7.59
8/22/08	7.74	7.64	7.19	7.32	7.20	7.61
8/29/08	7.68	7.77	7.24	7.49	7.23	7.38
9/2/08	7.75	7.64	7.06	7.55	7.40	7.62
9/5/08	7.07	7.06	7.06	7.57	7.11	7.40
9/9/08	7.61	7.62	7.15	7.52	7.02	7.20
9/12/08	7.40	7.54	7.10	7.32	7.00	7.33
9/16/08	7.66	7.35	7.12	7.24	7.14	7.11
9/20/08	7.67	7.47	7.08	7.26	7.35	7.16
9/24/08	7.74	7.95	7.14	7.31	7.22	7.22
9/27/08	7.78	7.88	7.03	7.43	6.99	7.77
9/30/08	7.60	7.72	7.00	7.37	7.34	7.61
10/3/08	7.08	7.05	7.05	7.47	7.18	7.29
10/7/08	7.40	7.34	7.11	7.45	7.04	7.60
10/10/08	7.21	7.20	7.20	7.65	6.91	7.29
10/14/08	7.42	7.32	7.22	7.29	6.98	7.21
10/17/08	7.67	7.69	7.18	7.52	6.89	7.39
10/21/08	7.07	7.26	7.16	7.40	7.20	7.22
	Site A	Site B	Site C	Site D	Site E	Site F
<b>Median</b>	7.53	7.48	7.12	7.42	7.16	7.34
<b>Mean</b>	7.48	7.47	7.11	7.39	7.14	7.38
<b>Stan Dev</b>	0.24	0.26	0.07	0.25	0.14	0.19
<b>Max</b>	7.78	7.95	7.24	7.80	7.40	7.77
<b>Min</b>	7.07	7.05	7.00	6.50	6.89	7.11

Table E.13 – Mean dissolved oxygen, temperature, pH, and depth for Sites C, G, and H.

	Site C	Site G	Site H
<b>DO - Influent (mg/L)</b>	0.200 ±0.9	0.212 ±0.05	0.234 ±0.11
<b>DO - Effluent (mg/L)</b>	0.565 ±0.45	0.276 ±0.09	0.254 ±0.25
<b>Temp. (°C)</b>	20.72 ±1.5	17.96 ±1.1	18.10 ±1.3
<b>Species Richness</b>	5	7	6
<b>pH</b>	7.16 ±0.7	7.05 ±0.5	7.13 ±0.4
<b>Depth (cm)</b>	54 ±2	50 ±1	52 ±4

APPENDIX F – QUALITY CONTROL AND CALIBRATION

Table F.1 – Quality control samples for triclosan test run on 8/22/08

Testing Day	8/12/2008			
Samples Collected	8/12/08	8/15/08	8/19/08	8/22/08
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.52	0.5	2	
S2 - Lab Blank	0.099	0.1	-0.1	

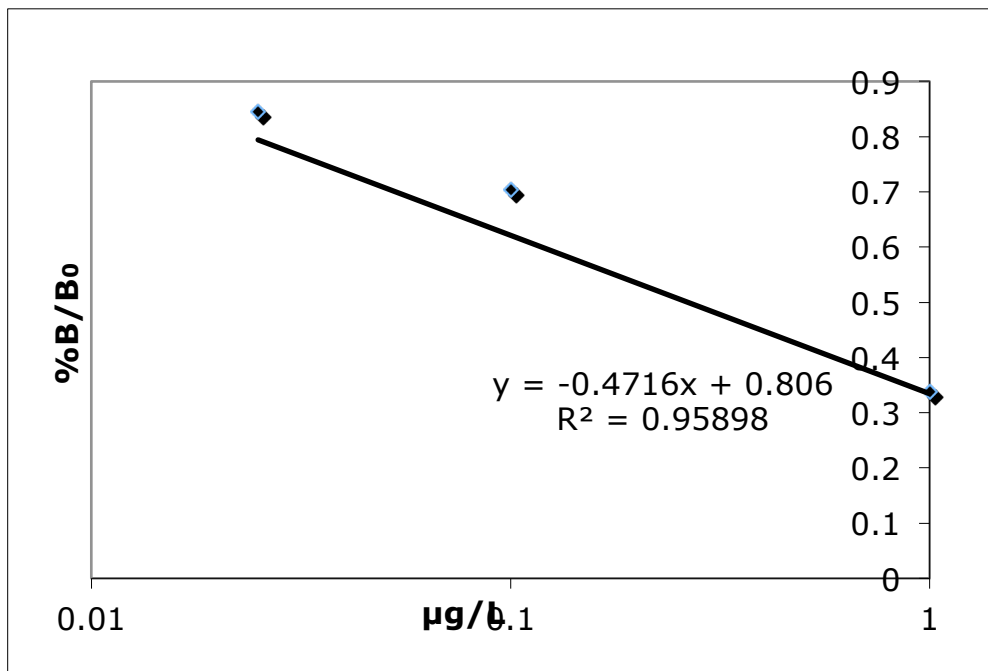


Figure F.1 – Calibration curve with the mean  $\%B/B_0$  from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.2 – Quality control samples for triclosan test run on 9/05/08.

Testing Day	9/5/08		
Samples Collected	8/29/08	9/2/08	9/5/08
	<b>Conc. µg/L</b>	<b>Known Conc. µg/L</b>	<b>% Error</b>
methanol	BDL		
DI	BDL		
Control	0.52	0.5	2.0
S2 - Lab Blank	0.103	0.1	0.3

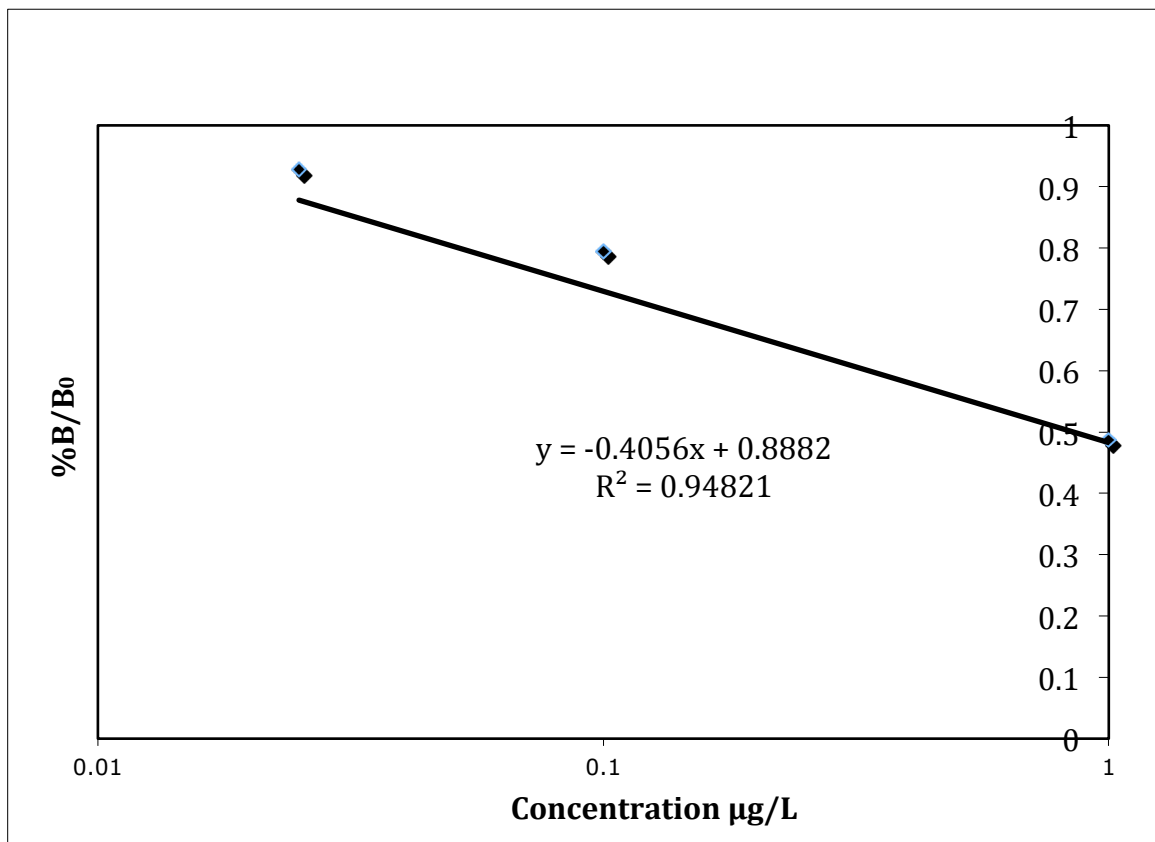


Figure F.2 – Calibration curve with the mean %B/B<sub>0</sub> from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.3 – Quality control samples for triclosan test run on 9/16/08.

Testing Day	9/16/08		
Samples Collected	9/9/08	9/12/08	9/16/08
	<b>Conc. µg/L</b>	<b>Known Conc. µg/L</b>	<b>% Error</b>
methanol	BDL		
DI	BDL		
Control	0.51	0.5	1.0
S1 - Lab Blank	0.027	0.025	0.3

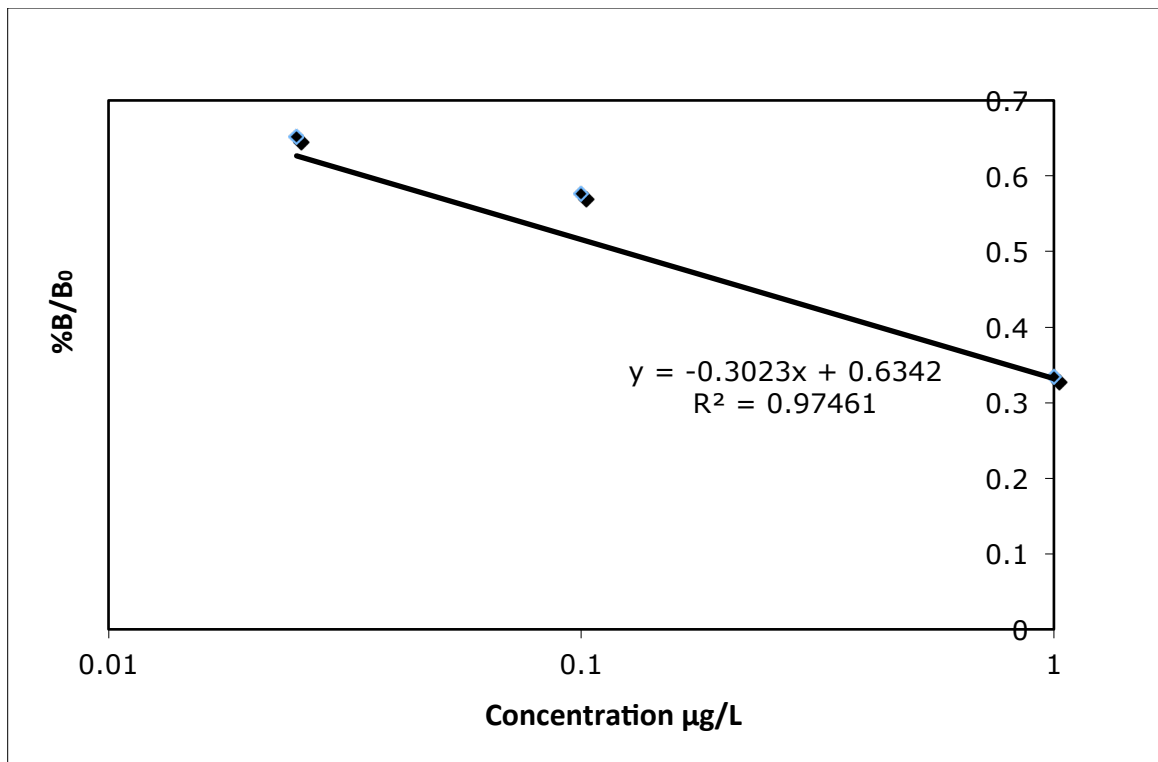


Figure F.3 – Calibration curve with the mean %B/B<sub>0</sub> from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.4 – Quality control samples for triclosan test run on 9/27/08

Testing Day	9/27/08		
Samples Collected	9/20/08	9/24/08	9/27/08
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>
methanol	BDL		
DI	BDL		
Control	0.51	0.5	1
S2 - Lab Blank	0.121	0.1	2.1

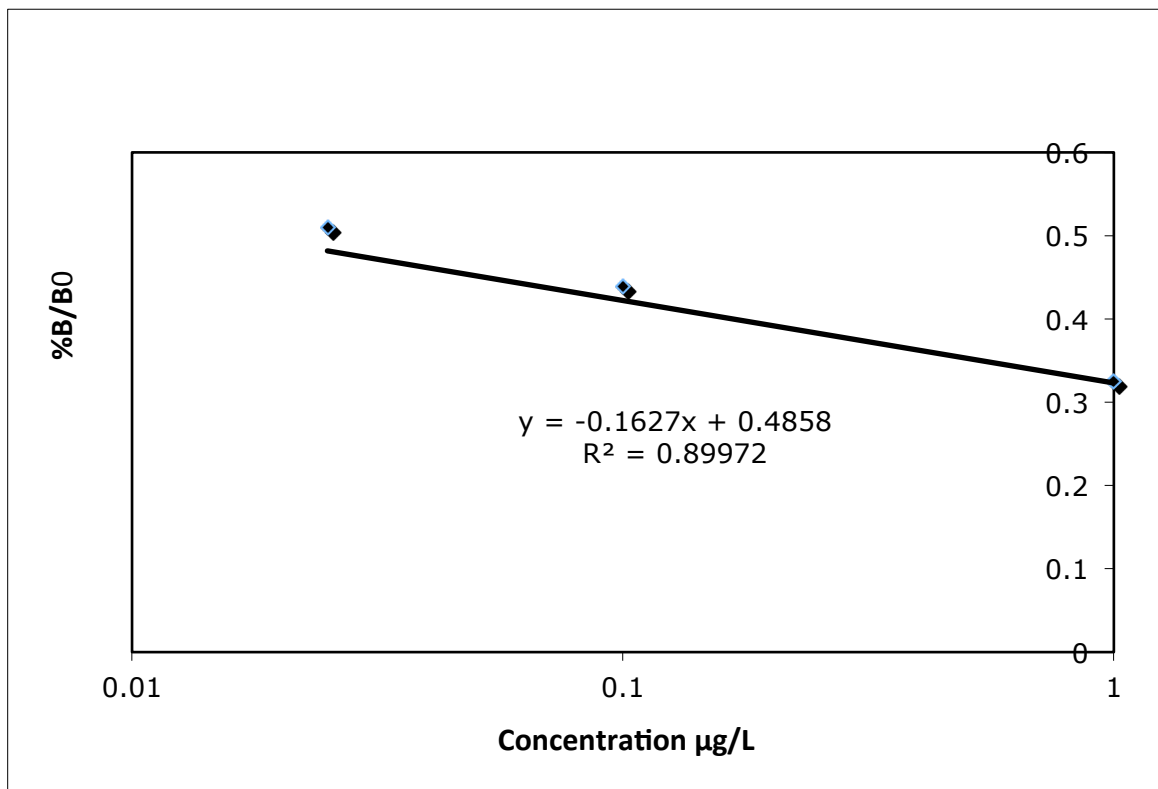


Figure F.4 – Calibration curve with the mean %B/B<sub>0</sub> from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.5 – Quality control samples for triclosan test run on 10/10/08

Testing Day	10/10/08			
Samples Collected	9/30/08	10/3/08	10/7/08	10/10/08
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.493	0.5	-0.7	
S3 - Lab Blank	0.956	1	-4.4	

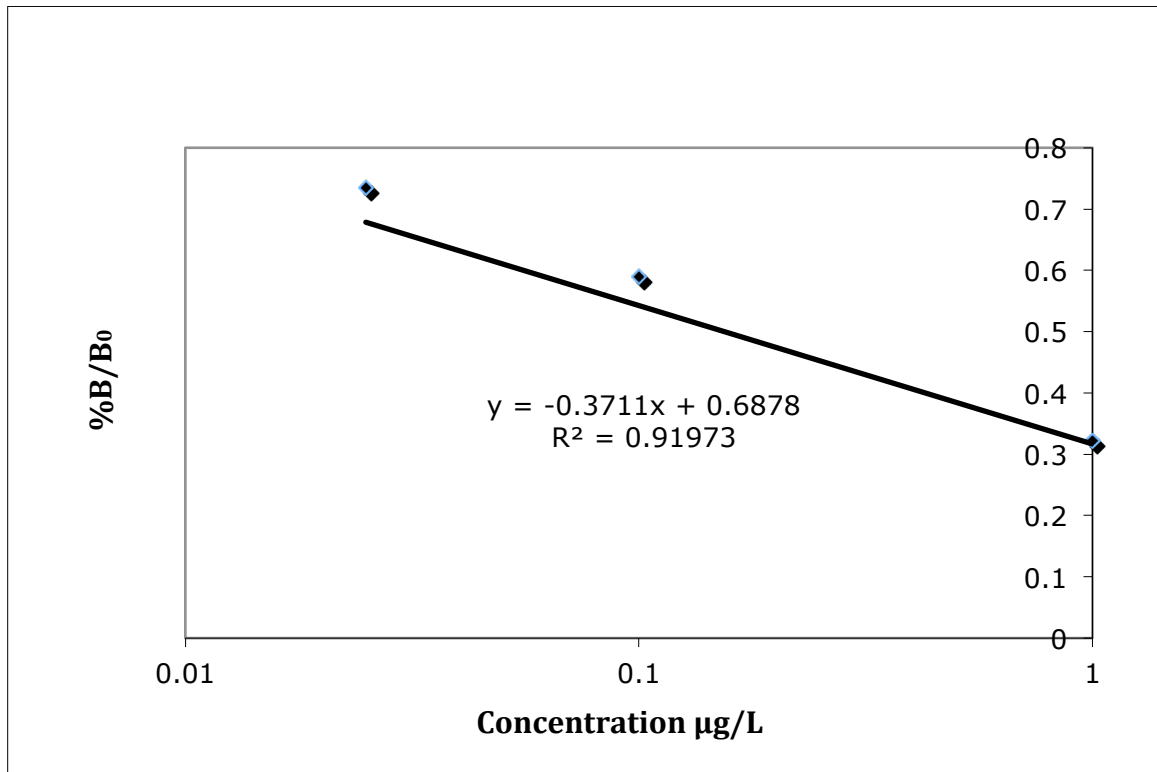


Figure F.5 – Calibration curve with the mean %B/B<sub>0</sub> from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.6 – Quality control samples for triclosan test run on 10/21/08.

Testing Day	10/21/08		
Samples Collected	10/14/08	10/17/08	10/21/08
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>
methanol	BDL		
DI	BDL		
Control	0.503	0.5	0.3
S2 - Lab Blank	0.225	0.25	-2.5

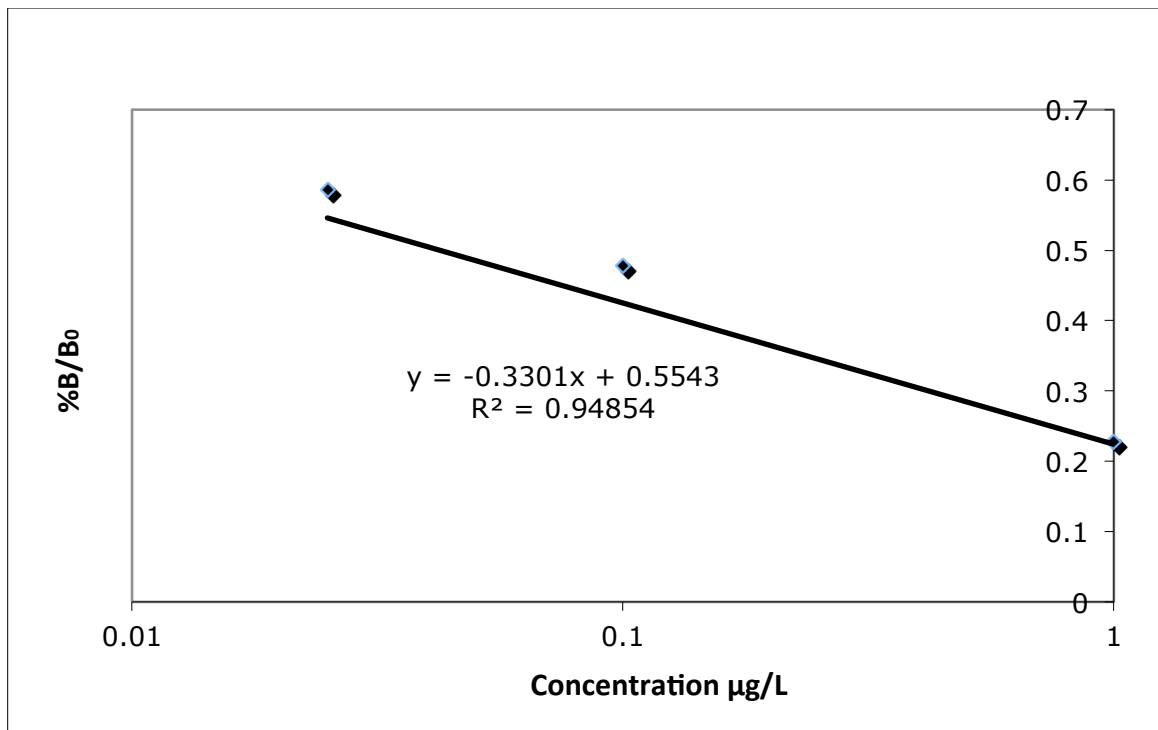


Figure F.6 – Calibration curve with the mean %B/B<sub>0</sub> from duplicate standard samples provided by Abraxis. The scale is logarithmic. The equation was used to obtain final concentrations for samples.

Table F.7- Quality Control Standards and Blanks for Iron, Phosphorus, Ammonia, Nitrite and Nitrate on sample collecting days for study on Reith Village Constructed Wetland described in Chapter 4.

Day			1-Jun-07	7-Jun-07	8-Jun-07	9-Jun-07	14-Jun-07	17-Jun-07	21-Jun-07	22-Jun-07
<b>Iron</b>	Standard 1	Percent Recovery	100	100	100	100	101	100	100	101
	Standard 2	Percent Recovery	99	100	99	99	98	100	100	99
	Blank		BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<b>Phosphorus</b>	Standard 1	Percent Recovery	100	100	99	101	100	102	100	100
	Standard 2	Percent Recovery	100	99	99	99	99	99	99	99
	Blank		BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<b>Ammonia</b>	Standard 1	Percent Recovery	99	100	100	100	100	100	100	99
	Standard 2	Percent Recovery	99	99	100	100	100	100	100	100
	Blank		BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<b>Nitrite</b>	Standard 1	Percent Recovery	100	100	100	101	100	101	101	101
	Standard 2	Percent Recovery	100	100	101	101	101	101	101	100
	Blank		BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<b>Nitrate</b>	Standard 1	Percent Recovery	98	98	100	100	100	100	100	100
	Standard 2	Percent Recovery	100	100	100	100	100	100	100	100
	Blank		BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Standard recovery procedure came from HACH DR/800 manual. BDL=Below Detection Limit. Acceptable recovery range was 10% variation.

Table F.8 – Duplicate sample runs for testing of influent and effluent water in the Rieth Village constructed wetland. Methods described in Chapter 5. Data is also summarized in Chapter 2.

Day			1-Jun-07	7-Jun-07	8-Jun-07	9-Jun-07	14-Jun-07	17-Jun-07	21-Jun-07	22-Jun-07	Mean	Std.Dev.
<b>Iron</b> (mg/L)	RV-IN	Run 1	0.058	0.177	0.060	0.043	0.011	0.050	0.118	0.116	0.08	0.06
		Run2	0.065	0.172	0.058	0.052	0.009	0.045	0.114	0.120		
	RV-OUT	Run 1	4.080	2.360	2.040	3.010	4.530	4.050	4.220	4.120	3.56	1.69
		Run2	4.250	2.420	1.960	2.920	4.640	3.980	4.130	4.220		
<b>Phosphorus</b> (mg/L)	RV-IN	Run 1	7.000	8.400	9.100	8.700	10.400	8.600	7.800	7.900	8.49	1.01
		Run2	6.890	8.530	8.950	8.650	10.750	8.490	7.610	8.030		
	RV-OUT	Run 1	0.120	0.110	0.800	0.250	0.200	0.270	0.220	0.610	0.32	0.25
		Run2	0.230	0.180	0.110	0.350	0.280	0.340	0.320	0.780		
<b>Ammonia</b> (mg/L)	RV-IN	Run 1	17.200	16.400	13.600	5.600	20.400	13.600	20.000	24.400	16.40	5.68
		Run2	16.800	17.100	12.900	5.300	21.000	13.400	20.400	25.000		
	RV-OUT	Run 1	7.200	3.600	0.800	2.400	8.400	0.800	5.800	11.800	5.10	3.91
		Run2	7.000	3.200	1.100	2.300	7.900	1.300	6.300	11.500		
<b>Nitrite</b> (mg/L)	RV-IN	Run 1	0.007	0.000	0.077	0.010	0.093	0.027	0.032	0.158	0.05	0.06
		Run2	0.007	0.005	0.083	0.017	0.089	0.025	0.040	0.139		
	RV-OUT	Run 1	0.013	0.076	0.016	0.033	0.041	0.002	0.002	0.000	0.02	0.03
		Run2	0.009	0.077	0.022	0.028	0.044	0.000	0.003	0.002		
<b>Nitrate</b> (mg/L)	RV-IN	Run 1	0.010	0.000	0.330	0.000	0.010	0.040	0.020	0.080	0.06	0.01
		Run2	0.014	0.008	0.290	0.003	0.010	0.041	0.027	0.085		
	RV-OUT	Run 1	0.020	0.180	0.110	0.090	0.550	0.010	0.000	0.000	0.12	0.19
		Run2	0.040	0.160	0.120	0.090	0.560	0.020	0.005	0.005		
<b>Water Temp</b>   RV (°C)		19	20	23	22	20	21.8	19	20	21	1	
<b>Water Level</b>   RV (cm)		22.5	22.5	21.5	21.0	22.0	22.0	21.5	21.0	21.8	0.6	

Table F.9 – Triplicate plate counts for E. coli (CFU/100 mL) in influent and effluent water samples taken from Rieth Village constructed wetland on June, 2007.

		Plate 1	Plate 2	Plate 3	Mean	SD
Influent	6/7/07	6600	7000	5600	6400	721
	6/14/07	43800	38700	55400	45967	8558
	6/21/07	34400	24300	18100	25600	8227
Effluent	6/7/07	1300	600	500	800	1002
	6/14/07	400	500	900	600	265
	6/21/07	100	400	100	200	173

Table F.10 – Quality control samples for triclosan tests run between 6/16/07 and 7/27/07 on constructed wetlands C, G, and H. Methods detailed in Chapter 2.

<b>Testing Date</b>		<b>6/16/07</b>		
	<b>Conc µg/L</b>	<b>Known Conc µg/L</b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.52	0.5	2	
S1 - Lab Blank	0.027	0.025	-0.1	

<b>Testing Date</b>		<b>6/23/07</b>		
	<b>Conc µg/L</b>	<b>Known Conc µg/L</b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.49	0.5	-1	
S3 - Lab Blank	1.01	1	-0.1	

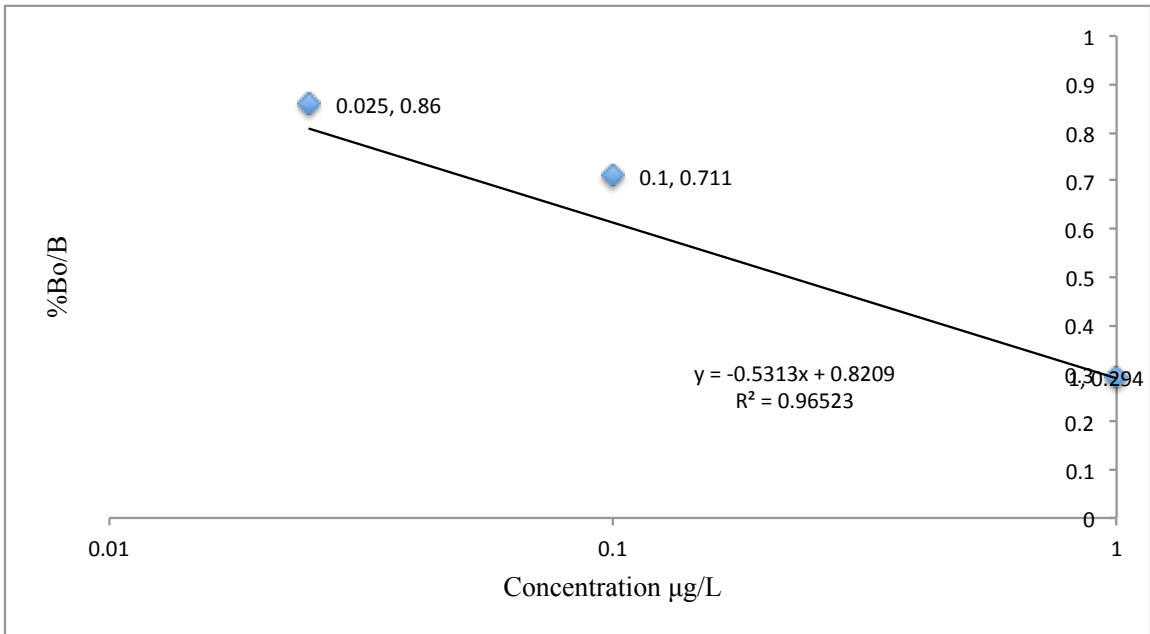
<b>Testing Date</b>		<b>6/30/07</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.5	0.5	0	
S2 - Lab Blank	0.09	0.1	0.2	

<b>Testing Date</b>		<b>7/7/07</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.5	0.5	0	
S3 - Lab Blank	0.97	1	0.2	

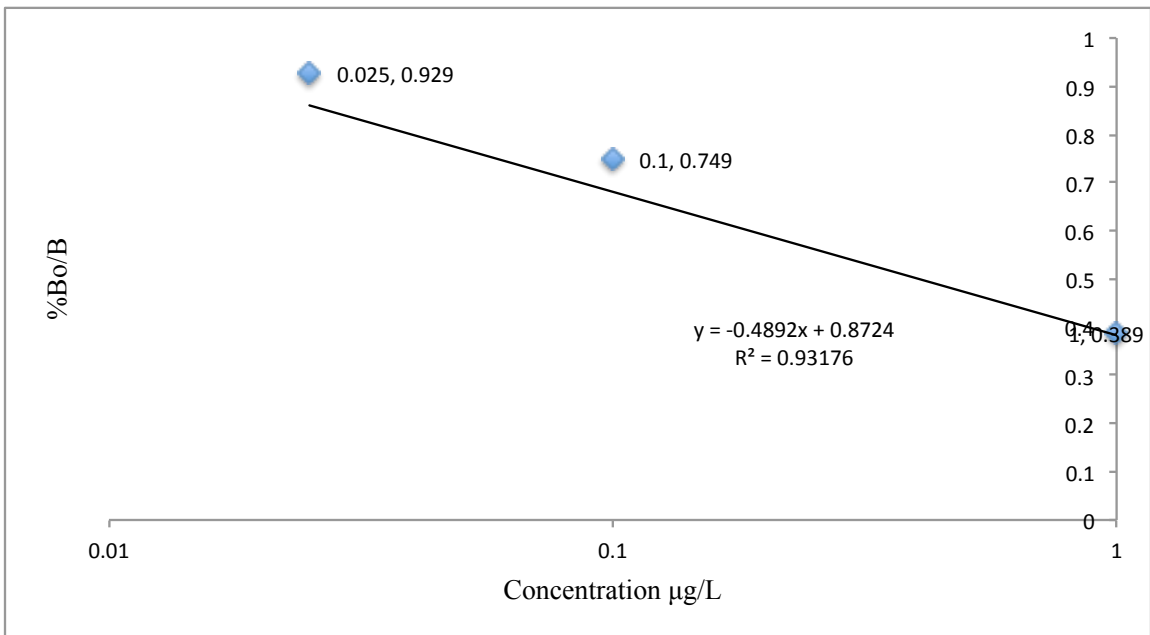
<b>Testing Date</b>		<b>7/14/07</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.52	0.5	2	
S3 - Lab Blank	0.098	1	1	

<b>Testing Date</b>		<b>7/21/07</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.52	0.5	2	
S1 - Lab Blank	0.021	0.025	-1	

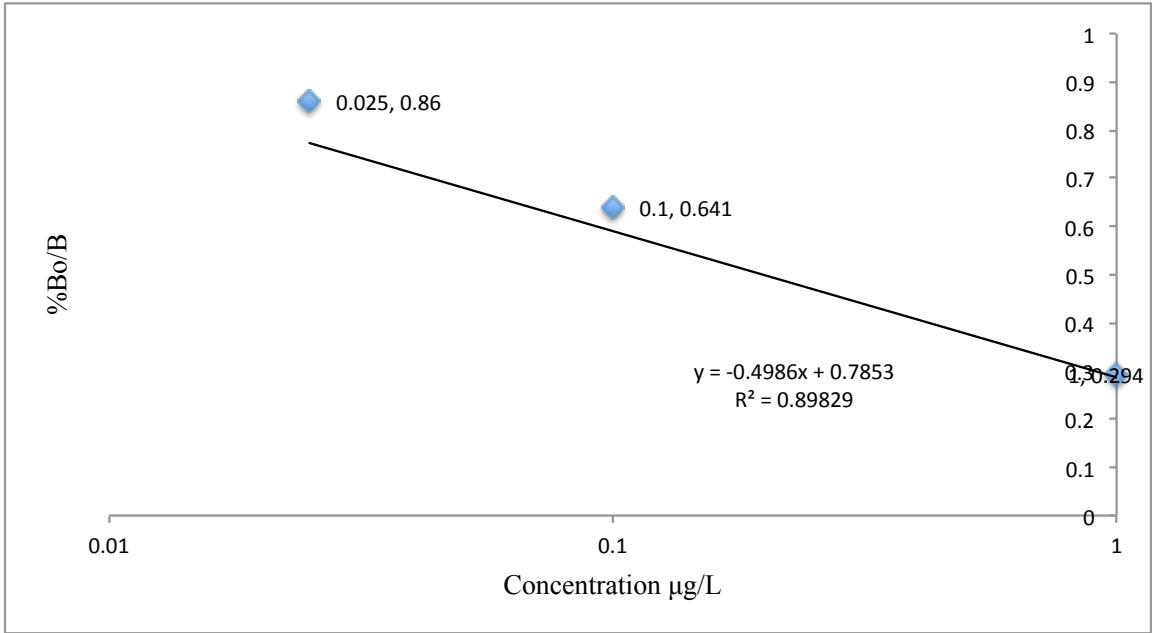
<b>Testing Date</b>		<b>7/27/07</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.5	0.5	0	
S2 - Lab Blank	0.095	0.1	-3	



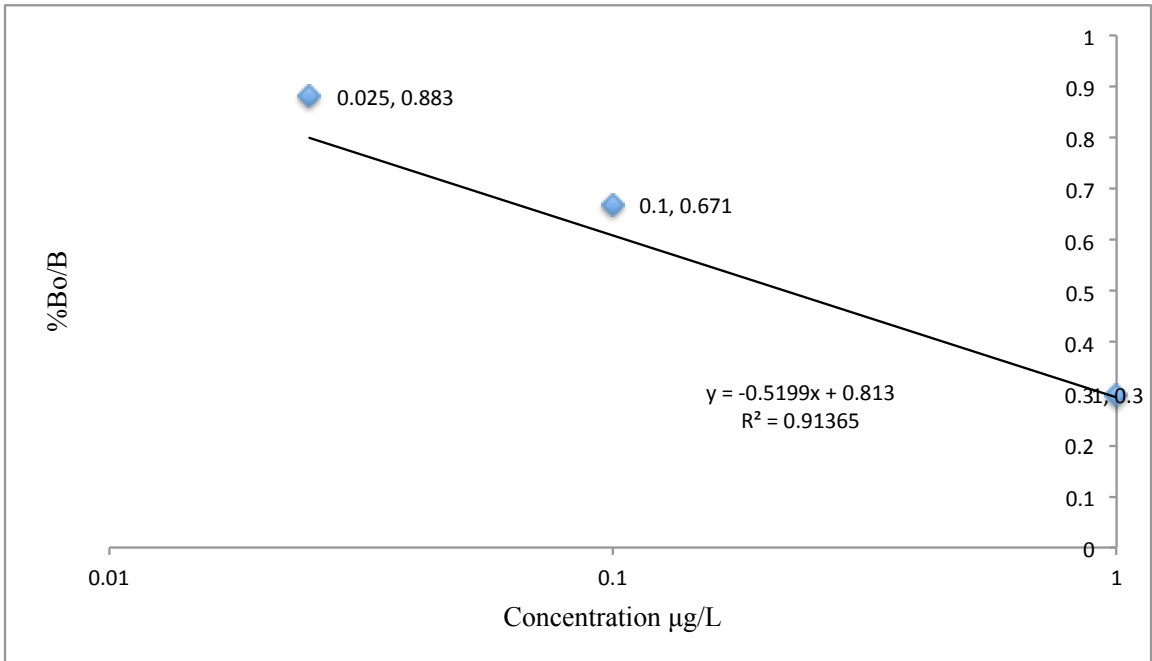
Triclosan Calibration Curve - 6/16/07



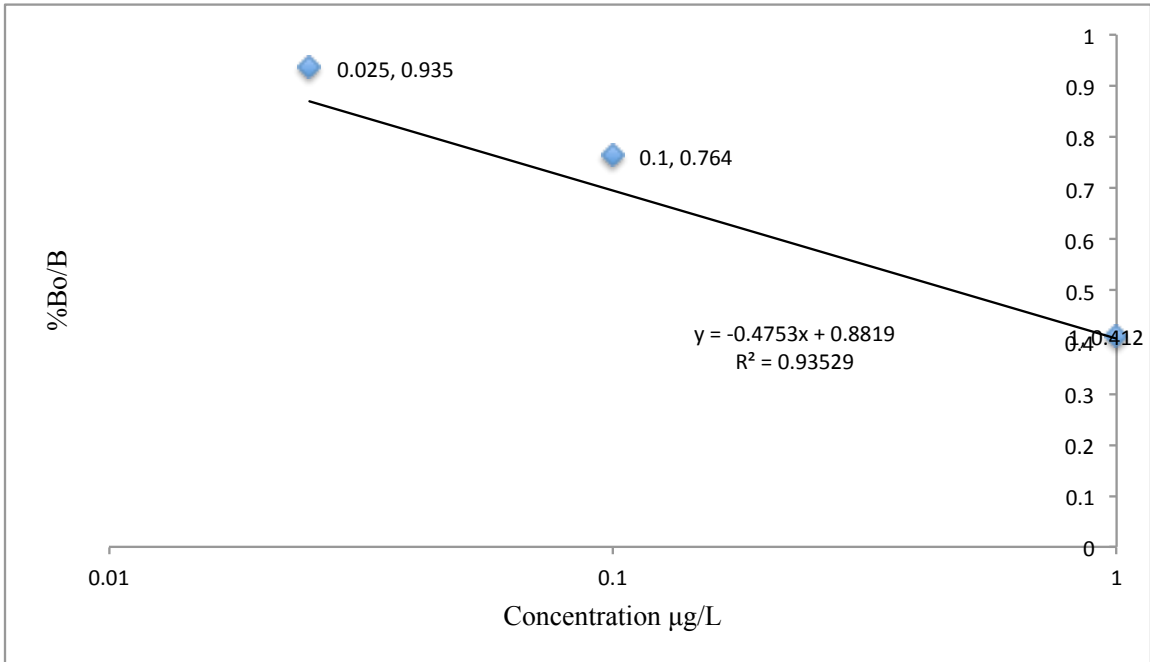
Triclosan Calibration Curve - 6/23/07



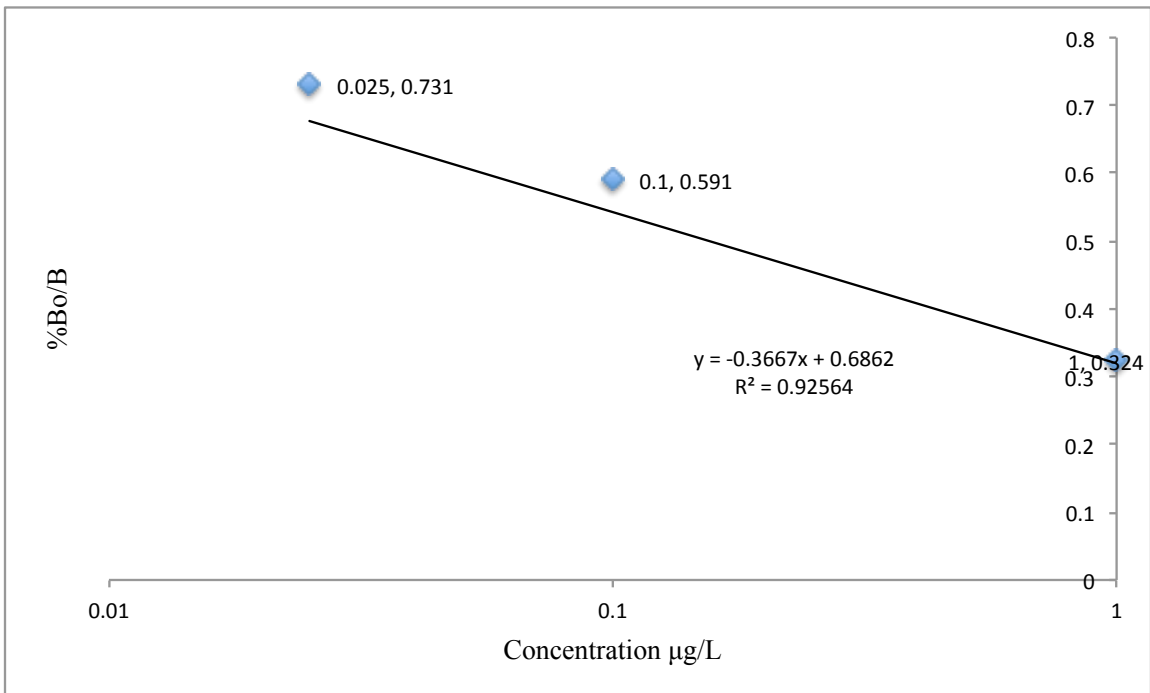
Triclosan Calibration Curve – 6/30/2007



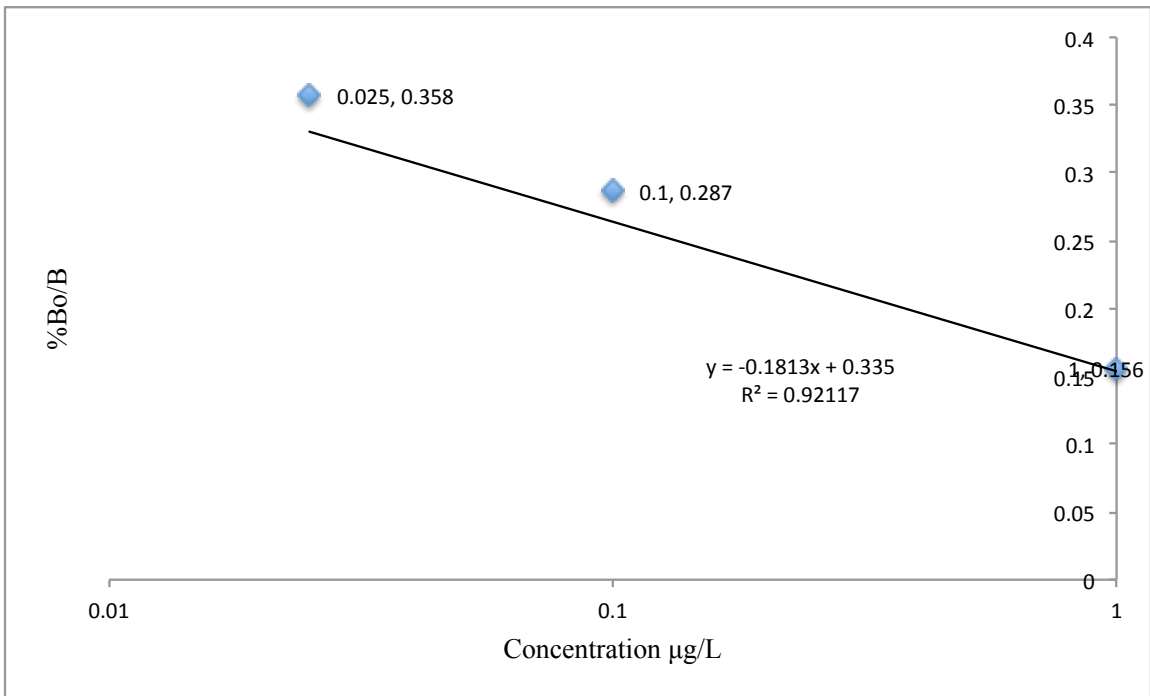
Triclosan Calibration Curve – 7/7/07



Triclosan Calibration Curve - 7/14/07



Triclosan Calibration Curve - 7/21/07



Triclosan Calibration Curve – 7/27/07

Figure F.7 – Calibration curves for triclosan tests run on constructed wetlands Sites G and H. Method details in Chapter 4.

Table F.11 - Quality control samples for triclosan tests run between 7/22/11 and 8/15/11 on laboratory container wetlands.

<b>Testing Date</b>		<b>7/22/11</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.49	0.5		-1
S1 - Lab Blank	0.011	0.025		-1.4
S3 - Lab Blank	1.01	1		1
S3 - Lab Blank	0.98	1		-2

<b>Testing Date</b>		<b>7/26/11</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.49	0.5		-1
S2 - Lab Blank	0.11	0.1		1
S3 - Lab Blank	1.03	1		3
S3 - Lab Blank	1.01	1		1

<b>Testing Date</b>		<b>8/1/11</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.51	0.5		1
S2 - Lab Blank	0.14	0.1		4
S3 - Lab Blank	0.96	1		-4
S3 - Lab Blank	0.98	1		-2

<b>Testing Date</b>		<b>8/15/11</b>		
	<b>Conc <math>\mu\text{g/L}</math></b>	<b>Known Conc <math>\mu\text{g/L}</math></b>	<b>% Error</b>	
methanol	BDL			
DI	BDL			
Control	0.54	0.5		4
S2 - Lab Blank	0.14	0.1		4
S3 - Lab Blank	0.99	1		-1
S3 - Lab Blank	1	1		0

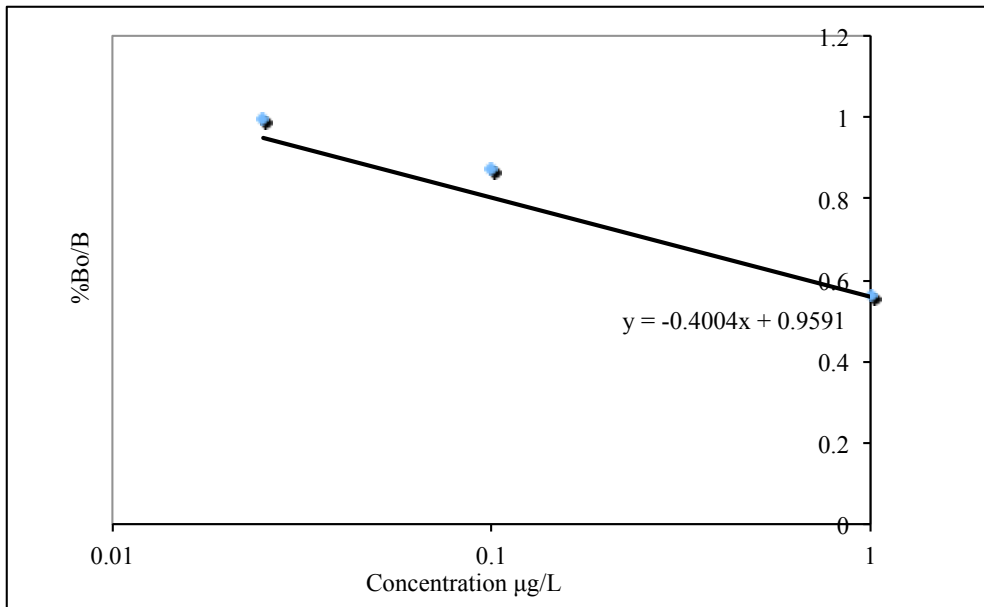


Figure F.8 – Calibration curves for triclosan tests run on laboratory container wetland 7/22/11. Method details in Chapter 3.

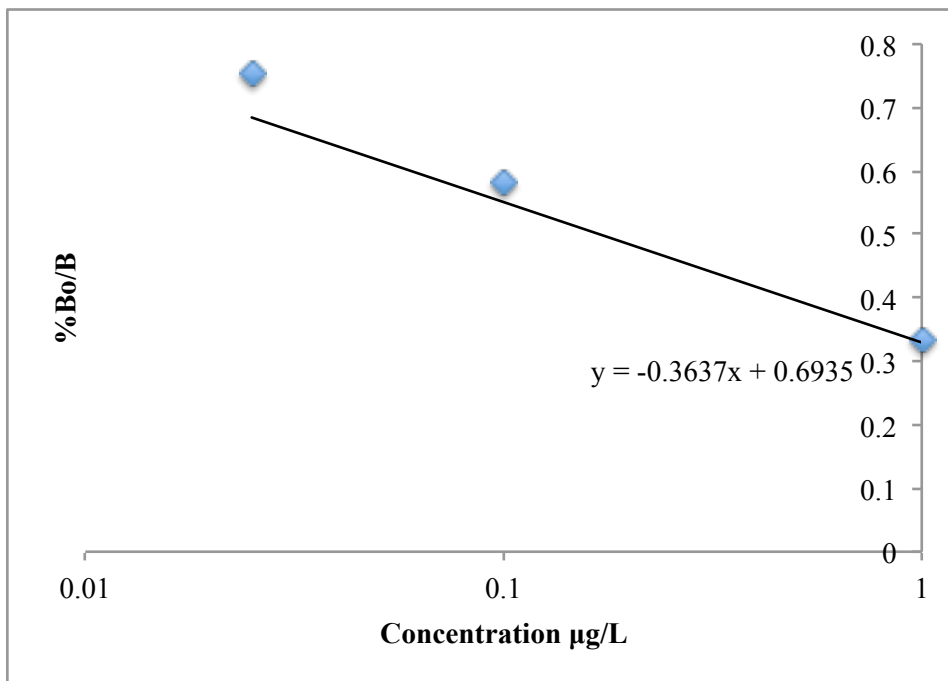


Figure F.9 – Calibration curves for triclosan tests run on laboratory container wetland 7/26/11. Method details in Chapter 3.

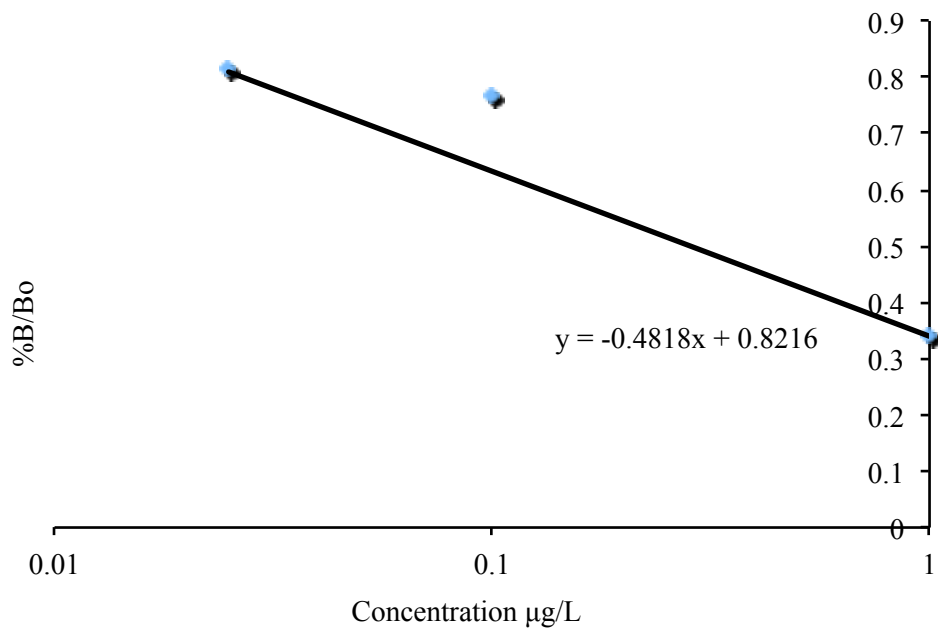


Figure F.10 – Calibration curves for triclosan tests run on laboratory container wetland 8/01/11. Method details in Chapter 3.

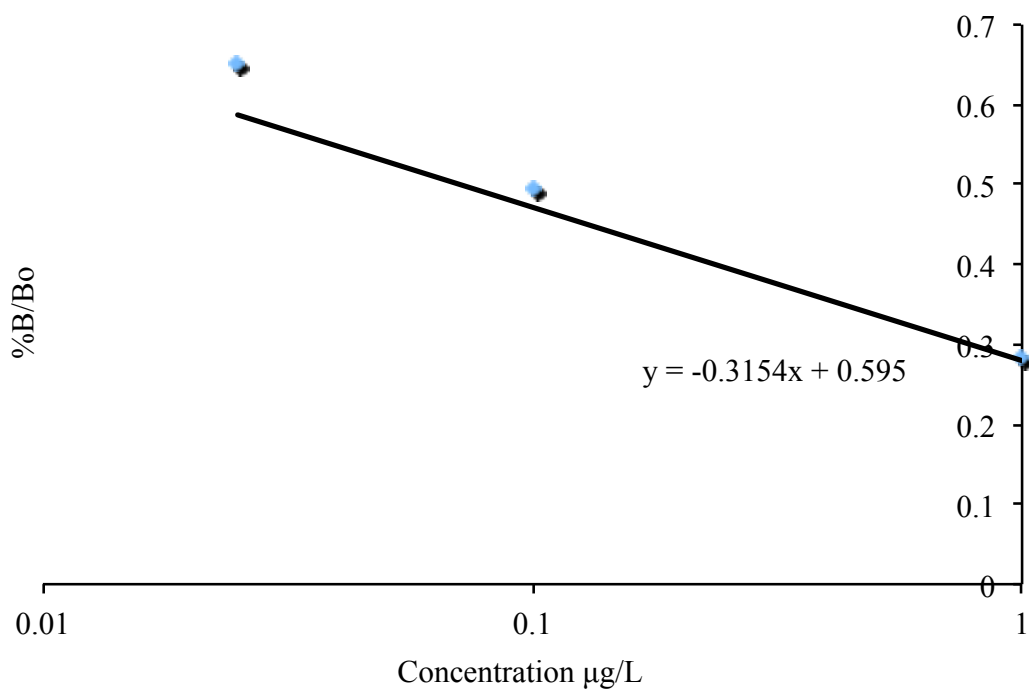


Figure F.11 – Calibration curves for triclosan tests run on laboratory container wetland 8/15/11. Method details in Chapter 3.

APPENDIX G – RESULTS FROM LABORATORY SCALE TESTS OF TRICLOSAN REMOVAL.

Table G.1 – Results for Triclosan study in laboratory containers with four different plant species. Details of methods in Chapter 3.

Container number	Species	7/18/11	7/22/11	7/26/11	8/1/11	8/15/11
		Triclosan Conc. (µg/L)	Triclosan Conc. (µg/L)	Triclosan Conc. (µg/L)	Triclosan Conc. (µg/L)	Triclosan Conc. (µg/L)
1	<i>carex comosa</i>	2	1.677	1.405	1.260	1.216
2	<i>carex comosa</i>	2	1.764	1.419	1.250	1.169
3	<i>carex comosa</i>	2	1.777	1.304	1.275	1.195
4	<i>Scirpus cyperinus</i>	2	1.732	1.409	1.244	1.232
5	<i>Scirpus cyperinus</i>	2	1.722	1.307	1.279	1.236
6	<i>Scirpus cyperinus</i>	2	1.750	1.400	1.267	1.264
7	<i>Schoenoplectus fluviatilis</i>	2	1.857	1.406	1.268	1.220
8	<i>Schoenoplectus fluviatilis</i>	2	1.751	1.445	1.296	1.226
9	<i>Schoenoplectus fluviatilis</i>	2	1.898	1.438	1.258	1.207
10	<i>scirpus atrovirens</i>	2	1.828	1.366	1.247	1.160
11	<i>scirpus atrovirens</i>	2	1.644	1.516	1.317	1.312
12	<i>scirpus atrovirens</i>	2	1.337	1.419	1.330	1.322
13	<i>Control</i>	2	1.788	1.453	1.263	1.241
14	<i>Control</i>	2	1.796	1.489	1.270	1.197
15	<i>Control</i>	2	1.837	1.515	1.256	1.199

Each value represents the mean of the triplicate samples.