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Co-Removal of Atrazine and Nitrate from Groundwater Using a Mulch Biofilm

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CO-REMOVAL OF ATRAZINE AND NITRATE FROM GROUNDWATER USING A MULCH BIOFILM

By

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CO-REMOVAL OF ATRAZINE AND NITRATE FROM GROUNDWATER USING A MULCH BIOFILM

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The popular broadleaf herbicide atrazine is often found in contaminated groundwater along with other agricultural chemicals, such as nitrate. Mulch biowalls, a passive treatment placed *in situ*, can inexpensively remediate groundwater by intercepting and treating a contaminant plume. Three types of organic mulch: cedar, cypress, and hardwood were evaluated for their ability to act as supporting materials for a biowall to simultaneously remove atrazine and nitrate from groundwater. Physical and chemical properties of the mulch were characterized. Cedar mulch had the highest organic carbon content, 996 mg/g. The adsorptive capacity of the mulch for atrazine and nitrate, in mono and binary adsorbate systems were evaluated in a series of isotherm experiments. There was no statistical difference in the ratio of q_e/C_e (equilibrium concentration on the mulch/equilibrium concentration in solution) for atrazine or nitrate among the three types of mulch, except for atrazine in the pairs of cedar-hardwood and cypress-hardwood in the binary adsorbate system. Atrazine adsorption appeared to exhibit a C-type isotherm, due to the range of concentrations examined; A wider range of atrazine concentrations may show a more distinct L-type isotherm. Atrazine adsorption

was not affected by the presence of nitrate. Nitrate adsorption did not clearly exhibit a specific isotherm type and was affected by surface properties of the mulch as well as the presence of atrazine. The adsorption behaviors of atrazine and nitrate were quantified from Langmuir and Freundlich isotherms. Atrazine adsorption was best modeled by the Freundlich isotherm, while nitrate adsorption was best modeled by the Langmuir. Qualitatively, cypress mulch exhibited the greatest sorption capacity for atrazine and nitrate and was selected to examine the feasibility of a mulch biowall using a laboratory-scale biotic column. The cypress column was not able to remove nitrate because the concentration of dissolved oxygen was too high, even after the addition of an external carbon source. The column was not able to remove atrazine because the concentration of nitrate was too high for bacterial degradation of the herbicide to occur.

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CHAPTER 1: INTRODUCTION

1.1 Background

Nebraska is a land of agriculture. Ninety three percent of Nebraska is farmland, and Nebraska ranks in the top ten states for crop production (Nebraska Agricultural Fact Card 2011). This substantial agricultural activity is made possible by the extensive use of fertilizers and pesticides to enhance production.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] is a popular broadleaf herbicide, typically used on corn. It is normally applied at 2.2 kg/hectare or less (Solomon et al. 1996). Between 30,000 and 34,000 tons of atrazine are used annually in the United States (Solomon et al. 1996; Hayes et al. 2002). However, about 10% of the atrazine applied washes off fields, moving away from target sites toward areas devoid of oxygen, like groundwater (Ma and Selim 1996; Gu et al. 2003). Forty five percent of groundwater contamination cases are attributed to point source contamination of atrazine (Silva et al. 2004).

More than 50% of United States population derives its primary drinking water from groundwater (Kross et al. 1992). Atrazine is the second most frequently detected pesticide in drinking water wells (U.S. EPA Office of Pesticide Programs 1993). In 2010, the Nebraska Department of Environmental Quality found that 5% of groundwater samples exceeded the reporting limit for atrazine (Nebraska Department of Environmental Quality, 2010). The maximum contaminant level for atrazine in drinking water is 3 μg/L, as set by the Environmental Protection Agency (EPA). Exposure to

atrazine causes endocrine disruption in frogs, rats, and humans (U.S. EPA Office of Pesticide Programs 1993; U.S. EPA Office of Pesticide Programs 2002; Villanueva et al. 2005).

Atrazine and nitrate are often found together in the groundwater of agricultural states (Ritter 1990). In 2010, the Nebraska Department of Environmental Quality found that 94% of groundwater samples exceeded the reporting limit for nitrate (Nebraska Department of Environmental Quality, 2010). The maximum contaminant level for nitrate in drinking water is 10 mg NO_3-N/L as nitrogen, as set by the EPA (Nebraska Department of Environmental Quality, 2010). Nitrate can cause methemoglobinemia, or "blue baby syndrome," because it interferes with the body's ability to carry oxygen in its red blood cells (Skipton and Hay 1998).

Literature has shown that researchers have tested several processes in the treatment of pesticide contamination in both soil and water including: chemical and biological treatment processes. Waria et al. (2009) used zero valent iron and ferrous sulfate to degrade atrazine chemically in soil. Soybean oil was also added to provide a carbon source for biological activity. Atrazine, initially at a concentration of 500 mg/kg soil, was reduced by 79% in 342 days. Tafoya-Garnica et al. (2009) used a fluidized bed reactor containing biological granular activated carbon to achieve high degradation rates. Modin et al. (2008) used a methane fed bioreactor intended to remove both atrazine and nitrate. However, atrazine removal was not successful (Modin et al. 2008). Bianchi et al. (2006) successfully used photolysis, photocatalysis (with TiO_2), and ozonation for atrazine degradation.

Processes such as these require the presence of a nutrient source, such as methane or soybean oil, and specialized treatment, such as ultraviolet radiation or biological activated carbon. These additions can greatly increase the cost of treatment, especially when the price of highly trained operators is factored in.

Passive treatment, such as a biowall, is inexpensive when compared to methods discussed above because it is placed *in situ*. Biowalls are bacteria supported on a natural substrate that is placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. Biowalls are low maintenance and can endure changes in operating conditions (Kao et al. 2001, Schipper et al. 2004; Seo et al. 2007). Biowalls supported on a natural substrate, such as mulch or peat moss, have been studied for naphthalene (Seo et al. 2007), tetrachloroethylene (Kao et al. 2001), and denitrification (Schipper et al. 2004; Ilhan et al. 2011), but rarely for atrazine removal. Ilhan et al. (2011) examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods.

Low concentrations of nitrate, ~1mM, do not interfere with atrazine degradation (Crawford et al. 1998, 2000). Some atrazine-degrading bacteria, such as *Pseudomonas* sp. ADP, can use nitrate as an electron acceptor under anoxic conditions (Shapir et al. 1998). However, when nitrate is present in excess, some atrazine-degrading bacteria may prefer to use nitrate as a source of nitrogen instead of atrazine (Hunter and Shaner 2010). This relationship may be dependent on the bacteria species present as well as the background concentration of nitrate.

1.2 Objective

The objective of this research is to examine a cost-effective and reliable biological treatment method for the co-removal of atrazine and nitrate from groundwater. Two approaches were established to achieve the objective. First, the adsorption capacities of three types of common gardening mulch for both atrazine and nitrate were examined. Second, the type of mulch exhibiting the largest adsorption capacity was used for further experimentation in a laboratory-scale biotic column experiment to examine the feasibility of implementation of this type of biowall in contaminated groundwater.

1.3 Thesis Organization

This thesis contains 5 chapters, references, and appendices. Chapter 2 provides a review of current literature relevant to this study, including advances in physical and biological remediation of atrazine as well as implications for large-scale bioremediation. Chapter 3 discusses an analysis of physical and chemical properties of the mulch and adsorption isotherms for atrazine and nitrate. Chapter 4 discusses a biotic column experiment that evaluated the ability of bacteria to simultaneously degrade atrazine and nitrate. Chapter 5 summarizes the conclusions and suggests directions for future study. Appendices include adsorption figures and other data.

CHAPTER 2: PHYSICAL AND BIOLOGICAL REMEDIATION OF ATRAZINE: A REVIEW

2.1 Background

Atrazine is one of the most widely used herbicides for the control of broad-leafed weeds. It was developed in Switzerland in 1958 by the Geigy Chemical Company, and became registered for use in the United States in 1959 (Solomon et al. 1996). Between 30,000 and 34,000 tons of atrazine are used annually in the United States, normally applied at 2.2-4.5 kg/ha (1.1-2.2 μg /g soil) (Yeomans and Bremner 1987; Solomon et al. 1996; Hayes et al. 2002).

However, about 10% of atrazine applied washes off fields, moving away from target sites toward areas devoid of oxygen, like groundwater (Ma and Selim 1996; Gu et al. 2003). The rest is retained in the soil; atrazine's vapor pressure is so low that volatilization is negligible (2.89E-7 mm of Hg at 25°C) and it is not photodegradable at wavelengths >300nm (Solomon et al. 1996). Forty five percent of groundwater contamination cases come from point source contamination of atrazine (Silva et al. 2004).

More than 50% of the United States population derives its primary drinking water from groundwater (Kross et al. 1992). The Environmental Protection Agency (EPA) has set the maximum contaminant level in drinking water for atrazine at 3 μg/L, whereas the European Union has set the level at 0.1 μg/L (Wilber et al. 1995; Faur et al. 2005; Zadaka et al. 2009). Atrazine is the second most frequently detected pesticide in drinking water wells (U.S. EPA Office of Pesticide Programs 1993). It has been found at levels

exceeding the maximum contaminant level because of its popularity and low biodegradability (Somasundaram and Coats 1990; Sene et al. 2010).

The degradation of atrazine occurs through one of two pathways; it can be dehalogenated to form hydroxyatrazine (HYA) or dealkylated to form deisopropylatrazine (DIA) or deethylatrazine (DEA). Without dehalogenation, the dealkylated metabolites still retain the phytotoxic properties and possibly the endocrine-disrupting potency of atrazine, making further degradation or removal of metabolites desirable (Boundy-Mills et al. 1997; Silva et al. 2004).

Atrazine is also frequently found in conjunction with its metabolites. In a national study of groundwater quality in the United States, 49.4% of sites where pesticides had been detected contained both atrazine and DEA. All but two of the sites conformed to drinking water criteria; yet, current drinking water criteria only enforce one compound at a time (Kolpin et al. 2000). Another study of vernal pools in protected areas in the United States found atrazine was the most frequently detected pesticide (53%), followed closely by DEA (47%), HYA (44%), and DIA (29%) (Battaglin et al. 2008). Synergistic effects from multiple compounds are unknown and cannot be predicted based on the toxicity of a single component (Marinovich et al. 1996).

However, regulations are changing to include metabolites. The European Union has set a limit of 0.5 μg/L for the combination of atrazine and its degradation products, known as Total Chloro-s-Triazine (TCT). The EPA is considering similar strict regulations for TCT (Faur et al. 2005; Jiang and Adams 2006). As these regulations reach the United States, further research should focus on elucidating the toxicity of the

metabolites, which is still largely speculative. Generally speaking, atrazine, DIA, DEA, and didealkylatrazine share a common mechanism of toxicity with respect to endocrine disruption (U.S. EPA Office of Pesticide Programs 1993; Jiang and Adams 2006). However, relative toxicity studies with bioluminescent bacteria have shown that DEA and DIA are less toxic than atrazine (Kross et al. 1992).

The toxicity of atrazine has been researched in a variety of animals. Studies on atrazine levels in fish species revealed that atrazine does not tend to bioconcentrate, like the infamous pesticide DDT (dichlorodiphenyltrichloroethane). Male frogs in water contaminated with greater than 0.1 μg atrazine/L show hermaphroditism and retarded gonadal development. In rodents, atrazine is embryotoxic and embryolethal, but not teratogenic (Villanueva et al. 2005). In adult rats, atrazine causes mammary gland tumors. Though this cancer mechanism is different in humans, it doesn't rule out the possibility of reproductive developmental effects by another mechanism (U.S. EPA Office of Pesticide Programs 2002). Health effects in humans from acute exposure to atrazine levels above the maximum contaminant level include "congestion of heart, lungs and kidneys; hypotension; antidiuresis; muscle spasms; weight loss; adrenal degeneration" (U.S. EPA Office of Pesticide Programs 1993).

Atrazine is not the only contaminant in natural waters. A study on leopard frogs by Hayes et al. (2006) used a low concentration (0.1 ppb) of a nine-pesticide mixture, including atrazine, to simulate a low runoff concentration. Tadpoles exposed to the mixture took a longer time to metamorphose, were smaller, and had weakened immune systems, making them vulnerable to predation and bacterial infections. Though the

toxicity of atrazine is relatively well characterized, the way it interacts in the environment with other pesticides or its own metabolites is still greatly unknown.

2.2 Objective

A wealth of research has occurred on various methods to enhance atrazine degradation. These methods fall into three main categories: physical, biological, and a combination of the two. However, much of this research, especially in the biological field, has only been done in a laboratory-scale setting. This review will investigate advances in physical and biological remediation of atrazine as well as implications for large-scale bioremediation.

2.3 Removal via Adsorption

2.3.1 Background

Adsorption occurs at a surface of a solid adsorbent, which forms chemical or physical bonds to remove a component, such as atrazine, from the fluid phase (Foo and Hameed 2010). The atrazine reaches the adsorbent after undergoing three types of diffusion. First, film diffusion moves the atrazine from the bulk phase to the adsorbent surface. Second, particle diffusion moves the atrazine to the interior of the adsorbent. Third, the atrazine is adsorbed onto the surface of the adsorbent (Chingombe et al. 2006). Compared to chemical or biological removal methods, adsorption has a low initial cost, offers flexibility and simplicity of operation, and doesn’t form harmful intermediates (Ahmad et al. 2010).

2.3.2 Objective

This section will discuss a range of adsorption research, beginning with how atrazine adsorption on soils is affected by soil properties, hydrolysis, and land application of wastewater. Next, the removal of atrazine from drinking water using activated carbon as well as natural materials will be discussed. The final section discusses the adsorption characteristics of atrazine metabolites.

2.3.3 Soil Adsorption

Atrazine enters soil environments through land application. Atrazine adsorption on soils is influenced by many factors, including: organic matter, pH, conductivity, alkalinity, suspended solids, dissolved salts, and water content (Seol and Lee 2000). This section will discuss the influence of pH and organic materials on soil adsorption and show how the structure of atrazine influences its affinity to soil. Lastly, soil remediation methods with activated carbon, hydrolysis, and wastewater application will be discussed.

A study by Clay and Koskinen (1990) showed that atrazine and hydroxyatrazine are more strongly adsorbed to soils at lower pH, 4, compared to a more neutral pH, 6, because atrazine and hydroxyatrazine are weak bases and have greater protonation at lower pH values. Atrazine desorption was hysteretic, which could have occurred for many reasons, including: equilibrium was not attained, precipitates, changes in desorption solution composition, degradation, or irreversible binding to soil.

Atrazine adsorbs rapidly to organic components of soils, especially polysaccharides, lignin, and humic substances (Ma and Selim 1996; Masaphy and Mandelbaum 1997). However, the origin of the organic matter influences the adsorption.

In a study by Laird et al. (1994), atrazine chemisorbed to the organic matter in coarse silicate clays, whereas atrazine only physisorbed to the organic matter in fine silicate clays, because the organic material in the finer clays had fewer organic functional groups.

Though Granular Activated Carbon (GAC) is more conventionally used for wastewater treatment, it has been used for soil remediation in the past. Gunther and Gunther (1970) suggested a rule of thumb for application rates of 200 lb/acre of activated carbon for every 1 lb/acre of atrazine. This is slightly higher than the 120 lb/acre suggested by Harvey (1973). Both application rates can be reduced with band applications or root dips (Gunther and Gunther 1970). However, the high rate of application required makes GAC feasible only for high value land or crops (Harvey 1973). Harvey (1973) also noted that freeze-thaw cycles were detrimental to the effectiveness of the activated carbon, which may limit its usefulness.

2.3.4 Dehalogenation of Atrazine

As stated in Section 2.1, dehalogentation of atrazine is highly desired due to the possible phytotoxicity and endocrine-disrupting potency of the halogenated metabolites (Boundy-Mills et al. 1997; Silva et al. 2004). Dehalogenation can occur through both chemical and biological processes.

Xu et al. (2001) tested the ability of freeze dried samples of sodium-saturated ferruginous smectite to adsorb atrazine. Reduced clay adsorbed 31% of the atrazine from solution, but further High-Performance Liquid Chromatography (HPLC) analysis revealed a high concentration of hydroxyatrazine. Xu et al. believes that the atrazine was hydrolyzed via a nucleophilic displacement of chlorine by hydroxide. Chemical

hydrolysis was favored in the reduced clay environment because a greater electron density in the alkaline environment (Xu et al. 2001). Armstrong et al. (1967) also noted the benefits of an alkaline environment as well as an acidic one. Alkaline hydrolysis occurred through a direct nucleophilic displacement. Acid hydrolysis occurred through protonation of a side chain or ring nitrogen atom and than nucleophilic displacement by water (Armstrong et al. 1967).

Hydrolysis can also occur biologically, with the enzyme AtzA. This enzyme was discovered by Mandelbaum et al. (1995) and characterized in the mid-nineties by deSousa et al. (1996). Both chemical and biological processes may be at work; Houot et al. (1998) hypothesized that increased formation of hydroxyatrazine was due to a combination of chemical and biological hydrolysis caused by a lower pH in soils from the addition of composted straw.

2.3.5 Stimulating Soil Microbial Activity with Wastewater

Addition of organic matter from wastewater treatment plant effluent causes an increase in the organic content of soil, which affects atrazine sorption (Barriuso et al. 1997; Masaphy and Mandelbaum 1997; Celis et al. 1998). For example, remediation with *Pseudomonas* sp. ADP was only 20% effective on soils that had been sprayed with treated wastewater, compared to 60-80% effective in soils without wastewater (Masaphy and Mandelbaum 1997). Conversely, addition of high concentrations (1058 mg of organic carbon/L) of dissolved organic matter from sewage sludge has the reverse effect: increasing desorption of atrazine, due to site competition or surface modification (Celis et al. 1998). However, in the presence of small concentrations of dissolved organic matter, up to 150 mg of organic carbon/L, atrazine adsorption is not suppressed (Seol and Lee

2000). The effects of wastewater application have important ramifications for the irrigation of farmland with effluent, and warrant further study.

This section has shown that the adsorption of atrazine on soils is strongly influenced by the presence of organic carbon as well as pH. Soil remediation with activated carbon is expensive. Recycling of wastewater treatment plant effluent onto soil may either decrease atrazine adsorption or increase it, depending on the concentration and properties of the organic material. Further research should examine the influences of soil properties on atrazine adsorption and the effects of irrigation with wastewater treatment plant effluent.

2.3.6 Activated Carbon Adsorption

Atrazine may enter drinking water through groundwater or runoff into surface water. Drinking water treatment plants typically use activated carbon treatments to remove atrazine or other residual compounds. This section will discuss the two most common types of activated carbon, Granular Activated Carbon (GAC) and Powdered Activated Carbon (PAC), as well as two variations: Biological Granular Activated Carbon (BGAC) and Activated Carbon Fibers (ACF).

Drinking water treatment plants use activated carbon to remove organics, residual inorganics, and taste/odor-causing compounds (Tchobanoglous et al. 2003). Activated carbon has been designated the best available technology for the removal of herbicides from drinking water by the EPA (Adams and Watson 1996). Activated carbon has a large porous surface area, controllable pore structure, low acid/base reactivity, and thermal stability. It has a low initial cost and high adsorption and regeneration capacities. It is

easy to control the dosage of activated carbon and it does not form any oxidation byproducts as in ozoneation (Adams and Watson 1996; Foo and Hameed 2010).

There are two types of activated carbon: Granular Activated Carbon (GAC) and Powdered Activated Carbon (PAC). GAC has larger particles with a diameter greater than 0.1 mm. In a water treatment process, it is contained in a pressurized contact basin. Conversely, PAC particles are smaller with a diameter less than 0.074 mm, and can be added at any point during the process (Tchobanoglous et al. 2003). Typically, PAC is added at the raw water intake, the rapid mix tank, or in a slurry contactor (Crittenden et al. 2005). Later in the process PAC must be settled out in a contacting basin or removed with filtration (Tchobanoglous et al. 2003). GAC requires less activated carbon, has easier handling, and can be regenerated, but has higher operation, maintenance, and capital costs. PAC has a low capital cost and offers flexibility of operation; however, it is hard to fully utilize its entire adsorption capacity and it requires an additional filtration procedure (Kyriakopoulos and Doulia 2006).

Both types of activated carbon can be designed to contain varying pore sizes. Pore size, as classified by the International Union of Pure and Applied Chemistry, can be seen in Table 2.1.

Table 2.1: Pore sizes of activated carbon (Ding et al. 2008)

Pore Type	**Size** *(Å)*
Macropores	>500
Mesopores	20-500
Secondary Micropores	8-20
Primary Micropores	<8

Atrazine is 9.6 x 8.4 x 3 Å and prefers primary or secondary micropores (Li et al. 2004a). However, in a drinking water treatment plant, atrazine is not the only target compound for removal, and must compete for adsorption sites with other compounds, including Natural Organic Matter (NOM). NOM is typically found at 3.7 ppm in natural waters, making it 1000 times greater than a typical concentration of atrazine (Kyriakopoulos and Doulia 2006; Zadaka et al. 2009). NOM affects atrazine adsorption in two ways: direct site competition and pore blockage (Zadaka et al. 2009).

NOM is larger than atrazine. If preloaded, it will block openings to smaller pores, reducing the surface area available for atrazine adsorption. Atrazine will have to move around the blockage or displace the NOM to adsorb. However, if NOM is in direct competition with atrazine, as in a batch reactor, pore blockage is not an issue, because atrazine can quickly adsorb to micropores before they are blocked (Li et al. 2003, 2004a).

Knappe et al. (1997) used RSSCT (Rapid Small Scale Column Tests) to examine the effect of NOM preloading on atrazine adsorption by GAC. Both virgin GAC and GAC that had been preloaded for 5 months effectively removed atrazine. However, a

longer preloading time, 20 months, was not successful due to enhanced adsorption and polymerization of NOM in the presence of oxygen. In a later study, Knappe et al. (1999) discovered that after preloading, there was no competition between NOM and atrazine for sites. Also, adsorption capacity could be increased by grinding preloaded GAC into PAC. The grinding increased the surface area by opening up pore space.

The PAC dose in a treatment plant is typically 1-2 mg/L for odor and taste control (Jiang and Adams 2006). If NOM is present, 10-16 times more PAC is required to achieve 90-99% removals of atrazine, because NOM has slower adsorption kinetics; NOM moves down the column quickly, preloading the bottom of the column before atrazine can get there (Li et al. 2003).

Ding et al. (2008) found that there was less site blockage in PAC with pores 15-50 Å. NOM favors this pore size, leaving smaller micropores unblocked for atrazine adsorption. Similarly, Li et al. (2003) found that PAC with a greater percentage of mesopores had better atrazine adsorption. Due to site competition, atrazine adsorption is not related to the total surface area, but rather to the number of available micropores (Ding et al. 2008). Thus, adsorption kinetics is more important than adsorption capacity.

There are many options to decrease the effect of NOM, including: pulse input of PAC, aeration, and optimizing the membrane cleaning interval. A pulse input of PAC results in a greater amount of contact time with a greater amount of PAC, lessening the effect of pore blocking materials (Li et al. 2004b). The adsorption capacities and lifespan of the PAC can be increased with intermittent high intensity aeration (2.7 L/min with a 2 second pause). These bubbles generate microscale high intensity eddies that shrink the

resistance of the boundary layer (Jia et al. 2006). Lastly, the performance of a small reactor can be optimized to avoid influence from pore blocking materials with a short membrane cleaning interval (MCI) and a low PAC dose (Li et al. 2004b).

Zhang and Emary (1999) used jar tests to simulate a drinking water treatment plant environment. PAC alone exhibited a 40-50% removal of atrazine. Typical drinking water treatment plant additions, such as alum coagulant or lime, had negligible effect on the atrazine removal. However, the combination of a lowered pH with sulfuric acid (5.8), alum coagulation, and PAC increased atrazine removal to over 60%. The lower pH increased the hydrophilic properties of atrazine and lowered the charge density on NOM, making atrazine more susceptible to removal by coagulation or adsorption.

GAC and PAC are the two most common types of activated carbon and are typically used in drinking water treatment plants. Two variations, more common in a laboratory setting, are Biological Granular Activated Carbon (BGAC) and Activated Carbon Fibers (ACF).

Herzberg et al. (2004) compared anaerobic atrazine degradation by *Pseudomonas* sp. ADP on an adsorbent medium (GAC) and non-adsorbent medium. The BGAC (Biological Granular Activated Carbon) column degraded more atrazine by two orders of magnitude than the column with non-adsorbent media, due to a "double flux" of atrazine through the biofilm and the adsorbent media. In a similar study, Feakin et al. (1995a) successfully used *Rodococcus rhodochrous* in a BGAC column for atrazine degradation. The authors hypothesized that atrazine adsorbed on the GAC is not bioavailable to the bacteria; atrazine must desorb into the liquid phase to become bioavailable (Feakin et al.

1995a). BGAC has limited application to drinking water treatment plants due to environmental regulations. In the United Kingdom, influent bacteria counts must be approximately equal to effluent bacteria counts. If bacterial counts are greater than 10^3, chlorination is advised (Feakin et al. 1995b).

Scientists that are specifically interested in examining pore blockage effects in activated carbon often turn to ACF (Activated Carbon Fibers). ACF are synthetic materials from polymeric substances. They are specifically engineered to have a uniform and continuous pore structure. GAC is exactly the opposite; It is made from impure, non-uniform feedstocks, it is not homogenous, and doesn't have continuous micropores (Pelekani and Snoeyink 2000). ACF have faster initial adsorption rates as well as a greater adsorption capacity than GAC (Faur et al. 2005). Pelekani and Snoeyink (2000) studied the competitive adsorption between atrazine and methylene blue (a compound of similar size to atrazine) on ACF. Similar to previous studies with NOM, the impact of preloading with the competing substance decreased as pore size increased. Also, increasing the volume of secondary micropores relative to primary micropores increased the adsorption of atrazine. This allowed atrazine to directly compete for sites, instead of finding primary micropores blocked by the competing substance.

In conclusion, activated carbon can be an effective tool for atrazine removal, provided enough micropores are not blocked by competing substances, such as NOM. BGAC have promise for use in the drinking water industry, but more experimentation is required to select robust strains of bacteria with higher survival rates. ACF are useful on a laboratory scale to examine pore blockage effects, but is too expensive for most real world applications. Activated carbon, though capable of removing 98% of atrazine, is

costly, leading researchers to examine cheaper alternatives: recycled activated carbon, modified soils, oil seed press cakes, switchgrass, and recycled materials.

2.3.7 Natural Materials Adsorption

PAC is typically used for six months to a year, and then it is discarded. Ghosh and Phillip (2005) found a way to reuse it as Powdered Waste Activated Carbon (PWAC). The PWAC removed 17.19 mg atrazine/g carbon, and when washed for reuse, removed 13.24 mg atrazine/g carbon. The PWAC also supported the growth of atrazine-degrading bacteria that grew on the surface of the activated carbon without causing a biofilm or a pressure drop (Ghosh and Phillip 2005).

Modified soils have been examined by Bottero et al. (1993) and Zadaka et al. (2008) for atrazine removal. Zeolites were not able to outperform activated carbon (Bottero et al. 1993). However, Zadaka et al. (2008) was able to achieve 93-96% removal rates of atrazine, outperforming activated carbon by 10%, with montmorillonite soils pre-adsorbed with 10% poly(4-vinylpyridine-*co*-styrene), or PVPco-S90%-mont. Also, the PVPco-S90%-montmorillonite was not as affected by addition of dissolved organic matter, was more structurally compatible with atrazine, and had a higher charge density than other modified soil.

Boucher et al. (2007) examined the adsorption of atrazine by oil seed press cakes. The press cakes adsorbed 58% of the atrazine, out-performing the seeds alone or ground seeds. This was due, in part, to a mass transfer effect; the oil particles were smaller in the press cakes and less blocked by other structures.

Atrazine adsorption on or near a field is highly desired to reduce runoff and groundwater contamination. The adsorption capacities of thatch and fresh switchgrass were evaluated in a laboratory setting to approximate the behavior of a vegetative filter. Both were able to effectively adsorb atrazine. The adsorption coefficients after 24 hours were 81.1 and 21.4 Lkg^{-1} for switchgrass and thatch, respectively. However, cut ends of switchgrass, not generally present in the field, may have skewed the adsorption data (Mersie et al. 2006). An earlier study by Mersie et al. (1999) used planted boxes of switchgrass instead of switchgrass cuttings. Bacterial degradation was faster in beds planted with switchgrass, and switchgrass plots successfully adsorbed atrazine. Similarly, Selim and Zhu (2005) found that sugarcane mulch residue left in the field after harvest exhibits strong atrazine retention, with a partitioning coefficient of 16.4 Lkg^{-1}.

Table 2.2 shows the wide variety of recycled materials that have been investigated as cheaper alternatives to activated carbon: wood charcoal, rubber granules, bottom ash, coconut fiber, and sawdust. Wood charcoal is the best alternative for adsorption, though it is not as efficient as activated carbon, with removal rates of 95-97%. However, rubber granules also have a high removal rate (82%) (Alam et al. 2000; Sharma et al. 2008). Alam et al. (2000) recommends rubber granules over wood charcoal because the disposal of the charcoal causes air pollution, whereas rubber granules can be recycled into rubberized asphalt.

Table 2.2: Comparison of natural materials for atrazine adsorption

Material	Initial Conc. of Atrazine	Time *(min)*	Amount Adsorbed	Percent Adsorbed	Source
Sugarcane mulch residue	3.37 mg/L	30240	47.87 mg/kg	53%	(Selim and Zhu 2005)
Sugarcane mulch residue	6.36 mg/L	30240	86.25 mg/kg	55%	(Selim and Zhu 2005)
Sugarcane mulch residue	12.34 mg/L	30240	160.3 mg/kg	57%	(Selim and Zhu 2005)
Sugarcane mulch residue	18.22 mg/L	30240	236.6 mg/kg	57%	(Selim and Zhu 2005)
Sugarcane mulch residue	24.30 mg/L	30240	310.5 mg/kg	57%	(Selim and Zhu 2005)
Sugarcane mulch residue	30.16 mg/L	30240	360.5 mg/kg	60%	(Selim and Zhu 2005)
Macro fungi *florida*	4 mg/L	240	2.472 mg/L	62%	(Alam et al. 2000)
Macro fungi *sajor caju*	4 mg/L	240	2.492 mg/L	62%	(Alam et al. 2000)
Saw dust	100 µg/L	100	73.25 µg/L	73%	(Sharma et al. 2008)
Bottom ash	4 mg/L	210	3.04 mg/L	76%	(Alam et al.2000)
Baggasse charcoal	50 µg/L	100	38.5 µg/L	77%	(Sharma et al. 2008)
Saw dust	50 µg/L	100	40.6 µg/L	81%	(Sharma et al. 2008)
Fly ash	100 µg/L	100	82.9 µg/L	83%	(Sharma et al. 2008)
Rubber granules	4 mg/L	100	3.32 mg/L	83%	(Alam et al. 2000)
Baggasse charcoal	100 µg/L	100	84.6 µg/L	85%	(Sharma et al. 2008)
Coconut fiber	50 µg/L	100	43.48 µg/L	87%	(Sharma et al. 2008)

Table 2.2 Continued: Comparison of natural materials for atrazine adsorption

Material	**Initial Conc. of Atrazine**	**Time** *(min)*	**Amount Adsorbed**	**Percent Adsorbed**	**Source**
Fly ash	50 µg/L	100	43.6 µg/L	87%	(Sharma et al. 2008)
Coconut charcoal	100 µg/L	100	93 µg/L	93%	(Sharma et al. 2008)
Wood charcoal	100 µg/L	100	95.5 µg/L	96%	(Sharma et al. 2008)
Wood charcoal	4 mg/L	45	3.82 mg/L	96%	(Alam et al. 2000)
Coconut charcoal	50 µg /L	100	47.8 µg/L	96%	(Sharma et al. 2008)
Coconut fiber	100 µg/L	100	96.29 µg/L	96%	(Sharma et al. 2008)
Wood charcoal	50 µg/L	100	48.7 µg/L	97%	(Sharma et al. 2008)

Alternatives for atrazine adsorption have varying degrees of effectiveness. However, none is equal to the power of activated carbon. Future research should focus on finding a cheap, fast, effective alternative for atrazine adsorption and should continue to elucidate the kinetics of atrazine adsorption.

2.3.8 Metabolite Adsorption

Atrazine is often found in conjunction with its metabolites in natural waters. Atrazine and its metabolites have different solubilities in water, as seen in Table 2.3. This affects their adsorption capacities, according to Lundelius rule: The extent of adsorption of a solute is inversely proportional to its solubility in the solvent (Adams and Watson 1996).

Table 2.3: Solubility of atrazine and its metabolites (Steinheimer 1993; Faur et al. 2005)

Compound	**Solubility in Water** *(mg/L)*
Atrazine	34.7
Deethylatrazine (DEA)	3200
Deisopropylatrazine (DIA)	670
Hydroxyatrazine (HYA)	7

DEA and HYA are the two most prevalent metabolites found in soils (Liu et al. 1996; Mudhoo and Garg 2011). As shown in Table 2.3, HYA is strongly adsorbed to soils. HYA has stronger adsorption because it has a higher protonation than atrazine at the same pH. However, HYA adsorption does not interfere with atrazine adsorption, because the two compounds prefer different types of sites (Ma and Selim 1996).

Faur et al. (2005) studied atrazine, DEA, and DIA adsorption on activated carbon fibers. They found that atrazine adsorbed the strongest, followed by DIA and DEA, confirming Lundelius rule. In binary systems, such as atrazine-DEA and atrazine-DIA,

the adsorbate of lower solubility, atrazine, was favored for adsorption, while the other, DIA or DEA, did not adsorb and had no influence on atrazine adsorption.

Standard drinking water treatment plant processes such as coagulation, flocculation, sedimentation, free chlorine, lime, or soda ash do not reduce the concentration of atrazine, DEA, or DIA. Ozone at a dose of 3-5 mg/L reduced TCT (Total Chloro s-Triazine) concentration in river water by 32% and in DI water by 70%, because of the persistence of the metabolites. Higher removals were seen with powdered activated carbon (0.55 m^3/g). In river water, 90% of TCT was removed with PAC concentrations of 20-50 mg/L and 80% was removed with PAC of 5 mg/L. However, using PAC concentrations more typical to treatment plants, 1-2 mg/L, only 40% removal could be achieved, due to the high solubilities of the metabolites and fowling by natural organic matter (Jiang and Adams 2006). A similar study showed using PAC to treat DEA and DIA requires 3.1-4.5 times more activated carbon than it would to treat atrazine alone, because of the higher solubilities of these metabolites (Adams and Watson 1996).

The varying solubilities of atrazine metabolites pose a special challenge for removal. As regulations change in the coming years to include metabolites, further research should focus on adsorption kinetics and examine cheaper adsorption materials.

2.3.9 Conclusions

The presence of organic matter, either in soil or in water, has a profound effect on the adsorption of atrazine. Further research should focus on what other properties affect adsorption, and which are most important to model. A widely applicable, accurate model for both atrazine adsorption and desorption has yet to be developed. Also, a suitable and

effective alternative for activated carbon for drinking water treatment is worthy of further study. Lastly, the adsorption properties of atrazine metabolites may be of concern, especially if regulations in the United States are adjusted to include TCT levels.

2.4 Removal via Bacteria

2.4.1 Background

Bacteria provide an alternative method for atrazine removal, either by degradation or mineralization. Atrazine degradation is the disappearance of the parent compound, atrazine, into intermediate compounds, or metabolites; atrazine mineralization is the complete transformation of atrazine and its metabolites into carbon dioxide (Ellis and Wackett 2011a, 2011b, 2011c).

In soils, the half-life of atrazine is 35-50 days with little mineralization of the s-triazine ring by indigenous bacteria (Topp 2001). The time required for indigenous bacteria to mineralize the s-triazine ring, thereby degrading atrazine into less toxic metabolites, has been estimated to be 60-360+days. Complete mineralization is estimated to occur only to less than 40% of applied atrazine. However, more rapid mineralization of atrazine has been reported in agricultural soils that frequently come in contact with atrazine. Repeated dosing of atrazine naturally selects bacteria with an enhanced ability to degrade atrazine (Alvey and Crowly 1996). Isolation of some of these indigenous atrazine-degrading bacteria began in the nineties and continues to the present day.

2.4.2 Objective

This section will discuss the spectrum of bacterial research, including a discussion of the effectiveness of indigenous bacterial consortia, specific isolates, and characterized

consortia. Next, isolated strains of bacteria that can co-remove both atrazine and nitrate will be discussed. The final two sections discuss the challenges with field application of bacteria, including efforts to stimulate field conditions in the laboratory, and creative solutions to combating the reduced effectiveness of applied bacteria in the field over time.

2.4.3 Uncharacterized Consortia

Indigenous soil bacteria can be acclimated to degrade atrazine, if given time for the proliferation of a degrading population that selects and expresses the right genes for degradation (Silva et al. 2004). In top soil, atrazine degradation occurs in 60 days, whereas, degradation in subsurface soils or in groundwater, takes significantly longer (U.S. Environmental Protection Agency 1988). Under aerobic conditions in soil, the half-life for atrazine is 3.6 ± 0.4 years; under denitrifying conditions, the half-life is over 500 years, suggesting that atrazine degradation is dependent on soil depth (Nair and Schnoor 1992; Kruger et al. 1993). However, a study by Wilber and Parkin (1995) found that atrazine degradation by a natural soil consortia was not different between aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions. However, degradation by the consortia was rapidly decreased under aerobic and nitrate-reducing conditions once the primary substrate, acetate, was depleted.

2.4.4 Isolates and Isolated Consortia

Isolated bacterial strains for atrazine mineralization have existed since the 1990's (Wackett et al. 2002). *Pseudomonas* sp. ADP was one of the first strains to be isolated. It was isolated and characterized by Mandelbaum et al. (1995) and continues to be studied due to its high removal efficiency. *Pseudomonas* sp. ADP can utilize atrazine as a

nitrogen source, but not as a carbon source. If a carbon source is added, such as citrate, removal efficiencies of 80% (Shapir et al. 1998) up to 95% (Katz et al. 2001) have been seen. The ratio for complete denitrification was calculated to be 5.11 g citrate g^{-1} NO_3-N (Katz et al. 2000).

However, the requirement of a carbon source puts *Pseudomonas* sp. ADP at a disadvantage compared to other isolates, such as *Pseudominobacter* sp. and *Nocardioides* sp. These isolates, especially *Pseudominobacter* sp., outperformed *Pseudomonas* sp. ADP because they can utilize atrazine both as a carbon and a nitrogen source (Topp 2001). Compounds with this capability have greater potential uses as bioremediation agents, because they do not require additional chemical stimulation.

Consortia are more common in nature than single strains and they can be more effective at atrazine removal. A consortia was isolated from atrazine degrading soil by Kolic et al (2007): *Arthrobacter* sp. AG1, *Arthrobacter keyseri* 12B, *Ochrobactrum* sp., and *Pseudomonas* sp. It was able to achieve 78% mineralization, because it shared carbon and nitrogen sources, and cross-fed metabolites.

A larger consortia was characterized by Smith et al (2005): *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Pseudomonas putida*, *Sphingomonas yaniokuyae*, *Nocardia* sp., *Rhizobium* sp., *Flavobacterium oryzihabitans*, and *Variovorax paradoxus*. The pivotal members of this consortia were *Nocardia* sp. and *Rhizobium* sp. *Nocardia* sp. was the only member that could use the enzyme TrzB to transform atrazine into hydroxyatrazine. Next, *Rhizobium* sp. used AtzB to transform hydroxyatrazine to the next product, *N*-ethylammelide, which all members could further degrade.

Characterization of natural consortia illuminated the metabolic pathway of atrazine, providing new bacteria that work either individually or together to perform fast and effective degradation. However, the impact of the natural bacteria that cannot be cultured must also be considered for their role in atrazine degradation (vanVeen 1999; Smith et al. 2005).

2.4.5 Co-removal of Atrazine and Nitrate

Atrazine and nitrate are often found together in groundwater in agricultural areas, so their simultaneous removal is often desired (Ritter 1990). Katz et al. (2001) used *Pseudomonas* sp. ADP in anoxic non-sterile reactors that had a high removal efficiency of atrazine, >95%, for the first month, and then lost effectiveness, 10-25%, due to competitive nitrifying bacteria that could not degrade atrazine. Herzberg et al. (2004) also saw a similar decrease in atrazine removal efficiency in a reactor filled with non-adsorptive media. However, this effect was not present in similar reactor with adsorptive media due to a "double flux" of atrazine through the biofilm and the adsorbent media.

Clausen et al. (2002) suggested that a high concentration of nitrate interferes with the degradation capabilities of *Pseudomonas* sp. However, this conclusion was based on a 14-day reaction time, which may not be long enough to see the full effect of an excess of nitrate.

Early reports by Cervelli and Rolston (1983) claimed that atrazine applied at 3 μg/g soil inhibited denitrification, specifically the reduction of N_2O to N_2. However, this was later disproved by Yeomans and Bremner (1987), who found no inhibition of denitrification when atrazine was applied at 5, 10, 25, or 100 μg/g soil.

Isolate M91-3 has been studied extensively for use in denitrification coupled with atrazine degradation in glass media columns by Crawford et al (2000). Atrazine degradation was achieved in both aerobic and anaerobic zones in the column. Low concentrations of nitrate, ~1 mM, did not interfere with atrazine degradation (Crawford et al. 1998, 2000). Also, the addition of glucose accelerated the anaerobic degradation of atrazine in the presence of nitrate (Crawford et al. 2000).

Hunter and Shaner (2010) used a double column containing a vegetable oil based denitrifying biobarrier followed by an aerobic reactor with an atrazine-degrading consortia to simulate nitrate and atrazine removal from groundwater. The denitrifying section removed 98% of the supplied nitrate and 30% of the atrazine, while the aerobic reactor removed the remaining 70% of the atrazine. Atrazine removal varied considerably in the denitrifying biobarrier, because of the interference of the nitrate, an easier source of nitrogen for column bacteria than atrazine.

As the above results show, co-removal of atrazine and nitrate is possible, though sometimes difficult, depending on the electron acceptor conditions, the presence of a carbon source, and the presence of other competing bacteria.

2.4.6 Biostimulation and Bioaugmentation

Laboratory studies using natural soils revealed the effects of bioaugmentation and biostimulation on indigenous bacteria. Bioaugmentation is the addition of non-indigenous microbial strains (i.e. *Pseudomonas* sp. ADP) to the environment for purposes of remediation. Biostimulation is the addition of chemicals (i.e. citrate) to the environment to stimulate naturally occurring bacteria for purposes of remediation. Biostimulation is

approved faster by government agencies, whereas, bioremediation, especially with genetically modified organisms, causes closer scrutiny (Wackett et al. 2002). Bioremediation is often preferred over physical or chemical remediation because it can be done *in situ* with lower costs and environmental impacts (Sturman et al. 1995; Newcombe and Crowley 1999).

Biostimulation has been researched with a number of materials: municipal solid waste compost, straw compost, rice hulls, sodium citrate, urea, Sudan hay, glucose, mannitol, acetic acid, and starch (Houot et al. 1998; Assaf and Harris 1994; Alvey and Crowley 1995; Chung et al. 1996; Getenga 2003). Rice hulls had the highest mineralization of atrazine, 88%, according to Alvey and Crowley (Alvey and Crowley 1995; Houot et al. 1998). More information about the effectiveness of these, and other materials can be found in Table 2.4.

In a study by Houot et al. (1998), municipal solid waste compost adsorbed 75% of applied atrazine, 1 kg/ha, making it unavailable for biodegradation. Conversely, in a study by Getenga (2003), municipal solid waste compost applied at 5000 ppm resulted in 55% mineralization of atrazine. It is unknown whether this effect is due to additional bacteria present in the compost or if the compost provided carbon and nitrogen for the soil bacteria to utilize.

Assaf and Harris (1994) found that their soil bacteria benefited from addition of mannitol, which produced a 17% increase in CO_2 evolution, suggesting a rate-limiting step in the metabolization of atrazine. Acetic acid additions to soil reduced the half-life of atrazine from 224 to 164 days. However, because only the level of atrazine was

measured, these values are not indicative of complete mineralization (Chung et al. 1996). The varied results from these studies show that further research in this area would benefit from a better understanding of bacterial community population dynamics to select the correct compound for biostimulation.

Silva et al. (2004) showed that biostimulation with citrate alone inhibited atrazine mineralization by indigenous bacteria, because their mineralization pathway may be different from that of *Pseudomonas* sp. ADP. However, bioaugmentation and biostimulation of *Pseudomonas* sp. ADP and citrate together reduced atrazine concentrations by 80% in 2 days. Rousseaux et al. (2003) found biostimulation and bioaugmentation with *Chelatobacter heintzii* Cit1 and sodium citrate was not effective in soils that already had an indigenous population of atrazine degrading bacteria. However, in soils without an indigenous population, biostimulation and bioaugmentation resulted in a 3-fold increase in mineralization capacity of atrazine.

Table 2.4: Comparison of biostimulation methods to encourage indigenous bacteria to mineralize atrazine

Bacteria Name	**Biostimulation**	**Initial Atrazine Conc.**	**Percent Mineralization**	**Time** *(days)*	**Addition Help or Hinder?**	**Source**
Natural Consortia	Only Compost	0.34 mg/kg	9%	250	hinder	(Barriuso et al. 1996)
Natural Consortia	Glucose	100 mg/kg	>10%	77	hinder	(Alvey and Crowley 1995)
Natural Consortia	Sudan Hay	100 mg/kg	>10%	77	hinder	(Alvey and Crowley 1995)
Natural Consortia	Sodium Citrate	100 mg/kg	>10%	77	hinder	(Alvey and Crowley 1995)
Natural Consortia	MSW Compost	10.9 mg/L	15%	150	neither	(Houot et al. 1998)
Natural Consortia	Composted Straw	10.9 mg/L	16%	150	neither	(Houot et al. 1998)
Natural Consortia	None	10.9 mg/L	24%	150	neither	(Houot et al. 1998)
Natural Consortia	None	100 ppm	31%	112	--	(Getenga 2003)
Natural Consortia	30% MSW Compost	0.34 mg/kg	34%	250	hinder	(Barriuso et al. 1996)
Natural Consortia	Mannitol	10 mg/kg	39%	326	neither	(Assaf and Harris 1994)
Natural Consortia	Urea	10 mg/kg	39%	326	neither	(Assaf and Harris 1994)
Natural Consortia	Mannitol and Urea	10 mg/kg	39%	326	neither	(Assaf and Harris 1994)
Natural Consortia	1000 ppm Compost	100 ppm	42%	112	help	(Getenga 2003)

Table 2.4 Continued: Comparison of biostimulation methods to encourage indigenous bacteria to mineralize atrazine

Bacteria Name	**Biostimulation**	**Initial Atrazine Conc.**	**Percent Mineralization**	**Time** *(days)*	**Addition Help or Hinder?**	**Source**
Natural Consortia	2500 ppm compost	100 ppm	46%	112	help	(Getenga 2003)
Natural Consortia	20 % MSW Compost	0.34 mg/kg	47%	250	hinder	(Barriuso et al. 1996)
Pseudomonas sp. ADP	None	30 ppm	50%	10	--	(Masaphy and Mandelbaum 1997)
Natural Consortia	5000 ppm compost	100 ppm	55%	112	help	(Getenga 2003)
Natural Consortia	Compost	100 mg/kg	59%	77	hinder	(Alvey and Crowley 1995)
Natural Consortia	10% MSW Compost	0.34 mg/kg	66%	250	hinder	(Barriuso et al. 1996)
Natural Consortia	None	100 mg/kg	73%	77	--	(Alvey and Crowley 1995)
Natural Consortia	Starch	100 mg/kg	75%	77	help	(Alvey and Crowley 1995)
Pseudomonas sp. ADP	Treated Wastewater	30 ppm	80%	10	help	(Masaphy and Mandelbaum 1997)
Natural Consortia	None	0.34 mg/kg	85%	250	--	(Barriuso et al. 1996)
Natural Consortia	Rice Hulls	100 mg/kg	88%	77	help	(Alvey and Crowley 1995)

As the above discussion and Table 2.4 show, the addition of natural materials can either help or hinder atrazine removal. The natural materials add new bacteria or fungi that may either compete with or assist local populations of atrazine-degrading microbes. The natural materials also may act as a source of nutrients for bacterial populations. However, atrazine may adsorb into pore space in the natural material, making it less bioavailable for degradation. The fastest mineralization rate was 80% in ten days with the addition of wastewater by Masaphy and Mandelbaum (1996). Rice hulls were the next most effective, with 88% mineralization in 77 days (Alvey and Crowley 1995).

2.4.7 From the Laboratory to the Field

Laboratory conditions are easier to control and are often less harsh to atrazine degrading bacteria than field conditions. Though some laboratories attempt to replicate conditions in the field, field conditions are hard to control such as: a non-uniform distribution of atrazine, the presence of other contaminants or metabolites, ambient temperature, and mass transport limitations for the contaminant, bacteria, and nutrients (Sturman et al. 1995; Strong et al. 2000; Silva et al. 2004).

One laboratory-scale study strived to replicate field conditions, using simulated rainwater, commercial herbicides, and earthworms to examine the survival rates and effectiveness of *Pseudomonas* sp. ADP. Chelinho et al. (2010) set up soil microcosms in the laboratory with earthworms (*Eisenia andrei*) and springtails (*Folsomia candida*) that were dosed with *Pseudomonas* sp. ADP and Atrazerba FL, a commercial herbicide containing atrazine. Both the herbicide and the bacteria were dispersed throughout the soil with simulated rainwater, to imitate field conditions. Mineralization of atrazine after

42 days of exposure was 99%, which was slower than similar studies without invertebrates, suggesting that the invertebrates may have contributed to a decline in the numbers of *Pseudomonas* sp. ADP.

Unfortunately, in field studies, bioaugmented bacteria often exhibit a low survival rate or lose their degradation ability over time. This could be due to a lack of available nutrients, competition with indigenous populations, or other conditions not favorable to bacterial growth (Sturman et al. 1995; Newcombe and Crowly 1999; Silva et al. 2004). Before introducing bacteria, vanVeen et al. (1999) recommends the use of microbiosensors to assess the soil environment for availability, distribution, and movement of soil nutrients. Thus, biostimulation, in addition to bioaugmentation, is recommended for field studies.

The natural fluctuations of soil conditions may stress bioaugmented bacteria. Inoculated bacteria survival is based on their ability to colonize soil particles. Stress on bioaugmented bacteria can be cushioned with a carrier, such as peat moss, to provided protected pore space and nutrients. Carriers must be nontoxic, biodegradable, and of consistent quality (vanVeen et al. 1999).

To overcome the loss of degradation ability, Newcombe and Crowly (1999) used a batch fermenter to deliver a bacterial consortia containing *Pseudomonas* sp. strain CN1 and *Clavibacter michiganese* ATZ1 to soils contaminated with 100 μg atrazine/g soil at different frequencies. In laboratory tests, soils that were inoculated once had a mineralization rate of 17%, but soils that were inoculated every three days had mineralization rates of 64%. In field tests, no significant mineralization occurred in soils

that were only inoculated once. However, 72% mineralization was seen in soils that had eight inoculations over 12 weeks. Lima et al. (2009) saw similar results with *Pseudomonas* sp. ADP and citrate. At low concentrations (6 μg/g soil) the citrate addition was unnecessary. However, at high concentrations (62 μg/g soil) a single inoculation mineralized 87% whereas the same single inoculation spread over three days mineralized 99%. This demonstrates the vast difference between laboratory and field conditions, and that repeated applications are one strategy to improve microorganism survival rates in the field.

Another strategy, suggested by Alvey and Crowly (1996), is to plant corn. Corn did not affect the mineralization rate of the bacterial consortia (*Pseudomonas* sp. strain CN1 and *Clavibacter michiganese* ATZ1), but it did increase the survival rate of the bacterial consortia. Survival of the bacterial consortia was 30 times higher in the planted soil compared to non-planted soil for low atrazine concentrations.

Once the enzymes responsible for atrazine degradation with *Pseudomonas* sp. ADP were illuminated, biochemists began creating their own bioaugmentation sources using chemically killed, recumbent organisms engineered to overproduce enzymes of interest. The first field-scale study of this in the United States was performed by Strong, et al. (2000) using *Escherichia coli* that had been engineered to produce AtzA, *atrazine chlorohydrolase*, the enzyme responsible for the dehalogenation of atrazine into hydroxyatrazine. In the laboratory studies, 84% of initial atrazine concentration was degraded, whereas field scale studies only achieved 77% degradation. This difference in performance is attributed to field conditions, which are harder to control, including distribution of atrazine and temperature.

The public may have a negative perception of bioaugmentation with exotic genetically-modified strains of bacteria (Shapir et al. 1998). However, this may be avoided by using a method proposed by Perumbakkam et al (2006). AtzA was delivered to two biofilm populations on a laboratory scale using plasmids: a mixed culture of indigenous bacteria and *Acinetobacter* sp. BD413. The gene augmentation was successful on both accounts, resulting in 80-85% degradation of 20 mg atrazine/L. Zhao et al. (2003) also used the AtzA gene to augment naturally occurring soil bacteria. The effectiveness of the AtzA gene was compared to *Pseudomonas* sp. ADP in both aged and un-aged soils. The AtzA gene resulted in faster degradation than by *Pseudomonas*; however, the degradation of both was slowed by aging.

The possibilities of inoculation of indigenous bacteria with necessary genes for degradation of contaminants is promising, especially if complete mineralization could be achieved. However, the cost of widespread use of engineered microorganisms is yet to be shown. Also, the economics and practicality of frequent re-inoculation must also be considered when designing large-scale projects (Topp 2001).

2.4.8 Aging

Remediation of older sites poses special challenges: the older the site, the greater the opportunity for atrazine or its metabolites to adsorb into inaccessible pore spaces, limiting the interaction with plants, animals, bacteria, and transport. Bound residues of atrazine are assumed to be unavailable, but they may not be truly unavailable to bacteria or other organisms (Barriuso et al. 2004). Therefore, bioavailability is best described for a specific organism and a specific mode of transport (Alexander 2000).

As a contaminant is sequestered in soil, its toxicity generally decreases with time, because the contaminant moves into the pore space or within the organic matter matrix. Desorption out of these two areas is very slow. However, risk of exposure is not completely eliminated because pockets of unadsorbed contaminant may still remain. Pockets like these are aged, but not sequestered (Alexander 2000).

By convention, pesticide concentrations in soil are measured as total concentration after extraction with harsh solvents. These solvents may be releasing more pesticide than is actually bioavailable, and may be causing unnecessary remediation in places where much of the contaminant is sequestered (Alexander 2000). Barriuso et al. (2004) proposed a milder extraction technique: a mix of calcium chloride and methanol. The introduction of *Pseudomonas* sp. ADP after the extraction didn't result in further degradation of atrazine, demonstrating that most of the bioavailable atrazine had been extracted. Milder extraction methods better simulate natural conditions and this approach could impact environmental regulations.

Bioavailability is an important consideration for remediation. It is influenced by the age of a site and whether a compound is truly sequestered, and will remain sequestered. Different soils may influence bioavailability differently. It is unknown which soil properties are most important to simulate in a laboratory setting. Differences in different types of soil, such as bulk soil and rhizosphere soil should be considered. Lastly, much research has been done with atrazine, but there are far fewer papers that examine the toxicity and bioavailability of its metabolites (Sturman et al. 1995; Chung and Alexander 1998; Alexander 1999; vanVeen et al. 1999; Alexander 2000).

2.4.9 Conclusions

Table 2.5 provides a summary of papers dealing with bacterial remediation. It is organized from increasing mineralization rates to increasing degradation rates. As Table 2.5 shows, *Pseudomonas* sp. ADP has the highest rates of mineralization, with the fastest rate, 7 days, attributed to Lima et al. (2009) However, the effectiveness of *Pseudomonas* sp. ADP ranges from no degradation at all (Pearson et al. 2006) to 100% degradation (Katz et al. 2001) to 98% mineralization (Lima et al. 2009). Natural consortia, share a similar range from no degradation (Pearson et al. 2006) to 100% degradation (Smith et al. 2005) to 84% mineralization (Alvey and Crowley 1996). Bioaugmented natural consortia are not as effective, ranging from 50% degradation (Topp 2001) to 80% mineralization (Silva et al. 2004).

The fastest mineralization rates, under 10 days, were accomplished with, *Pseudomonas* sp. ADP amended with citrate, achieving 75-98% mineralization (Shapir et al. 1998; Kolic et al. 2007; Lima et al. 2009). Perumbakkam et al. (2006) achieved the fastest degradation rates, only a few hours, with their novel delivery of a plasmid carrying the AtzA gene to a natural consortia. Hopefully, with future work on gene delivery methods and the atrazine metabolic pathway, this high degradation rate can be translated into a high mineralization rate. High mineralization rates from both *Pseudomonas* sp. ADP and natural consortia take about a month to achieve (Alvey and Crowley 1996; Strong et al. 2000; Katz et al. 2001; Chelinho et al. 2010)

The wealth of information on bacterial mineralization of atrazine leaves many questions unanswered. As more strains are discovered, a unified method to compare the atrazine removal ability of different strains will be necessary. The interaction between

members of an atrazine-degrading consortia as well as nutrient requirements should be examined to illuminate under what circumstances bioaugmentation, biostimulation, or gene addition can be most effective. Also, the continued persistence of atrazine in the environment calls for a better understanding and replication of field conditions in the laboratory. Lastly, remediation of older sites requires a more specific definition of bioavailability that should be incorporated into future environmental regulations.

Table 2.5: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	Electron Donor	Initial Atrazine Concentration	Percent Mineralization or Degradation		Time *(days)*	Source
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		100 mg/kg	17%	mineralization	35	(Newcombe and Crowley 1999)
Natural consortia	Citrate	0.5 mg/kg soil	21%	mineralization	106	(Rousseaux et al. 2003)
Pseudomonas sp. ADP + natural consortia		168.7 µg/g soil	31%	mineralization	7	(Silva et al. 2004)
Pseudomonas sp. ADP		100 mg/kg	35%	mineralization	35	(Newcombe and Crowley 1999)
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		100 mg/kg	38%	mineralization	84	(Newcombe and Crowley 1999)
Chelatobacter heintzii Cit1+natural consortia	Citrate	0.5 mg/kg soil	50%	mineralization	60	(Rousseaux et al. 2003)
Natural consortia		0.37 ppm	50%	mineralization	1095	(Nair and Schnoor 1992)
Natural consortia		0.37 ppm	50%	mineralization	11680	(Nair and Schnoor 1992)
Natural consortia		168.7 µg/g soil	54%	mineralization	67	(Silva et al. 2004)
Pseudomonas sp. ADP	Citrate	2.8 µmol/L	63%	mineralization	14	(Clausen et al. 2002)
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		100 mg/kg	64%	mineralization	35	(Newcombe and Crowley 1999)

Table 2.5 Continued: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	**Electron Donor**	**Initial Atrazine Concentration**	**Percent Mineralization or Degradation**		**Time** *(days)*	**Source**
Pseudomonas sp. ADP	Citrate	1500 ppm	70%	mineralization	21	(Mandelbaum et al. 1995)
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		3 mg/kg soil	71%	mineralization	28	(Alvey and Crowley 1996)
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		100 mg/kg	72%	mineralization	84	(Newcombe and Crowley 1999)
Pseudomonas sp. ADP	Phosphate	0.01ppm	75%	mineralization	4	(Shapir et al. 1998)
Natural consortia	Citrate	0.5 mg/kg soil	75%	mineralization	30	(Rousseaux et al. 2003)
Chelatobacter heintzii Cit1+natural consortia	Citrate	0.5 mg/kg soil	75%	mineralization	30	(Rousseaux et al. 2003)
Chelatobacter heintzii Cit1	Citrate	0.5 mg/kg soil	75%	mineralization	30	(Rousseaux et al. 2003)
Recombinant *E. Coli* bred to express atzA+natural consortia	Phosphate	6700 ppm	77%	mineralization	56	(Strong et al. 2000)
Pseudomonas sp. ADP	Citrate and Phosphate	10 ppm	78%	mineralization	15	(Shapir et al. 1998)

Table 2.5 Continued: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	Electron Donor	Initial Atrazine Concentration	Percent Mineralization or Degradation		Time *(days)*	Source
Arthrobacter sp. AG1+*Arthrobacter keyseri* 12B+*Ochrobactrum* sp. + *Pseudomonas* sp.	Citrate	500 mg/L	78%	mineralization	6	(Kolic et al. 2007)
Pseudomonas sp. ADP + natural consortia	Citrate	337.4 µg/g	80%	mineralization	18	(Silva et al. 2004)
Natural consortia	Phosphate	17100 ppm	84%	mineralization	35	(Strong et al. 2000)
Clavibacter michiganese, *Pseudomonas* sp., and *Cytophaga* sp.		3 mg/kg soil	84%	mineralization	28	(Alvey and Crowley 1996)
Pseudomonas sp. ADP	Citrate	200,000 g/hectare	87%	mineralization	7	(Lima et al. 2009)
Pseudomonas sp. ADP		100 mg/kg	90%	mineralization	35	(Newcombe and Crowley 1999)
Pseudomonas sp. ADP	Citrate	200,000 g/hectare	98%	mineralization	7	(Lima et al. 2009)
Pseudomonas sp. ADP	Glucose	10 µg/L	0%	degradation	108	(Pearson et al. 2006)
Pseudomonas sp. ADP	Glucose	10 µg/L	0%	degradation	108	(Pearson et al. 2006)
Natural consortia	Glucose	10 µg/L	0%	degradation	108	(Pearson et al. 2006)
Natural consortia	Glucose	10 µg/L	0%	degradation	108	(Pearson et al. 2006)

Table 2.5 Continued: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	**Electron Donor**	**Initial Atrazine Concentration**	**Percent Mineralization or Degradation**		**Time** *(days)*	**Source**
Consortia		20 µg/g	0.02%	degradation	30	(Goswami and Green 1971)
Pseudomonas sp. ADP		1500 ppm	17%	degradation	21	(Mandelbaum et al. 1995)
Pseudomonas sp. ADP	Citrate and Phosphate	12.5 mg/L	18%	degradation	103	(Katz et al. 2001)
Pseudomonas sp. ADP	Citrate and Phosphate	0.1 mg/L	18%	degradation	103	(Katz et al. 2001)
Consortia from Sludge	Dextrose	5 mg/L	45%	degradation	5	(Ghosh and Phillip 2004)
Natural consortia	Acetic Acid	10 mg/L	50%	degradation	164	(Chung et al. 1996)
Natural consortia		100 ppm	50%	degradation	1.25	(Mandelbaum et al. 1993)
Nocardioides sp. strain C190+natural consortia		10 mg/L medium	50%	degradation	3	(Topp 2001)
Pseudaminobacter strains (C147 or C195) +natural consortia		10 mg/L medium	50%	degradation	5	(Topp 2001)
M91-3		21.6 mg/L	50%	degradation	6	(Crawford et al.1998)
Pseudomonas sp. ADP + natural consortia		10 mg/L medium	50%	degradation	10	(Topp 2001)
Natural consortia		3.2 kg/hectare	50%	degradation	14	(Somasundaram and Coats 1990)

Table 2.5 Continued: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	Electron Donor	Initial Atrazine Concentration	Percent Mineralization or Degradation		Time *(days)*	Source
Natural consortia		2.2 kg/hectare	50%	degradation	21	(Somasundaram and Coats 1990)
Natural consortia		12500 µg/kg	50%	degradation	38	(Seybold et al. 2001)
Natural consortia		718 µg/kg	50%	degradation	86	(Seybold et al. 2001)
Natural consortia		5 µg/g	50%	degradation	87	(Kruger et al. 1993)
Natural consortia		5 µg/g	50%	degradation	87	(Kruger et al. 1993)
Natural consortia + plasmid containing atzA		20 mg/L	90%	degradation	0.0625	(Perumbakkam et al. 2006)
Acinetobacter sp. strain BD413+plasmid containing atzA		20 mg/L	90%	degradation	0.0625	(Perumbakkam et al. 2006)
Pseudomonas sp. ADP	Citrate	22 mg/kg soil	98%	degradation	42	(Chelinho et al. 2010)
Pseudomonas sp. ADP	Citrate	44 mg/kg soil	99%	degradation	42	(Chelinho et al. 2010)
Pseudomonas sp. ADP	Citrate and Phosphate	12.5 mg/L	100%	degradation	30	(Katz et al. 2001)
Pseudomonas sp. ADP	Citrate and Phosphate	0.1 mg/L	100%	degradation	30	(Katz et al. 2001)

Table 2.5 Continued: Comparison of bacteria that have been used to remediate atrazine

Bacteria Name	**Electron Donor**	**Initial Atrazine Concentration**	**Percent Mineralization or Degradation**		**Time** *(days)*	**Source**
Agrobacterium tumefaciens, Caulobacter crescentus, Pseudomonas putida, sphingomonas yaniokuyae, Nocardiodes sp., *Rhizobium* sp., *Flavobacterium oryzihabitans, Variovorax paradoxus*	Glucose	250 µg/g	100%	degradation	4	(Smith et al. 2005)

2.5 Conclusions

This review highlighted some of the literature surrounding atrazine remediation. Physical methods, such as adsorption on soil, activated carbon, or other natural materials can be effective if competition for active sites can be kept to a minimum. Biological methods, a relatively new technique, can be fast and effective if the conditions are favorable. The continued persistence of atrazine and its metabolites in the environment as well as ever-changing regulations will continue to require creative solutions and more accurate laboratory simulations in the future.

CHAPTER 3: EVALUATION OF MULCH PROPERTIES FOR ATRAZINE CONTAMINATED GROUNDWATER REMEDIATION

3.1 Background

Atrazine is a popular broadleaf herbicide, typically used on corn (Solomon et al. 1996). Atrazine has a moderate solubility in water (34.7 mg atrazine/L at 25˚C), and a slow biodegradation rate (Faur et al. 2005). After application, atrazine can slowly infiltrate through soil to groundwater (Ma and Selim 1996; Gu et al. 2003). In 2010, the Nebraska Department of Environmental Quality found that 5% of groundwater samples exceeded the reporting limit for atrazine (Nebraska Department of Environmental Quality, 2010).

The maximum contaminant level (MCL) for atrazine in drinking water is 3 μg/L, as set by the EPA (Wilber et al. 1995). The European Union has not only set the MCL at 0.1 μg/L, but has also banned the use of atrazine due to its persistence in the environment (Wilber et al. 1995; Faur et al. 2005; Sass and Colangelo 2006; Zadaka et al. 2009). Atrazine was the most frequently detected pesticide (53%) in of water samples from vernal pools in protected areas in the United States (Battaglin et al. 2008). Exposure to atrazine causes endocrine disruption in frogs, rats, and humans (U.S. EPA Office of Pesticide Programs 1993, 2002; Villanueva et al. 2005).

Indigenous soil bacteria, when exposed to atrazine over long periods of time, may gradually increase their capacity to degrade it. Specific strains have been isolated and characterized from indigenous consortia and reapplied for remediation via bioaugmentation (Mandelbaum et al. 1995; Alvey and Crowly 1996; Newcombe and Crowley 1999; Silva et al. 2004; Smith et al. 2005; Kolic et al. 2007; Lima et al. 2009).

Unfortunately, survival rates of isolated strains of bacteria in the field are low, making multiple reapplications necessary (Silva et al. 2004; Newcombe and Crowly 1999; Sturman et al. 1995).

Bacteria supported on a substrate, or a biowall, avoids the hassle of re-application rates and is better able to endure changes in operating conditions (vanVeen et al. 1997). *In situ* treatments like these can be placed to intercept the contaminant plume to prevent the spread of further contamination. Biowalls consist of bacteria supported on a natural substrate, placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. This treatment is inexpensive when compared to pump and treat methods, especially when the supporting substrate is cheap and abundant, like mulch (Kao et al. 2001; Schipper et al. 2004; Seo et al. 2007). Substrates made of natural materials have been used as a supporting material for biowalls to remove naphthalene (Seo et al. 2007) and tetrachloroethylene (Kao et al. 2001), but rarely for atrazine. Ilhan et al. examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods (Ilhan et al. 2011).

Atrazine and nitrate often coexist in groundwater (Ritter 1990). In 2010, the Nebraska Department of Environmental Quality found that 94% of groundwater samples exceeded the reporting limit for nitrate (Nebraska Department of Environmental Quality, 2010). The MCL for nitrate is 10 mg NO_3-N/L ("Basic Information" 2012). Co-removal of atrazine and nitrate, often desired in agricultural states like Nebraska, is possible with

biological treatment if a carbon source is provided. This carbon source can be added separately, or could be part of the biowall itself (Schipper et al. 2004).

3.2 Objective

The objectives of this study were twofold. The first objective was to identify the physical and chemical properties of three types of common gardening mulch. The second objective was to characterize the adsorptive capacity of the mulch for atrazine and nitrate with a series of isotherm experiments.

3.3 Materials and Methods

3.3.1 Organic Mulch

Three types of common gardening mulch were selected as possible substrates for biofilm support: cedar, cypress, and hardwood. The mulch was purchased from a local gardening supplies store, Earl May (Lincoln, NE, USA).

Mulch samples were prepared using the method reported by Seo et al. (Seo et al. 2007), with modifications. Mulch samples were dried in a fume hood overnight, ground for 30 seconds using a Black and Decker food processor for 30 seconds, and sieved to a #10 mesh size (2 mm). To remove the influence of natural bacteria and fungi, the mulch samples were autoclaved twice before they were finally dried in a 105°C oven.

3.3.2 Physical and Chemical Properties of the Mulch

The pH, conductivity, water content, organic content, and cation exchange capacity (CEC) of each mulch was measured to determine the best kind of mulch to use as a supporting material for a biowall. The pH, conductivity, and organic content (Loss-On-Ignition method) were determined using techniques from the Methods of Soil

Analysis (Klute et al. 1994; Weaver et al. 1994). The pH and conductivity measurements were taken after 10 grams of mulch sample mixed with 100 mL of nanopure water for 10 minutes. The organic content (Loss-On-Ignition method) measurements were performed using a weight difference in mulch samples before and after ignition in a muffle furnace (550°C).

The water content was determined by taking the difference in weights between unprepared mulch and after drying it in a 105°C oven overnight.

The values for organic content (High Range COD and Low Range COD) were found using the reactor digestion method with high and low range COD digestion vials, respectively, available from HACH chemicals (Loveland, CO, USA).

The Cation Exchange Capacity (CEC) was determined using a modification of the barium chloride method (Ross 1995; Ciesielski and Sterckeman 1997), wherein the concentrations of barium and magnesium ions were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS), respectively, rather than by accurate weighing. The barium chloride and the magnesium sulfate were purchased from Fisher Scientific (Waltham, MA, USA). In this method, the adsorbed barium exchanges with magnesium and precipitates as $BaSO_4$. Briefly, 2.5 g of mulch sample was shaken with 30 mL of a 0.1 M $BaCl_2$ solution for one hour. The supernatant was collected and analyzed by ICP-MS. The mulch samples were equilibrated with 30 mL of 2 mM $BaCl_2$. Lastly, the mulch samples were shaken with 0.02M $MgSO_4$ for two hours.

Each datum point represents an average of three samples. The mulch properties were compared with SigmaPlot 12 (Systat Software, San Jose, CA, USA) using a one-way analysis of variance (ANOVA) with a significance level of α=0.05.

3.3.3 Isotherm Experiments

The adsorptive capacity of mulch for removal of atrazine, nitrate, and both atrazine and nitrate was quantified by conducting isotherm experiments. Sodium nitrate was purchased from Fisher Scientific (Waltham, MA, USA). Atrazine was purchased from Chemservice (West Chester, PA, USA). Amber glass bottles with Teflon caps were used to minimize the effect of light on the samples. The bottles were filled with varying weights of mulch and filled to the rim with selected concentrations of solution to avoid any headspace (Environmental Protection Agency 1992). The bottles were then sealed with Parafilm, capped with a Teflon cap, and again sealed with Parafilm. Control tests were conducted to ensure that the mulch samples did not contain atrazine or nitrate and atrazine or nitrate were not adhering either to the glass container or to the filter paper.

Weights and concentrations were selected so that the final concentration of a chemical in solution would be either less than 90% or greater than 10% of the initial solution concentration. The mono adsorbate data were obtained for concentration ranges of 0.5-20 mg NO_3-N/L and 5-10 mg atrazine/L. The nitrate adsorption data were obtained using two concentration ranges, 0.5-3.4 mg NO_3-N/L and 0.5-20 mg NO_3-N/L. The lower range reflects the median background concentration of nitrate in Nebraska Groundwater in 2009, 4.7 mg NO_3-N/L ("Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater" 2011).

Binary adsorbate data were obtained for two concentrations: 7 and 3.5 mg NO_3-N/L, paired with 5 and 2.5 mg atrazine/L, respectively. The nitrate concentration was selected based on the average background concentration of nitrate in Nebraska groundwater in 2009, 7.8 mg NO_3-N/L ("Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater" 2011).

The initial atrazine concentration in solution for both the mono and binary adsorbate isotherms is more than a thousand times higher than typical background concentrations in groundwater, which are typically less than 1.5 µg atrazine/L (Nebraska Department of Environmental Quality, 2010). Higher concentrations were used to increase the concentration gradient and ensure more reliable adsorption data.

The bottles were tumbled at 18 rpm for 5 days. The equilibrium time was selected based on a literature review (Seo et al. 2007). After 5 days, the solutions were filtered with a Millipore filtration apparatus using a Whatman GF/A filter (Fisher Scientific, Waltham, MA, USA) to remove particulate matter, and concentrations of the chemicals were determined, as described in Section 3.2.4. The adsorption capacity of nitrate and atrazine were calculated from the Langmuir and Freundlich data.

The equation for the Langmuir isotherm is given as

$$\frac{1}{q_e} = \frac{1+K_L C_e}{q_{max}\, K_L C_e} \tag{1}$$

or, in a linearized form,

$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{C_e}{q_{max}} \tag{2}$$

where q_e is the amount of adsorbed compound on the mulch at equilibrium (mg/kg), q_{max} is the maximum adsorption capacity (mg/kg), K_L is the Langmuir constant (L/mg), and C_e is the concentration of compound in solution at equilibrium (mg/L) (Tchobanoglous, et al. 2003).

The equation for the Freundlich isotherm is given as

$$q_e = K_F C_e^{1/n} \quad (3)$$

or, in a linearized form,

$$\log(q_e) = \log(K_F) + \frac{1}{n}\log(C_e) \quad (4)$$

where K_F ((mg/kg)(mg/L)$^{-n}$) and n are constants representing sorption capacity and intensity, respectively (Tchobanoglous, et al. 2003).

Data were discarded if the equilibrium concentration in solution was greater than 90% or less than 10% of the initial solution concentration to ensure that the adsorption quantity was accurate. A 95% confidence interval was used by Sigma Plot 12 (Systat Software, San Jose, CA, USA) to test for possible outliers before fitting to adsorption models. The ratio of q_e/C_e and the average value of isotherm coefficients were compared

between data sets using a one way analysis of variance (ANOVA) with a significance level of 0.05 in SigmaPlot 12.

3.3.4 Instrumental Analysis

Nitrate was analyzed using a Dionex Ion Chromatograph (ICS-90) (Sunnyvale, CA, USA) with a Dionex AS40 Autosampler. Sample size was 5 mL. The column was a 4x250mm IonPac AS14. An isocratic mobile phase consisted of 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$. The data were analyzed with Chromeleon v. 6.7, Build 1820.

Atrazine was analyzed using a Waters Alliance 2695 High-Performance Liquid Chromatograph (HPLC) (Milford, MA, USA) connected to a Waters 2996 Photodiode Array (PDA) detector. The sample size was 25 μL and was injected at 1 mL/min. The mobile phase was a gradient of water and methanol, as shown in Figure 3.1. The column type was Kromasil 100-5C8 and was 4.6 m in length and 250 mm in diameter. The column temperature was 50°C. The detector wavelength was set at 222 nm. The data were analyzed with Waters Empower Software Build #1154.

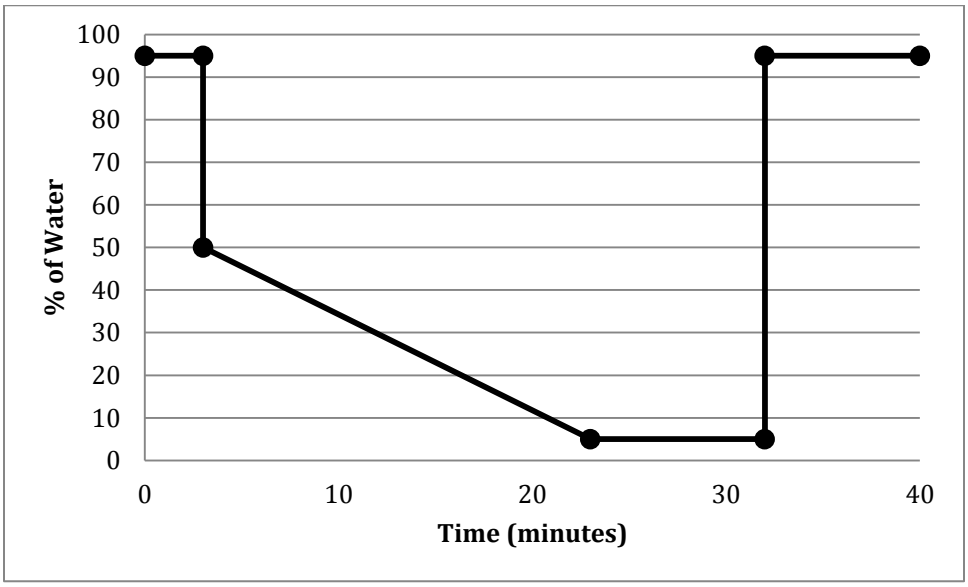

Figure 3.1: High-performance liquid chromatography gradient for atrazine analysis

3.4 Results

3.4.1 Physical and Chemical Properties of the Mulch

The pH, conductivity, water content, organic content, and cation exchange capacity (CEC) were measured to determine the best kind of mulch to use as a supporting material for a biowall (Table 3.1). Each data point represents an average of three samples.

Table 3.1: Physical and chemical properties of cedar, cypress, and hardwood mulch. Superscripts indicate pairings of statistical significance.

	Cedar	**Cypress**	**Hardwood**
pH	6.73 ± 0.73^{a}	5.32 ± 0.02^{a}	5.98 ± 0.51
Conductivity (μS/cm)	129.1 ± 25.3	99.2 ± 6.5	105.8 ± 17.1
Water content (%)	44.89 ± 5.14	53.82 ± 3.98	40.42 ± 7.68
Organic content (mg/g)			
Loss-On-Ignition	976.95 ± 2.52^{b}	996.35 ± 2.16^{b}	984.17 ± 0.9^{b}
Low Range COD	1186.67 ± 323.93^{c}	$2000 \pm 144.22^{c,d}$	1253.33 ± 106.92^{d}
High Range COD	1246.73 ± 118.34	1301.33 ± 370.91	1005.08 ± 2.8
Cation Exchange Capacity (meq/100g)	7.97 ± 1.45	6.73 ± 0.68	7.23 ± 1.25

Loss-on-Ignition was the only property measured that showed a statistically significant difference between all three types of mulch. The pH difference between cedar and cypress mulch is statistically significant and the Low Range COD difference between the pairs of cedar-cypress and cypress-hardwood is statistically significant. More accurate measuring equipment or methods could be used to verify these statistics in the future.

Qualitatively, among the three types of mulch, cedar mulch has the highest pH, conductivity, and CEC, whereas cypress mulch has the lowest values. Seo et al. (2009) demonstrated a similar pattern: mulches with a high conductivity also exhibited a high CEC. This association can be expected, because CEC is a measure of the ability of the material to exchange cations and electrical conductivity is a measure of the ion content of a solution (Henry 1997).

Henry (1997) reported that CEC was inversely proportional to water content. This is shown in Table 3.1; qualitatively, cypress has the highest water content and the lowest

CEC. Research by the Virginia Extension Board concluded the inverse: High CEC was linked to high organic and water content in clay soils (Grisso et al. 2009). This is most likely because mulch has different properties than clay soil. Further investigation is needed to verify this.

Surface area measurements were beyond the scope of this limited project. However, the surface areas of the cypress and hardwood mulch were assumed to be similar to results from Seo et al. (2009). The authors performed a Brunauer, Emmett, and Teller (BET) isotherm revealing that cypress and hardwood mulch had a surface area of 11-18 m^2/g and 25-32 m^2/g), respectively.

3.4.2 Isotherm Experiments: Mono Systems

Isotherm experiments were performed to quantify the adsorptive capacity of cedar, cypress, and hardwood mulch for the mono systems of atrazine and nitrate, as well as the binary system of atrazine-nitrate. The raw data for the mono-system isotherms for atrazine and nitrate are given in Appendices A and B, respectively.

The raw isotherm data were graphed in Appendices A and B for atrazine and nitrate, respectively, to determine which of the Giles isotherm types would best describe the data (Giles et al. 1974a). The atrazine figures, from Appendix A, are linear, thus appearing to show that atrazine is exhibiting C-type, or constant partitioning, adsorption. However, Calvet (1989) suggests that an L-type, or Langmuir adsorption, better describes atrazine adsorption. The atrazine isotherm appears to be a C-type, likely due to the range of concentrations examined; a wider range of atrazine concentrations may show a more distinct L-type isotherm.

The raw isotherm data for nitrate, as seen in Appendix B, do not show a distinct isotherm type. Nitrate should be exhibiting a H-type, or high affinity, adsorption (Giles et al. 1974b). Nitrate is a negatively charged ion that has a high affinity for positively charged sites on the surface of the mulch. However, organic materials generally don't have a high number of positive sites. The number of positive sites can be increased with surface treatments, such as acidification (Cays-Vesterby 2009).

Atrazine adsorption from the aqueous phase was correlated using the Langmuir isotherm, as shown in Equation 2, and the Freundlich isotherm, as shown in Equation 4. The mono atrazine isotherm results are shown in Table 3.2. The corresponding figures for atrazine can be found in Appendix C. Nitrate adsorption from the aqueous phase was also correlated using the Langmuir and Freundlich isotherms. The isotherm results for nitrate are shown in Table 3.3 for all concentrations, and in Table 3.4 for C_o<3.4 mg NO_3-N/L. The corresponding figures for nitrate for all concentrations and for C_o<3.4 mg NO_3-N/L can be found in Appendices D and E, respectively. Based on the R^2 values, Tables 3.2, 3.3, and 3.4 show that the Freundlich isotherm best describes atrazine adsorption and the Langmuir isotherm best describes nitrate adsorption.

Table 3.2: Langmuir and Freundlich constants for mono atrazine isotherm

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	-1.79	-0.03	0.30	11	0.79	0.85	0.91	12
Cypress	1.16	0.06	0.16	12	0.05	1.04	0.94	12
Hardwood	-0.65	-0.06	0.73	9	0.07	0.84	0.94	11

Table 3.3: Langmuir and Freundlich constants for mono nitrate isotherm

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.12	-4.53	0.92	12	-0.02	2.45	0.87	8
Cypress	0.18	2.11	0.90	22	-0.001	1.47	0.02	8
Hardwood	0.13	-1.94	0.98	17	-0.01	1.93	0.62	11

Table 3.4: Langmuir and Freundlich constants for mono nitrate isotherm for C_o<3.4 mg NO_3-N/L

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.34	-5.53	0.86	9	-0.10	3.73	0.14	7
Cypress	0.06	-3.55	0.81	17	-0.03	1.54	0.09	8
Hardwood	0.17	-5.86	0.79	12	-0.04	1.99	0.12	9

A statistical analysis of the ratio of the equilibrium concentration of a compound on the mulch and the equilibrium concentration of a compound in solution (q_e/C_e) was performed for each system using a one way ANOVA. The analysis revealed that there was no significant difference between the three types of mulch for either atrazine or nitrate adsorption. There was not a significant difference between the q_e/C_e ratio for the entire nitrate range and the small (C_o<3.4 mg NO_3-N/L) nitrate range. A statistical analysis comparing the average values of coefficients for all nitrate concentrations and for C_o<3.4 mg NO_3-N/L found the difference was not significant, for either the Freundlich or Langmuir isotherms.

Qualitatively, Tables 3.2 and 3.3 show that cypress mulch has the highest adsorption capacity for nitrate, 0.18 mg/g (q_{max}), and atrazine, 1.04 (mg/g)(mg/L)$^{-n}$ (K_F), while cedar and hardwood have lesser, similar values. At low concentrations of nitrate, as in Table 3.4, cedar mulch has the highest adsorption capacity for nitrate, 0.06 mg/g (q_{max}). The sorption capacity of atrazine on cedar and hardwood mulch, 0.8 (mg/g)(mg/L)$^{-n}$, was the same as the value found by Alam et al. (2000) for adsorption of atrazine on wood charcoal.

3.4.3 Isotherm Experiments: Binary Systems

The binary system of atrazine-nitrate was modeled similarly to the mono systems, using both Freundlich and Langmuir isotherms. The raw binary data for atrazine and nitrate can be seen in Appendices F and G, respectively.

The raw isotherm data were graphed in Appendices F and G for atrazine and nitrate, respectively, to determine which of the Giles isotherm types would best describe

the data (Giles et al. 1974a). Figures in Appendix F show that atrazine is still exhibiting linear adsorption, approximating the L-type, even in the presence of nitrate. Nitrate figures in Appendix G are nearly vertical, showing that nitrate adsorption is highly dependent on surface property variations of the mulch.

The binary atrazine results are shown in Table 3.5. The corresponding figures for atrazine can be seen in Appendix H. Binary nitrate isotherm results with initial concentrations of 7 and 3.5 mg NO_3-N/L can be seen in Tables 3.6 and 3.7, respectively. The corresponding figures for nitrate, C_o=7 and 3.5 mg NO_3-N/L, can be found in Appendices J and K, respectively. Based on the R^2 values, Tables 3.5, 3.6, and 3.7 show that the Freundlich isotherm continues to best describe atrazine adsorption, whereas neither the Langmuir nor the Freundlich isotherm best describes nitrate adsorption for all much types.

Table 3.5: Langmuir and Freundlich constants for atrazine in the binary isotherm

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.45	0.24	0.58	7	0.06	1.08	0.92	10
Cypress	0.32	0.40	0.65	11	0.05	1.09	0.83	11
Hardwood	1.38	0.05	0.16	10	0.07	1.00	0.94	10

Table 3.6: Langmuir and Freundlich constants for nitrate in the binary isotherm with an initial concentration of 7 mg NO_3-N/L

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.03	-0.32	0.02	5	0.07	0.56	0.04	5
Cypress	0.01	-0.23	0.89	5	-0.29	34.5	0.82	5
Hardwood	0.006	-0.21	0.85	6	-0.23	24.45	0.79	5

Table 3.7: Langmuir and Freundlich constants for nitrate in the binary isotherm with an initial concentration of 3.5 mg NO_3-N/L

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.01	-0.47	0.74	7	-0.30	7.04	0.96	6
Cypress	0.03	-0.87	0.36	6	-0.02	1.28	0.33	6
Hardwood	0.004	-0.41	0.94	5	-0.82	203.66	0.99	6

A statistical analysis of the ratio of equilibrium concentration of a compound on the mulch and the equilibrium concentration of a compound in solution (q_e/C_e) was performed for each system using a one way ANOVA. The analysis revealed that the only significant difference between mulch types was during binary atrazine adsorption for the pairs of cypress-hardwood and cedar-hardwood. There was not a significant difference between the three types of mulch in the binary nitrate systems.

The differences in the q_e/C_e ratio between the mono and binary atrazine isotherms were significant for all systems. Also, the q_e/C_e ratio between the small concentration (C_o<3.4 mg NO_3-N/L) mono and the entire nitrate isotherm were significantly different from both binary nitrate systems, 3.5 and 7 mg NO_3-N/L.

In a binary system, the compound with lower solubility, atrazine, is favored for adsorption and that the co-adsorbate, nitrate, in this case, has no influence on adsorption (Faur et al. 2005). This relationship can be seen graphically in Figure 3.2. A similar comparison was done by Faur et al. (2005) with atrazine and deethylatrazine or deisopropylatrazine. When binary and mono systems for atrazine adsorption on cypress mulch are graphed together, the slope is nearly the same, as seen in Equations 5 and 6. Figures for cedar and hardwood mulch can be seen in Appendix I.

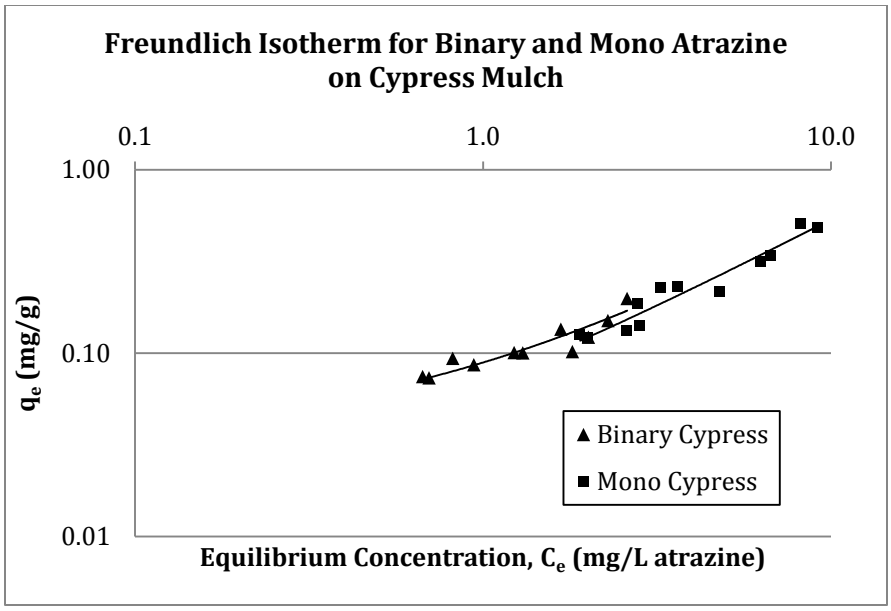

Figure 3.2: Freundlich adsorption isotherm for atrazine on cypress mulch in the presence and absence of nitrate

The equations for the best fit lines in Figure 3.2 are as follows:

In the mono cypress (absence of nitrate), the equation is:

$$\log(q_e) = 0.0517\log(C_e) + 0.0184 \quad (5)$$

where q_e is the amount of atrazine adsorbed on the mulch at equilibrium (mg/g) and C_e is the concentration of atrazine in solution at equilibrium (mg/L). This equation has an R^2 of 0.94.

In the binary cypress (presence of nitrate), the equation is:

$$\log(q_e) = 0.0511\log(C_e) + 0.0378 \quad (6)$$

This equation has an R^2 of 0.83.

A statistical analysis comparing the average values of the Langmuir and Freundlich coefficients for atrazine in the mono and binary systems (Tables 3.2 and 3.5) indicated that they are not significantly different.

Qualitatively, as in the mono system for atrazine, cypress mulch has the highest adsorption capacity, 1.09 $(mg/g)(mg/L)^{-n}$ (K_F). Also, cypress mulch has the lowest value of sorption intensity (1/n) for atrazine in both the mono and binary systems. For low values of 1/n, less energy is required for the adsorbate to adsorb on the surface; this low energy barrier results in faster adsorption (American Water Works Association 1999; Hristovski et al. 2009).

The binary nitrate isotherms were obtained for two initial concentrations: 7 and 3.5 mg NO_3-N/L, paired with concentrations of 5 and 2.5 mg atrazine/L, respectively. The Langmuir and Freundlich isotherm data can be seen in Tables 3.6 and 3.7 for 7 and 3.5 mg NO_3-N/L, respectively. A statistical analysis comparing the ratio of q_e/C_e between the 7 mg NO_3-N/L system and the 3.5 mg NO_3-N/L system revealed that the apparent differences were not statistically significant. Additionally, a statistical analysis comparing the average values of the coefficients for the two concentrations revealed that they were not significant for the Langmuir or the Freundlich isotherm.

Comparison of the average values of the Langmuir coefficients for nitrate in the mono and binary systems (Tables 3.3, 3.4, 3.6, and 3.7) revealed a significant difference in maximum adsorption capacity (q_{max}), but not the Langmuir constant (K_L), when the entire mono nitrate system is compared to both binary systems. The reverse is true when the small (C_o<3.4 mg NO_3-N/L) mono system is compared to both binary systems: the

maximum adsorption capacity (q_{max}) is not significantly different, but the Langmuir constant (K_L) is different. The Langmuir constant is related to the energy of adsorption and is proportional to the adsorption bond (American Water Works Association 1999). Therefore, the presence of atrazine affects the capacity for nitrate adsorption at high concentrations of nitrate, but at low concentrations of nitrate, the energy of nitrate adsorption is affected. A statistical analysis comparing the average values of the Freundlich coefficients for nitrate in the mono and binary systems systems (Tables 3.3, 3.4, 3.6, and 3.7) revealed that there was no significant difference between coefficients.

Graphically, as seen in Appendices J and K, both the Langmuir and Freundlich isotherms for binary nitrate are nearly vertical, regardless of starting concentration. In contrast, the mono nitrate behavior, as seen in Appendices D and E, exhibits smaller slopes at low concentrations for the Freundlich isotherm, and linear behavior for the Langmuir isotherms. This change of adsorption behavior in the presence of atrazine implies that atrazine is affecting nitrate adsorption. The differences between the binary and mono systems are most apparent in the Freundlich isotherm when binary and mono data are graphed together (Appendix L). This sudden increase in slope may have been caused by cation effects (Fawcett and Sellan 1977), variations in surface organic functional groups (Laird et al. 1994), other surface composition differences between samples or, most likely, the blocking of desirable nitrate sites with atrazine.

Qualitatively, the Langmuir isotherms for nitrate in the binary system reveal that, at the lower concentration (3.5 mg NO_3-N/L), the cypress mulch had the highest adsorption capacity (q_{max}), 0.03 mg/g. However, at the higher concentration (7 mg NO_3-N/L) the cedar mulch had the highest adsorption capacity (q_{max}), 0.03 mg/g. In both

cases, the hardwood mulch had the lowest adsorption capacity (q_{max}), 0.004 mg/g for 3.5 mg NO_3-N/L and 0.006 mg/g for 7 mg NO_3-N /L. Qualitatively, the Freundlich isotherms for nitrate in the binary system suggest that cypress has the highest adsorption capacity (K_F) for nitrate at 7 mg NO_3-N/L, 34.5 $(mg/g)(mg/L)^{-n}$. However, hardwood has the highest adsorption capacity (K_F) for nitrate at 3.5 mg NO_3-N/L, 203.66 $(mg/g)(mg/L)^{-n}$.

3.5 Conclusions

The mulch characterization tests (Table 3.1) showed qualitatively that cypress had the lowest electrical conductivity of the three types of mulch. Electrical conductivity changes the charge of the surface and competes for available surface sites, thereby influencing adsorption (Xu et al. 2009).In contrast, Henry (1997) states that higher conductivity is a result of migration of ions into solution, thus, freeing sites for adsorption (Henry 1997). However, this claim was based on research of the adsorption of seawater into clay soils, not herbicides and soils, as in Xu et al. (2009). Further investigation of the relationships between organic content, water content, CEC, conductivity, and adsorption in different materials would enhance future adsorption research.

The mulch characterization tests also showed that cypress mulch had significantly higher organic carbon content than other types of mulch. Atrazine adsorbs rapidly and preferentially to materials with a higher organic content (Ma and Selim 1996; Masaphy and Mandelbaum 1997; Xu et al. 2009). Therefore, cypress mulch, with the lowest electrical conductivity and high organic carbon content, should have the highest sorption capacity.

Although the isotherm experiments showed that the differences in the ratio of q_e/C_e among the three types of mulch were not significant, qualitatively, cypress exhibited the highest adsorption capacity for atrazine. For atrazine systems, cypress had the lowest 1/n value and the highest K_F value in the Freundlich isotherms, showing that it rapidly adsorbs atrazine and has a high capacity for the adsorption of atrazine, respectively. In most nitrate systems, a Langmuir isotherm analysis showed that cypress had the highest q_{max} value, meaning that it had a comparatively higher sorption capacity for nitrate.

Atrazine adsorption exhibited what appeared to be a C-type isotherm, however, a literature review revealed that, in general, the L-type better describes atrazine adsorption (Calvet 1989). Atrazine adsorption continued to exhibit L-type isotherms in the binary system, and was not affected by the presence of nitrate. Based on Giles et al. (1974b), nitrate adsorption was expected to show H-type isotherm behavior. However, nitrate adsorption did not exhibit a specific isotherm type because it was highly affected by surface properties of the mulch and the presence of atrazine.

Although the isotherm data shows that cypress is the best choice for atrazine adsorption, it does not equal the sorption capacity of activated carbon. Adams et al. (1996) found K_F and 1/n values of 467 $(mg/g)(L/mg)^{1/n}$ and 0.44 for Calgon F-200 activated carbon, which are 450 times that of the mulch, making activated carbon a more economical choice. However, this comparison is based on adsorption data alone, without the influence of a bacterial biofilm, as would be present in actual implementation of a mulch-based biowall for atrazine removal.

Based on its physical and chemical properties and its capacity for adsorption of atrazine and nitrate in mono and binary systems, cypress mulch was determined to be the best substrate to support bacterial growth in a biowall experiment.

CHAPTER 4: ATRAZINE CONTAMINATED GROUNDWATER REMEDIATION WITH A MULCH BIOWALL

4.1 Background

The extensive use of the broadleaf herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and its persistence in soil and groundwater is a worldwide concern. Atrazine and nitrate are often found together in groundwater of agricultural states (Ritter 1990).

Ongoing research on remediation techniques for pesticide contamination includes chemical and biological treatment processes. Waria et al. (2009) used zero valent iron and ferrous sulfate to degrade atrazine chemically in soil. Soybean oil was also added to provide a carbon source for biological activity. Atrazine, initially at a concentration of 500 mg/kg soil, was reduced by 79% in 342 days. Tafoya-Garnica et al. (2009) used a fluidized bed reactor containing biological granular activated carbon to achieve high degradation rates. Modin et al. (2008) used a methane-fed bioreactor intended to remove both atrazine and nitrate. However, atrazine removal was not successful. Bianchi et al. (2006) successfully used photolysis, photocatalysis (with TiO_2), and ozonation for atrazine degradation. Processes such as these require the presence of a nutrient source, such as methane or soybean oil, and specialized treatment, such as ultraviolet radiation or biological activated carbon. These additions may be worthwhile for short time periods, but would be costly over time, due to materials and operations costs.

Passive treatments are much more cost effective and require little specialized equipment. A permeable reactive barrier, or biowall, can be placed to intercept a contaminated groundwater plume, forming a bioreactive zone. Biowalls consist of

bacteria supported on a natural substrate, placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. Biowalls are typically made of cheap, abundant materials that perform remediation using a combination of bacterial growth and adsorption. *In situ* treatments such as these are low maintenance and can endure changes in operating conditions (Kao et al. 2001; Kalin 2004; Seo et al. 2007).

Biowalls have been tested extensively for denitrification. They can be placed either directly in aquifers or in vadose zones above aquifers (Kao et al. 2001; Kalin 2004). They can also be used to treat water from subsurface tile drainage (Ilhan et al. 2011). Denitrification requires a carbon source, which can be obtained from the organic content of the barrier itself, or added separately, as in Hunter (Schipper et al. 2004; Hunter 2009).

Biowalls supported on a natural substrate, such as mulch or peat moss, have been studied for naphthalene (Seo et al. 2007) and tetrachloroethylene (Kao et al. 2001) removal, but rarely for atrazine. Ilhan et al. examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods (Ilhan et al. 2011).

4.2 Objective

The objective of this research was to examine the feasibility of implementing a cypress mulch biowall with a laboratory-scale biotic column designed to remove atrazine and nitrate. It was anticipated that the cypress mulch, which is inexpensive and available

in Nebraska, would act both as a supporting material and a carbon source for denitrification.

4.3 Materials and Methods

4.3.1 Organic Mulch

Cypress mulch was selected as the supporting substrate for the biowall, based on physical and chemical analysis in comparison with cedar and hardwood mulch, as well as isotherm experiments, as described in Section 3. Physical and chemical properties of cypress mulch include a pH of 5.32±0.02, an electrical conductivity of 99.2±6.5µS/cm, a water content of 53.82±3.98%, and a cation exchange capacity of 6.73±0.68 meq/100g.

Cypress mulch was prepared using a modification of the method of Seo et al. (2007), as described in Section 3.3.1.

4.3.2 Isotherm Experiments

The adsorptive capacity of the mulch for removal of atrazine and nitrate was quantified with isotherm experiments, as described in Section 3. Various weights of mulch were paired with various concentrations of atrazine and nitrate, placed in brown glass bottles, and tumbled at 18 rpm for 5 days. Adsorption data was fitted to Freundlich and Langmuir isotherms. Qualitatively, for atrazine adsorption in the binary system, cypress mulch had the lowest 1/n value, 0.05, and the highest K_F value, 1.09 (mg/g)(mg/L)-n, meaning that it rapidly adsorbs atrazine and has a relatively high capacity for the adsorption of atrazine, respectively. Qualitatively, for nitrate adsorption in the binary system, cypress mulch had the highest q_{max} value, 0.03 mg/g, meaning that it has a relatively high capacity for nitrate adsorption.

4.3.3 Column Set Up

A laboratory-scale biotic column was used to simulate implementation of a biowall. The column and clamps were purchased from Custom Glassblowing of Louisville, Kentucky, USA and was the same model used by Seo et al. (2007). Figure 4.1 shows a schematic of the column set up. The column is 30 cm in length and has a 3.8 cm inner diameter. The body of the column has five sample ports and there are two additional ports on each of the end caps, for a total of seven sample ports.

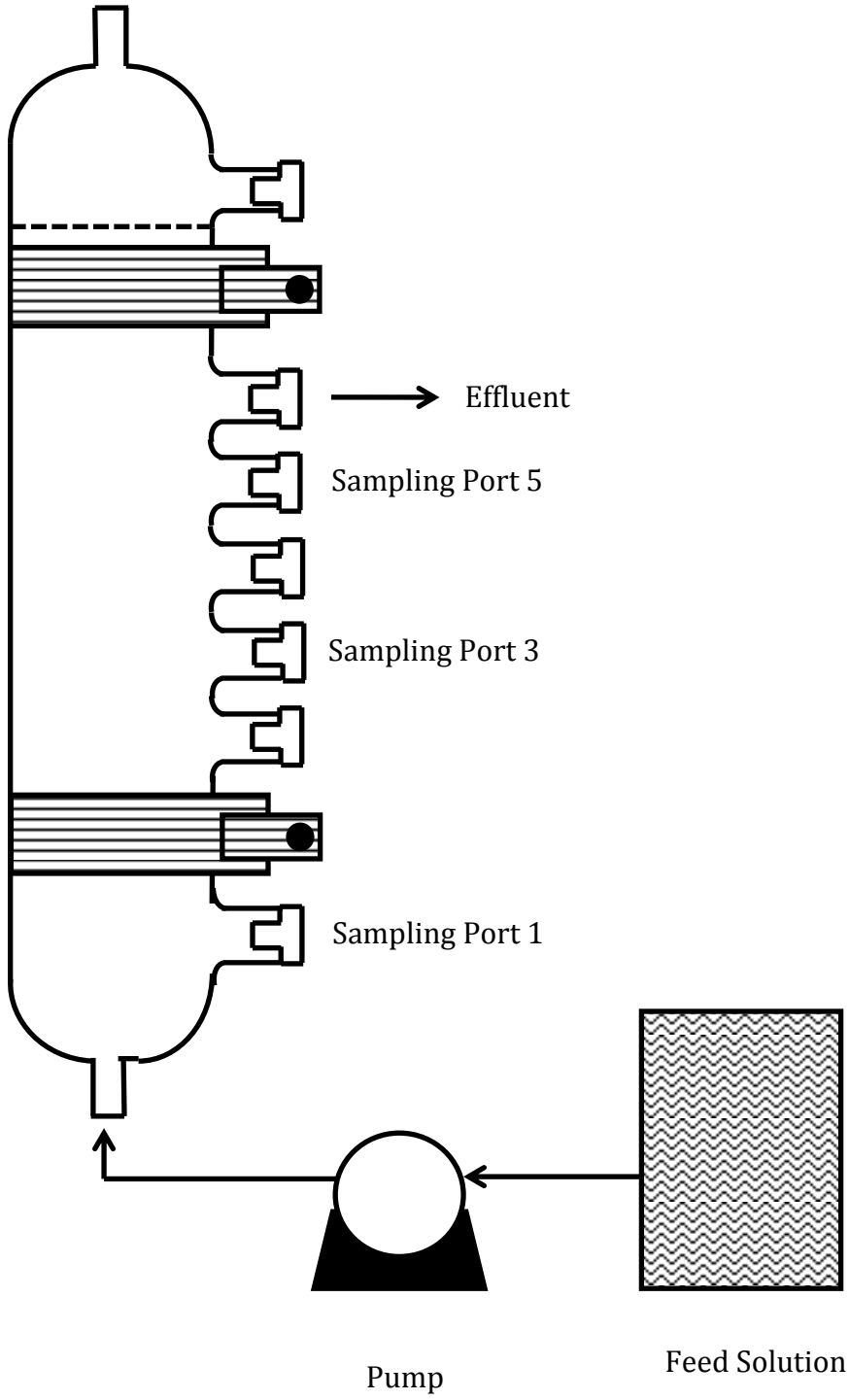

Figure 4.1: A schematic of the column set up

Tygon tubing from Cole-Palmer (Vernon Hills, IL, USA) was used for connections. The tubing was attached to the pump using plastic hose barbs from Ace Hardware (Lincoln, NE, USA). Each of the seven sample ports were plugged with septa from Sigma-Aldrich (St. Louis, MO, USA). Both the column and the connecting tubing were wrapped in aluminum foil to keep out light. The feed solution was wrapped in towels to keep out light. A piece of vinyl tubing (Ace Hardware, Lincoln, NE, USA) connected the effluent port to a hose barb, and then to Tygon tubing, which ran to the waste container.

The glass groundwater feed container was 22 L in volume and was capped with a green neoprene stopper from Sigma-Aldrich (St. Louis, MO, USA) to limit evaporation. A hose barb imbedded in the neoprene stopper allowed the Tygon tubing to enter the groundwater feed container. The waste container was a 113 L drum also affixed with a neoprene stopper with an imbedded hose barb to allow the passage of fluids.

Forty-five grams of cypress mulch was added to the column. The mulch was held in the column at the influent and effluent ports with a fiberglass mesh purchased at Baker Hardware (Lincoln, NE, USA). The column was seeded with 4 L of primary effluent from the Teresa Street wastewater treatment plant (Lincoln, NE, USA) pumped at 2.5 mL/min. This flow rate was chosen based on a literature review (Seo et al. 2007). Next, the column was fed with a simulated groundwater solution, whose composition can be seen in Table 4.1. The nitrate concentration was chosen based on the average background concentration of nitrate in Nebraska Groundwater in 2009, 7.8 mg NO_3-N/L ("Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater " 2011). The

composition of the remainder of the groundwater was selected based on a literature review (Dahab and Sirigina 1994; Nebraska Department of Environmental Quality, 2010).

The atrazine concentration in the column is a thousand times higher than the typical background concentration in groundwater, which is typically less than 1.5 µg atrazine/L (Nebraska Department of Environmental Quality, 2010). Atrazine is an unfavorable nitrogen source for bacteria (Clausen et al. 2002; Hunter and Shaner 2010). Using an initial concentration of atrazine that is the same order of magnitude as the initial concentration of nitrate was intended to encourage utilization of atrazine by bacteria.

Table 4.1: Chemical composition of synthetic groundwater solution (after Dahab and Sirigina 1994)

Compound	**Amount** (*mg/L*)
KH_2PO_4	150
K_2HPO_4	32.5
$FeSO_4 \cdot 7H_2O$	0.816
Na_2MoO_4	0.2365
$MnSO_4 \cdot 7H_2O$	0.1565
$CaCl_2 \cdot 6H_2O$	0.526
Na_2SO_3	250
$CoCl_2 \cdot 6H_2O$	1.052
$NaNO_3$	42.5 (7 mg NO_3-N/L)
Atrazine	1

Sodium nitrate, dipotassium phosphate, ferrous sulfate, calcium chloride, and cobalt chloride were purchased from Fisher Scientific (Waltham, MA, USA). Atrazine was purchased from Chemservice (West Chester, PA, USA). Sodium sulfite was purchased from Sigma Aldrich (St. Louis, MO, USA). The monopotassium phosphate, was purchased from EM Science (Gibbstown, NJ, USA). The sodium molybdate was

purchased from Strem chemicals (Newburyport, MA, USA). The magnesium sulfide was purchased from Mallinckrodt chemicals (St. Louis, MO, USA).

The potassium phosphate buffers stabilized the pH. The ferrous sulfate, sodium molybdate, magnesium sulfide, and calcium chloride provided minerals necessary for bacterial growth. The sodium sulfite and cobalt chloride were added at twice their stoichiometric concentration in an effort to keep the dissolved oxygen level below 1 mg O_2/L to promote denitrification.

The synthetic groundwater solution was mixed in a 22 L glass container and fed to the column from the bottom. Dissolved oxygen and pH readings were taken biweekly from the influent and effluent ports. Samples were also taken biweekly using a syringe from Becton, Dickinson, and Company (Franklin Lakes, NJ, USA) from ports 1 (influent), 3, 5, and 6 (effluent), as shown in Figure 4.1. The sample size was 12 mL. The samples were filtered with a Millipore filtration apparatus with a Whatman GF/A filter (Fisher Scientific, Waltham, MA, USA) to remove particulate matter. Concentrations of nitrate and atrazine were determined, as described in Section 4.3.4.

The average concentration of both atrazine and nitrate taken from each sample port were compared in SigmaPlot 12 (Systat Software, San Jose, CA, USA) using a one way analysis of variance (ANOVA) with a significance level of 0.05.

4.3.4 Instrumental Analysis

Nitrate was analyzed with a Dionex Ion Chromatograph and atrazine was analyzed with a Waters Alliance 2695 High-Performance Liquid Chromatography (HPLC), as described in Section 3.2.4.

Dissolved oxygen was measured using an YSI 5010 probe attached to an YSI 5100 meter. The pH was measured using an 8102 BNUWP probe attached to an Orion 4 star meter from Thermo Scientific.

4.4 Results

The column ran for three months with an average influent pH of 6.64 and average dissolved oxygen of 2.23 mg O_2/L. The raw column data is shown in Appendix M. The oxygen scavengers, sodium sulfite and cobalt chloride, added at twice their stoichiometric concentrations, were not sufficient to overcome the daily diffusion of oxygen from the atmosphere into the feed solution. Oxygen rich conditions are not conducive to denitrification, because oxygen is desired over nitrate as an electron acceptor. Anoxic conditions are required for denitrification to occur, meaning a dissolved oxygen level below 0.5 mg O_2/L (van Haandel and van der Lubbe 2007).

During the course of the experiment, the cypress mulch did not degrade sufficiently to serve as an electron donor for denitrification. The rate of decomposition of mulch is inversely proportional to the ratio of lignin to nitrogen. The ratio of lignin to nitrogen is 125 in cypress mulch, making it very resistant to decomposition (Duryea et al. 1999). Atrazine makes a poor electron donor for nitrate reduction because only the carbon atoms in the side chains are a readily available energy source (Katz et al. 2000, 2001). Cypress mulch, as found in the mulch characterization experiments in Section 3.4.1, was the most acidic of the three types of mulch examined, with a pH of 5.32. Acidic conditions are not desirable for biological activity (Seo et al. 2007) and may have limited biofilm development.

Acetic acid was added on day 61 to serve as a carbon source to encourage denitrification. Using the suggested ratio of carbon to nitrogen of 1:1.45 for denitrification from Dahab and Lee (1988), 24.3 mL/L of glacial acetic acid was added. The acid was neutralized with 17,535 mg/L sodium hydroxide before being added to the groundwater solution. Both acetic acid and sodium hydroxide were purchased from EM Science (Gibbstown, NJ, USA).

The addition of acetic acid brought the influent dissolved oxygen down to 1.6 mg O_2/L, which is still too aerobic for denitrification. Additionally, the amount of acid added may not have been sufficient to satisfy the energy requirements of both aerobic and anaerobic bacteria.

Measured concentrations of atrazine and nitrate in the influent and effluent are shown in Figures 4.2 and 4.3, respectively. The influent concentrations of atrazine and nitrate were 1 mg atrazine/L and 7 mg NO_3-N/L, respectively. Note that on day 61, acetic acid was added as a carbon source.

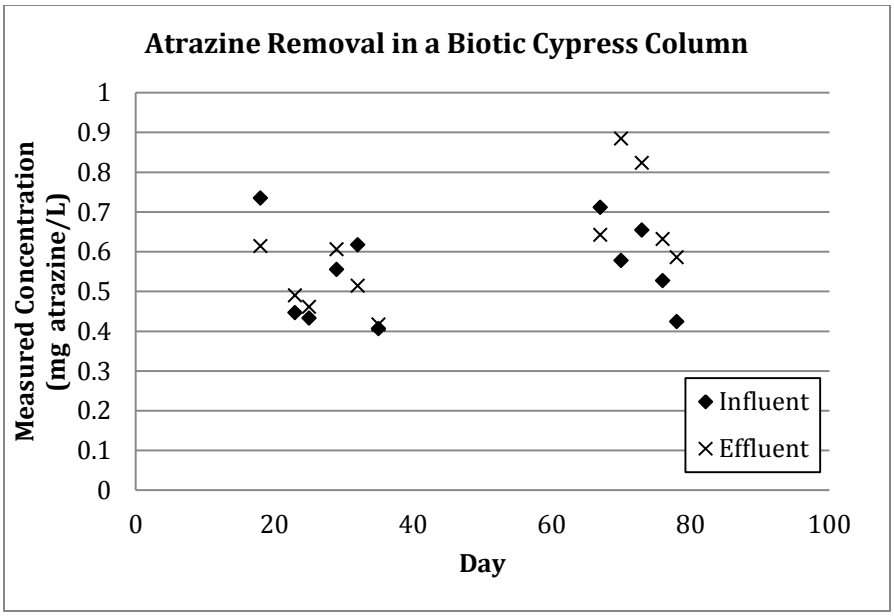

Figure 4.2: Measured atrazine concentrations in the influent and effluent ports of a biotic cypress column

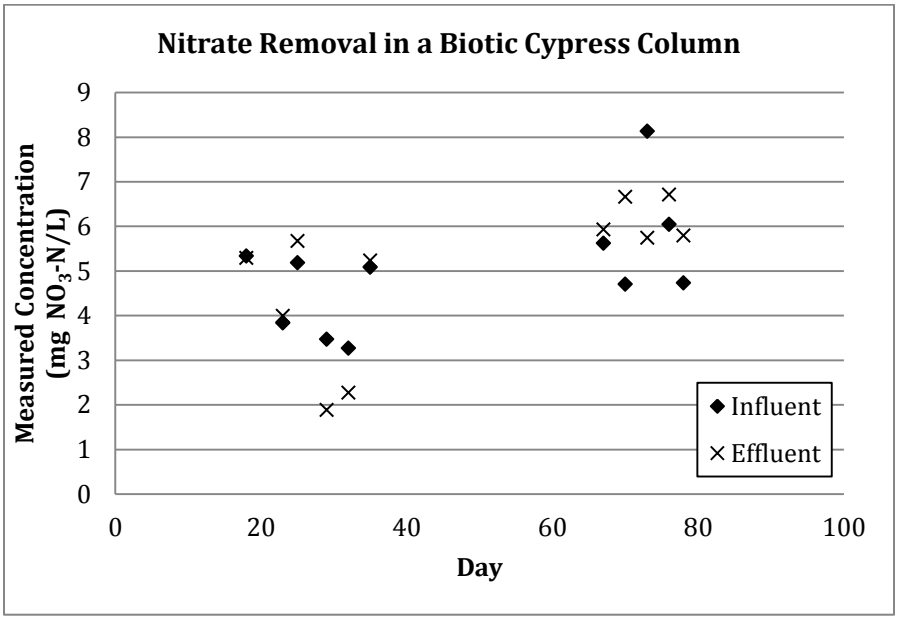

Figure 4.3: Measured nitrate concentrations in the influent and effluent ports of a biotic cypress column

Figures 4.2 and 4.3 show fluctuations in both influent and effluent concentrations, including times when the effluent concentration exceeds the influent concentration. A statistical analysis comparing average concentrations measured from different column ports found that these fluctuations are not significant. Graphs comparing the data from sample ports 3 and 5 can be found in Appendix N.

Denitrification is not affected by the presence of atrazine (Yeomans and Bremner 1987; Ilhan et al. 2011). However, atrazine degradation can be affected by the presence of nitrate. When present in excess, nitrate provides a more readily accessible source of nitrogen for atrazine-degrading bacteria, thus inhibiting the degradation of atrazine (Clausen et al. 2002; Hunter and Shaner 2010). Physical removal of atrazine, as in Ilhan et al. (2011), was not occurring, because the adsorption capacity of the mulch was exhausted early in the experiment.

4.5 Conclusions

The column failed to remove atrazine and nitrate, even with the addition of an external carbon source. Further investigation to determine what combination of natural materials can provide a viable carbon source and support a biowall in a field situation is recommended.

The oxygen concentration was too high for nitrification to occur and the nitrate concentration was too high for atrazine degradation to occur. There are three ways to avoid problems like these: a double column system (Hunter and Shaner 2010), a low concentration of nitrate (Crawford et al. 1998), or an adsorptive media (Herzberg et al. 2004).

Hunter and Shaner (2010) used a two-column silica sand system containing first, a vegetable oil based denitrifying biobarrier followed by an aerobic reactor with an atrazine degrading consortia. The reactors were fed with 3 mg atrazine/L and 5 mg NO_3-N/L. The denitrifying section removed 98% of the supplied nitrate and 30% of the atrazine, while the aerobic reactor removed the remaining 70% of the atrazine. The double column system sustained high removal rates of both nitrate and atrazine even when the nitrate concentration was spiked to 50 mg NO_3-N/L.

Crawford et al. (1998) used isolate M91-3 for denitrification coupled with atrazine degradation in glass media columns. The column was fed with 0.1 mM atrazine and 1 mM NO_3-N, and kept under anoxic conditions with continuous sparging of nitrogen gas. Atrazine degradation was achieved in the column, therefore the authors concluded that low concentrations of nitrate, ~1 mM, do not interfere with atrazine degradation.

Katz et al. (2001) used *Pseudomonas* sp. ADP in anoxic non-sterile reactors filled with glass spheres that had a 90% removal efficiency of nitrate. The removal efficiency of atrazine was high, >95%, for the first month, and then lost effectiveness, to 10-25%. This discrepancy was due to the influence of competitive nitrifying bacteria that could not degrade atrazine. Herzberg et al. (2004) also saw a similar decrease in atrazine removal efficiency in a reactor filled with non-adsorptive media. However, this effect was not present in a similar reactor with adsorptive media due to a “double flux” of atrazine through both the adsorptive media and the biofilm.

These authors, and many others, have successfully utilized biotic columns in the laboratory to study remediation of atrazine, nitrate, and other compounds. Laboratory

conditions are easier to control and are often less harsh to bacteria. In a field-scale situation, factors such as a non-uniform distribution of atrazine, the presence of other contaminants or metabolites, ambient temperature, and mass transport limitations for the contaminant, bacteria, and nutrients may influence the effectiveness of a given remediation method (Silva et al. 2004; Strong et al. 2000; Sturman et al. 1995). Knowing which of these processes have the greatest effect on remediation effectiveness, and therefore, most important for laboratory-scale simulation, would enhance future research.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

This study investigated the ability of a mulch biowall to remove atrazine and nitrate from contaminated groundwater. First, the physical and chemical properties of three types of mulch were characterized. Next, the adsorption capacity of the mulch for atrazine and nitrate was analyzed in a series of isotherm experiments. Finally, the feasibility of implementing a cypress mulch biowall with a laboratory-scale biotic column designed to remove atrazine and nitrate was evaluated. From this research, the following conclusions were made:

Mulch Characterization

- Cypress mulch had a significantly higher organic carbon content than cedar or hardwood mulch.
- Qualitatively, cypress mulch had the lowest pH, electrical conductivity, and cation exchange capacity.

Isotherm Experiment

- Based on the ratio of q_e/C_e (equilibrium concentration on the mulch over equilibrium concentration in solution), there was no statistical difference between the three types of mulch except for the pairs of cedar-hardwood and cypress-hardwood in the system of binary atrazine adsorption.
- The ratio of q_e/C_e was statistically different between mono and binary systems.

- . Atrazine adsorption appeared to exhibit a C-type isotherm, due to the range of concentrations examined; A wider range of atrazine concentrations may show a more distinct L-type isotherm.
- Nitrate does not significantly influence the adsorption of atrazine.
- Nitrate adsorption was highly dependent on mulch surface properties and did not exhibit a specific type of isotherm.
- Atrazine influenced nitrate adsorption.
- In the binary system, average values of Langmuir coefficients for nitrate adsorption were significantly different, indicating that atrazine is affecting the capacity of nitrate adsorption (q_{max}) at high concentration of nitrate, but at low concentrations of nitrate (C_e<5), the energy of nitrate adsorption (K_L) is affected.
- Qualitatively, cypress mulch exhibited the highest capacity for adsorption of atrazine and nitrate.
- Cypress was selected as the best substrate to support bacterial growth in a biotic column.

Column Experiment

- Nitrate removal was not effective because daily diffusion of oxygen into the feed container resulted in conditions unsuitable for denitrification.
- Atrazine removal was not effective because the nitrate concentration was too high.

5.2 Recommendations for Future Research

The findings presented here could be enhanced with future research.

- Previous studies, as well as the findings presented here, imply relationships between two or more of the following: organic content, water content, cation exchange capacity, conductivity, and adsorption. Further investigation in a wide variety of materials may be able to identify exactly which are related and why.
- The influence of atrazine on nitrate adsorption was most likely due to differences in surface properties between the three types of mulch. Further investigation, including a surface area measurement, via the Brunauer, Emmett, and Teller (BET) method, would confirm this.
- Cedar mulch did not degrade sufficiently to provide a carbon source for denitrification. Further investigation to determine what combination of natural materials can provide a viable carbon source and support a biowall in a field situation is recommended.

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APPENDICIES

Appendix A: Raw Data for Mono Atrazine System

The following tables include the raw data for the mono Langmuir and Freundlich isotherms for atrazine. Tables A1 and A3 shows the Langmuir and Freundlich data, respectively, after a statistical analysis removing data points that had a final solution concentration either greater than 90% or less than 10% of the initial solution concentration and that fell outside a 95% confidence interval. The removed data points can be seen in Tables A2 and A4, respectively. Control tests were performed to ensure: 1. The mulch did not contain atrazine, 2. The atrazine was not adhering to the glass sample containers, and 3. The filters were not altering the concentration of atrazine. The data from the control tests can be seen in Table A5. The raw data is plotted in Figures A1-A3.

Table A1: Raw data for mono atrazine Langmuir isotherm after statistical analysis

Mulch Type	Initial Conc.	Mulch Weight	Liquid Volume	Final Conc. (C_e)	Conc. on Mulch (q_e)	C_e/q_e	Date
	mg atrazine/L	*grams*	*mL*	*mg atrazine/L*	*mg/g*	*g/L*	
Cedar	10	0.1505	42.4	7.685	0.65	11.78	9.12b
Cedar	5	0.2506	42.2	3.756	0.21	17.93	9.12b
Cedar	5	0.5004	41.2	3.062	0.16	19.19	9.12b
Cedar	15	0.5011	41.4	8.565	0.53	16.11	9.12a
Cedar	5	0.7505	40.9	2.639	0.13	20.51	9.12b
Cedar	5	1.0003	40.6	2.067	0.12	17.36	9.12b
Cedar	15	1.0024	40.3	6.034	0.36	16.74	9.12a
Cedar	15	1.5	39.4	4.925	0.26	18.61	9.12a
Cedar	15	2.0008	37.9	3.834	0.21	18.13	9.12a
Cedar	10	2.5021	37.2	1.895	0.12	15.73	9.12b
Cedar	15	2.5041	37.2	3.003	0.18	16.85	9.12a
Cypress	5	0.1509	42.7	3.770	0.35	10.83	9.12b
Cypress	5	0.2513	42.4	3.631	0.23	15.73	9.12b
Cypress	5	0.5018	41.9	2.779	0.19	14.98	9.12b
Cypress	10	0.5021	42.4	6.273	0.31	19.93	8.26
Cypress	5	0.7505	41.4	2.596	0.13	19.58	9.12b
Cypress	5	1.0015	40.9	1.902	0.13	15.04	9.12b
Cypress	10	1.0017	41.4	4.799	0.21	22.33	8.26
Cypress	15	1.0045	41	6.726	0.34	19.92	9.12a
Cypress	15	1.5018	39.6	4.028	0.29	13.92	9.12a
Cypress	10	2.0006	39.4	2.820	0.14	19.95	8.26
Cypress	15	2.0082	39	3.236	0.23	14.16	9.12a
Cypress	10	2.5095	37.8	2.007	0.12	16.67	9.12b
Hardwood	10	0.1504	42.7	8.047	0.55	14.51	9.12b
Hardwood	10	0.2507	42.3	7.369	0.44	16.60	9.12b
Hardwood	5	0.5019	41.9	3.274	0.14	22.71	9.12b
Hardwood	5	0.7502	41.1	2.905	0.11	25.31	9.12b
Hardwood	5	1.0002	40.5	2.344	0.11	21.79	9.12b
Hardwood	15	1.0024	40.8	6.476	0.35	18.67	9.12b
Hardwood	15	1.5012	40.1	4.517	0.28	16.13	9.12b
Hardwood	15	2.5014	37.6	3.804	0.16829	22.61	9.12b
Hardwood	10	2.5019	37.9	2.681	0.110872	24.18	9.12b

Table A2: Raw data for mono atrazine Langmuir isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*grams*	*mL*	*mg atrazine/L*	*mg/g*	*g/L*	
Cedar	5	0.0513	42.5	4.379	0.51	8.51	9.12b
Cedar	5	0.1516	42.6	3.776	0.34	10.98	9.12b
Cedar	10	0.2503	42.3	6.781	0.54	12.47	9.12b
Cedar	10	0.5011	42.2	6.668	0.28	23.77	8.26
Cedar	10	1.0045	40.6	4.896	0.21	23.74	8.26
Cedar	10	2.0007	38.1	3.152	0.13	24.16	8.26
Cypress	10	0.1512	42.7	8.207	0.51	16.20	9.12b
Cypress	10	0.2534	42.3	6.898	0.52	13.32	9.12b
Cypress	5	0.499	43	4.284	0.06	69.40	9.12b
Cypress	15	0.501	41.3	9.153	0.48	18.99	9.12a
Cypress	15	2.5042	28	9.250	0.06	143.86	9.12b
Hardwood	5	0.0507	42.9	4.253	0.63	6.73	9.12b
Hardwood	5	0.1503	42.2	4.029	0.27	14.79	9.12b
Hardwood	5	0.2501	42.2	3.632	0.23	15.74	9.12b
Hardwood	15	0.5011	42.1	7.088	0.66	10.66	9.12b
Hardwood	10	0.5041	41.7	7.166	0.23	30.57	8.26
Hardwood	10	1.0029	41.8	5.187	0.20	25.86	8.26
Hardwood	15	2.0019	38.7	2.813	0.24	11.94	9.12b
Hardwood	10	2.0033	36.5	3.863	0.11	34.55	8.26

Table A3: Raw data for mono atrazine Freundlich isotherm after statistical analysis

Mulch Type	**Initial Concentration**	**Mulch Weight**	**Liquid Volume**	**Final Conc.(C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg atrazine/L	*grams*	*mL*	*mg atrazine/L*	*mg/g*	
Cedar	10	0.1505	42.4	7.685	0.652	9.12b
Cedar	10	0.2503	42.3	6.781	0.544	9.12b
Cedar	5	0.2506	42.2	3.756	0.210	9.12b
Cedar	5	0.5004	41.2	3.062	0.160	9.12b
Cedar	15	0.5011	41.4	8.565	0.532	9.12a
Cedar	5	0.7505	40.9	2.639	0.129	9.12b
Cedar	5	1.0003	40.6	2.067	0.119	9.12b
Cedar	15	1.0024	40.3	6.034	0.360	9.12a
Cedar	15	1.5	39.4	4.925	0.265	9.12a
Cedar	15	2.0008	37.9	3.834	0.212	9.12a
Cedar	10	2.5021	37.2	1.895	0.121	9.12b
Cedar	15	2.5041	37.2	3.003	0.178	9.12a
Cypress	10	0.1512	42.7	8.207	0.506	9.12b
Cypress	5	0.2513	42.4	3.631	0.231	9.12b
Cypress	15	0.501	41.3	9.153	0.482	9.12a
Cypress	5	0.5018	41.9	2.779	0.185	9.12b
Cypress	10	0.5021	42.4	6.273	0.315	8.26
Cypress	5	0.7505	41.4	2.596	0.133	9.12b
Cypress	5	1.0015	40.9	1.902	0.127	9.12b
Cypress	10	1.0017	41.4	4.799	0.215	8.26
Cypress	15	1.0045	41	6.726	0.338	9.12a
Cypress	10	2.0006	39.4	2.820	0.141	8.26
Cypress	15	2.0082	39	3.236	0.228	9.12a
Cypress	10	2.5095	37.8	2.007	0.120	9.12b
Hardwood	5	0.1503	42.2	4.029	0.273	9.12b
Hardwood	10	0.1504	42.7	8.047	0.555	9.12b
Hardwood	5	0.2501	42.2	3.632	0.231	9.12b
Hardwood	10	0.2507	42.3	7.369	0.444	9.12b
Hardwood	5	0.5019	41.9	3.274	0.144	9.12b
Hardwood	5	0.7502	41.1	2.905	0.115	9.12b
Hardwood	5	1.0002	40.5	2.344	0.108	9.12b
Hardwood	15	1.0024	40.8	6.476	0.347	9.12b
Hardwood	15	1.5012	40.1	4.517	0.280	9.12b
Hardwood	15	2.5014	37.6	3.804	0.168	9.12b
Hardwood	10	2.5019	37.9	2.681	0.111	9.12b

Table 4: Raw data for mono atrazine Freundlich isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Concentration**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg atrazine/L	*grams*	*mL*	*mg atrazine/ L*	*mg/g*	
Cedar	5	0.0513	42.5	4.379	0.515	9.12b
Cedar	5	0.1516	42.6	3.776	0.344	9.12b
Cedar	10	0.5011	42.2	6.668	0.281	8.26
Cedar	10	1.0045	40.6	4.896	0.206	8.26
Cedar	10	2.0007	38.1	3.152	0.130	8.26
Cypress	5	0.1509	42.7	3.770	0.348	9.12b
Cypress	10	0.2534	42.3	6.898	0.518	9.12b
Cypress	5	0.499	43	4.284	0.062	9.12b
Cypress	15	1.5018	39.6	4.028	0.289	9.12a
Cypress	15	2.5042	28	9.250	0.064	9.12b
Hardwood	5	0.0507	42.9	4.253	0.632	9.12b
Hardwood	15	0.5011	42.1	7.088	0.665	9.12b
Hardwood	10	0.5041	41.7	7.166	0.234	8.26
Hardwood	10	1.0029	41.8	5.187	0.201	8.26
Hardwood	15	2.0019	38.7	2.813	0.236	9.12b
Hardwood	10	2.0033	36.5	3.863	0.112	8.26

Table A5: Control data for mono atrazine isotherm

Mulch Type	Initial Concentration	Mulch Weight	Liquid Volume	Final Concentration (C_e)	Date
	mg atrazine/L	*grams*	*mL*	*mg atrazine/L*	
Cedar	0	1.0035	40.7	0.00	9.12b
Cypress	0	1.0019	40.8	0.00	8.26
Hardwood	0	1.0028	41	0.00	8.26
No Mulch	10	0	43.1	10.28	8.26
No Mulch	5	0	42.7	4.86	9.12b
No Mulch	15	0	43.1	14.74	9.12b
No Mulch, Filtered	10	0	42.9	9.31	9.12b
No Mulch, Unfiltered	10	0	42.9	10.05	9.12b

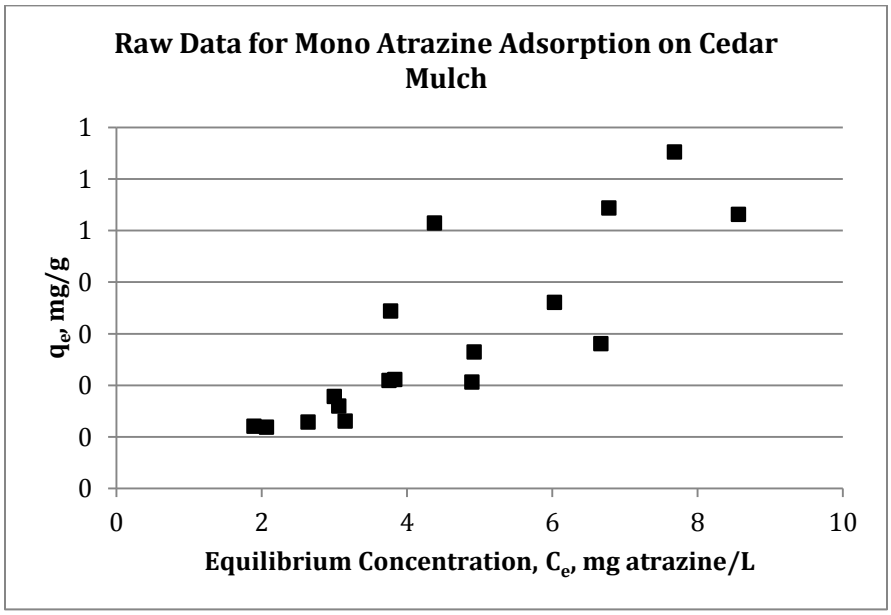

Figure A1: Raw data for mono atrazine isotherm on cedar mulch

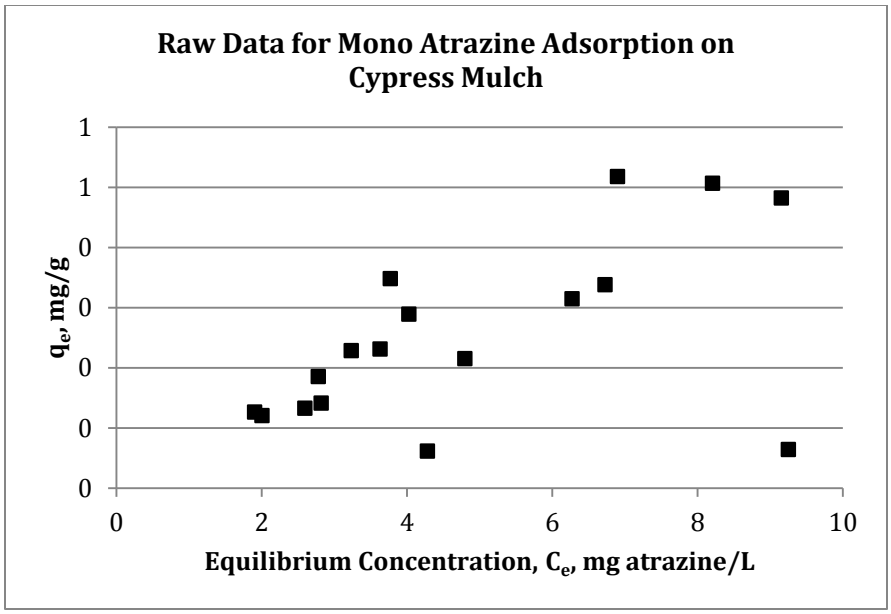

Figure A2: Raw data for mono atrazine isotherm on cypress mulch

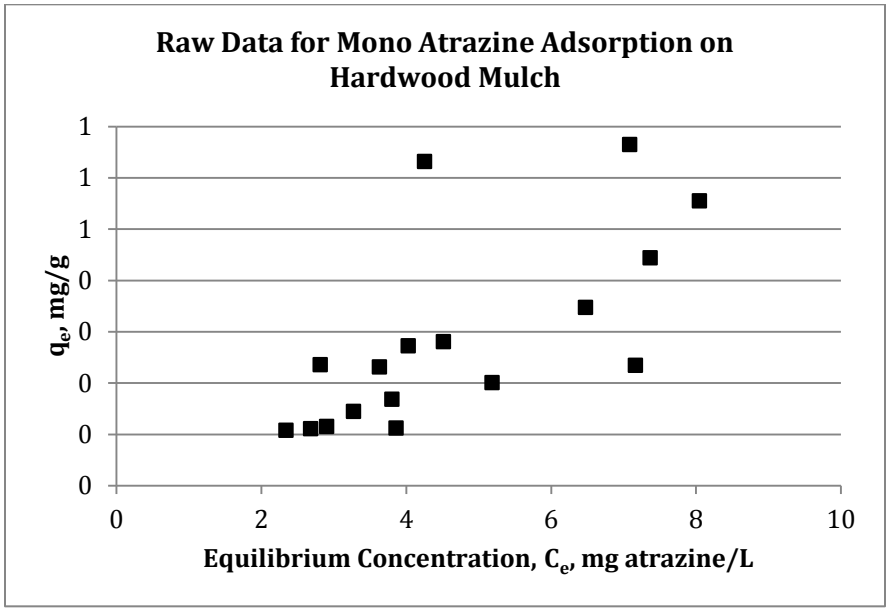

Figure A3: Raw data for mono atrazine isotherm on hardwood mulch

Appendix B: Raw Data for Mono Nitrate System

The following tables include the raw data for the mono isotherm for nitrate. Nitrate was analyzed using two isotherms, Langmuir and Freundlich. Tables B1 (Langmuir) and B5 (Freundlich) shows the data after a statistical analysis removing data points that had a final solution concentration either greater than 90% or less than 10% of the initial solution concentration and that fell outside a 95% confidence interval (90-10 Rule). The data points that were removed based on the 90-10 rule can be seen in Table B2. The data points that were removed based on a 95% confidence interval can be seen in Table B3 (Langmuir) and Table B6 (Freundlich). Control tests were performed to ensure: 1. The mulch did not contain nitrate, 2. The nitrate was not adhering to the glass sample containers, and 3. The filters were not altering the concentration of nitrate. The data from the control tests can be seen in Table B4. The raw data is plotted in Figures B1-B3.

A smaller range of final nitrate concentration (C_o<3.4 mg NO_3-N/L)was also analyzed using both Langmuir and Freundlich isotherms. Tables B7 (Langmuir) and B9 (Freundlich) shows the data after a statistical analysis removing data points that had a final solution concentration either greater than 90% or less than 10% of the initial solution concentration and that fell outside a 95% confidence interval. The data points that were removed based on a 95% confidence interval can be seen in Table B8 (Langmuir) and B10 (Freundlich).

Table B1: Raw data for mono nitrate Langmuir isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	0.68	0.0512	253.8	0.48	0.98	0.49	8.30
Cedar	0.68	0.15	253.6	0.43	0.42	1.03	8.23
Cedar	1.58	0.15	252.3	1.19	0.66	1.81	7.26
Cedar	0.68	0.2506	253	0.25	0.43	0.58	8.23
Cedar	1.58	0.2519	254.6	1.04	0.55	1.90	7.26
Cedar	3.39	0.5009	253.7	2.54	0.43	5.92	8.1
Cedar	1.58	0.5014	253.5	0.72	0.44	1.65	7.26
Cedar	3.39	1.0012	251.5	2.26	0.28	7.98	8.1
Cedar	20.33	1.3306	39.5	16.32	0.12	137.26	8.30
Cedar	3.39	2.002	248.1	0.97	0.30	3.24	8.1
Cedar	3.39	6.0025	239.5	2.06	0.05	38.89	8.1
Cedar	3.39	8.004	236	1.65	0.05	32.21	8.1
Cypress	0.68	0.0501	253.8	0.53	0.75	0.71	8.23
Cypress	0.68	0.1528	253.4	0.4	0.46	0.87	8.23
Cypress	0.68	0.2503	251.5	0.33	0.35	0.95	8.30
Cypress	3.39	0.5	252.3	2.63	0.38	6.88	8.1
Cypress	1.58	0.5008	252.4	0.99	0.30	3.32	7.26
Cypress	0.68	0.5017	251.7	0.15	0.26	0.57	8.23
Cypress	1.58	0.7501	252.2	0.73	0.29	2.55	7.26
Cypress	0.68	0.7503	252.05	0.23	0.15	1.53	8.30
Cypress	20.33	1.0001	40.7	15.33	0.20	75.41	8.30
Cypress	3.39	1.0013	251.1	2.39	0.25	9.55	8.1
Cypress	15.00	1.0016	40.8	11.16	0.16	71.35	11.15
Cypress	1.58	1.0029	251.5	0.64	0.24	2.71	7.12
Cypress	20.33	1.3303	40.2	14.61	0.17	84.59	8.30
Cypress	3.39	2	249.6	0.83	0.32	2.60	8.1
Cypress	1.58	2.0019	250.8	0.41	0.15	2.80	7.26
Cypress	1.58	2.0032	249.6	0.79	0.10	8.02	7.12
Cypress	3.39	4.0011	246.2	2.51	0.05	46.48	8.1
Cypress	1.58	4.0013	246.3	0.35	0.08	4.62	7.12
Cypress	1.58	4.0025	246.8	0.71	0.05	13.22	7.26
Cypress	1.58	5.0016	252.9	0.31	0.06	4.82	7.26

Table B1 Continued: Raw data for mono nitrate Langmuir isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cypress	3.39	6.0032	241.5	1.44	0.08	18.38	8.1
Cypress	1.58	7.0017	240.1	0.38	0.04	9.23	7.26
Hardwood	0.68	0.0506	253.2	0.59	0.44	1.35	8.30
Hardwood	0.68	0.1504	253.4	0.45	0.38	1.17	8.30
Hardwood	1.58	0.1514	254.1	1.3	0.47	2.76	7.26
Hardwood	1.58	0.2508	253.4	1.26	0.32	3.89	7.26
Hardwood	0.68	0.2523	253.4	0.33	0.35	0.95	8.23
Hardwood	1.58	0.5001	253.4	0.68	0.46	1.49	7.26
Hardwood	3.39	0.5011	251.7	2.51	0.44	5.69	8.1
Hardwood	0.68	0.5018	252.2	0.14	0.27	0.52	8.23
Hardwood	1.58	0.7504	251.8	0.49	0.37	1.34	7.26
Hardwood	20.33	1	40.6	17.27	0.12	139.23	8.30
Hardwood	3.39	1.0007	251.7	2.33	0.27	8.76	8.1
Hardwood	1.58	1.0009	251.3	0.85	0.18	4.63	7.26
Hardwood	1.58	1.002	251.2	0.82	0.19	4.30	7.12
Hardwood	20.33	1.3308	40.4	15.77	0.14	114.04	8.30
Hardwood	1.58	1.5022	251	0.51	0.18	2.85	7.26
Hardwood	3.39	2.0016	249	1.83	0.19	9.44	8.1
Hardwood	3.39	4.0018	242.5	1.19	0.13	8.94	8.1

Table B2: Raw data for mono nitrate Langmuir isotherm removed based on the 90-10 rule

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	9.49	0.5011	42	9.66	-0.01	-658.95	9.21
Cedar	0.68	0.5035	250.8	0.03	0.32	0.09	8.23
Cedar	1.58	0.7501	252.2	0.01	0.53	0.02	7.26
Cedar	0.68	0.753	251.8	0.01	0.22	0.04	8.23
Cedar	12.24	1.0005	40.8	11.25	0.04	278.71	11.15
Cedar	1.58	1.0009	251.7	0.02	0.39	0.05	7.12
Cedar	15.00	1.001	40.3	14.79	0.01	1749.35	11.15
Cedar	1.58	1.0027	251.6	0.01	0.39	0.03	7.26
Cedar	0.68	1.0111	251.9	0	0.17	0.00	8.23
Cedar	20.33	1.5013	39.2	18.39	0.05	363.95	9.21
Cedar	9.49	1.502	39.7	9.16	0.01	1066.02	9.21
Cedar	1.58	2	249.9	0.03	0.19	0.15	7.12
Cedar	20.33	2.5012	37.2	18.36	0.03	628.16	9.21
Cedar	1.58	4.0008	230.1	0.12	0.08	1.43	7.12
Cedar	3.39	4.0025	244.5	0	0.21	0.00	8.1
Cypress	1.58	1.0002	251.1	0.04	0.39	0.10	7.26
Cypress	9.49	1.5015	40.1	8.62	0.02	373.10	9.21
Cypress	20.33	1.5035	39.5	18.36	0.05	355.61	9.21
Cypress	20.33	2.0019	39.6	18.36	0.04	472.29	9.21
Cypress	9.49	2.0027	38.7	8.9	0.01	787.17	9.21
Hardwood	9.49	0.5033	41.7	8.96	0.04	205.95	9.21
Hardwood	12.24	1.0005	41.1	11.37	0.04	318.20	11.15
Hardwood	9.49	1.0016	40.5	8.75	0.03	294.38	9.21
Hardwood	12.24	1.5004	39.7	11.65	0.02	746.47	11.15
Hardwood	9.49	1.5007	39.3	9.16	0.01	1075.94	9.21
Hardwood	1.58	2.0014	250	0	0.20	0.00	7.12
Hardwood	20.33	2.0025	38.5	18.48	0.04	520.92	9.21
Hardwood	20.33	2.5016	27.6	18.33	0.02	832.69	9.21
Hardwood	1.58	4.0009	245.6	0.01	0.10	0.10	7.12

Table B3: Raw data for mono nitrate Langmuir isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	20.33	0.6703	41.2	15.69	0.28	55.07	8.30
Cedar	20.33	1.0008	40.7	15.68	0.19	83.00	8.30
Cedar	9.49	1.0056	40.4	8.53	0.04	222.30	9.21
Cedar	12.24	1.5001	39.6	10.98	0.03	330.15	11.15
Cedar	15.00	1.5013	38.8	12.54	0.06	197.24	11.15
Cedar	20.33	2.0002	38.4	18.15	0.04	434.63	9.21
Cypress	20.33	0.1517	47.6	17.4	0.92	18.96	9.21
Cypress	9.49	0.3303	42	7.78	0.22	35.88	8.30
Cypress	20.33	0.67	41.7	15.79	0.28	55.94	8.30
Cypress	9.49	0.6707	41.4	8.17	0.08	100.65	8.30
Cypress	12.24	1.0035	40.9	10.08	0.09	114.51	11.15
Cypress	9.49	1.0068	41.2	7.56	0.08	95.97	9.21
Cypress	12.24	1.5025	40.4	10.64	0.04	247.34	11.15
Cypress	15.00	1.503	39.7	13.05	0.05	253.36	11.15
Cypress	3.39	8.001	237.4	2.46	0.03	89.39	8.1
Hardwood	9.49	0.1701	42.4	7.64	0.46	16.61	8.30
Hardwood	9.49	0.2509	4.4	7.35	0.04	196.30	8.30
Hardwood	9.49	0.3312	41.7	7.89	0.20	39.29	8.30
Hardwood	20.33	0.6703	41.1	17.17	0.19	88.75	8.30
Hardwood	15.00	1.0013	40.9	12.87	0.09	147.92	11.15
Hardwood	15.00	1.5019	40.1	12.72	0.06	208.95	11.15
Hardwood	20.33	1.5025	39.9	17.94	0.06	283.23	9.21
Hardwood	3.39	6.0022	241	2.31	0.04	53.39	8.1
Hardwood	3.39	8.0016	236.5	1.99	0.04	48.18	8.1

Table B4: Control data for mono nitrate isotherm

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Initial Conc.**
	mg NO_3-N/L	*grams*	*mL*	*mg NO_3-N/L*	
Cedar	0	1.3318	39.4	0	8.30
Cedar	0	2.0004	249.2	0	7.12
Cypress	0	1.3302	40.1	0	8.30
Cypress	0	2.0005	249.9	0	7.12
Hardwood	0	1.3303	40.2	0	8.30
Hardwood	0	2.0004	249.2	0	7.12
No Mulch	1.58	0	253.9	1.39	7.12
No Mulch	1.58	0	254	1.44	7.26
No Mulch	3.39	0	252.5	3.09	8.1
No Mulch	0.68	0	251.3	0.6	8.23
No Mulch	9.49	0	42.4	9.35	8.30
No Mulch	20.33	0	42.4	18.76	8.30
No Mulch	20.33	0	43.4	20.79	9.21
No Mulch	9.49	0	42.7	10.16	9.21
No Mulch	12.24	0	42.8	12.24	11.15
No Mulch	15.00	0	42.7	14.76	11.15
No Mulch, Filtered	0.68	0	254.2	0.58	8.30
No Mulch, Unfiltered	0.68	0	254.2	0.62	8.30

Table B5: Raw data for mono nitrate Freundlich isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	0.68	0.15	253.6	0.43	0.42	1.03	8.23
Cedar	0.68	0.2506	253	0.25	0.43	0.58	8.23
Cedar	1.58	0.5014	253.5	0.72	0.44	1.65	7.26
Cedar	3.39	1.0012	251.5	2.26	0.28	7.98	8.1
Cedar	20.33	1.3306	39.5	16.32	0.12	137.26	8.30
Cedar	15.00	1.5013	38.8	12.54	0.06	197.24	11.15
Cedar	20.33	2.0002	38.4	18.15	0.04	434.63	9.21
Cedar	3.39	2.002	248.1	0.97	0.30	3.24	8.1
Cypress	0.68	0.5017	251.7	0.15	0.26	0.57	8.23
Cypress	0.68	0.7503	252.05	0.23	0.15	1.53	8.30
Cypress	20.33	1.0001	40.7	15.33	0.20	75.41	8.30
Cypress	15.00	1.0016	40.8	11.16	0.16	71.35	11.15
Cypress	12.24	1.0035	40.9	10.08	0.09	114.51	11.15
Cypress	9.49	1.0068	41.2	7.56	0.08	95.97	9.21
Cypress	20.33	1.3303	40.2	14.61	0.17	84.59	8.30
Cypress	1.58	2.0019	250.8	0.41	0.15	2.80	7.26
Hardwood	0.68	0.1504	253.4	0.45	0.38	1.17	8.30
Hardwood	0.68	0.2523	253.4	0.33	0.35	0.95	8.23
Hardwood	0.68	0.5018	252.2	0.14	0.27	0.52	8.23
Hardwood	1.58	0.7504	251.8	0.49	0.37	1.34	7.26
Hardwood	20.33	1	40.6	17.27	0.12	139.23	8.30
Hardwood	1.58	1.0009	251.3	0.85	0.18	4.63	7.26
Hardwood	15.00	1.0013	40.9	12.87	0.09	147.92	11.15
Hardwood	1.58	1.002	251.2	0.82	0.19	4.30	7.12
Hardwood	20.33	1.3308	40.4	15.77	0.14	114.04	8.30
Hardwood	20.33	1.5025	39.9	17.94	0.06	283.23	9.21
Hardwood	3.39	2.0016	249	1.83	0.19	9.44	8.1

Table B6: Raw data for mono nitrate Freundlich isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Vol.**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	0.68	0.0512	253.8	0.48	0.98	0.49	8.30
Cedar	1.58	0.15	252.3	1.19	0.66	1.81	7.26
Cedar	1.58	0.2519	254.6	1.04	0.55	1.90	7.26
Cedar	3.39	0.5009	253.7	2.54	0.43	5.92	8.1
Cedar	20.33	0.6703	41.2	15.69	0.28	55.07	8.30
Cedar	20.33	1.0008	40.7	15.68	0.19	83.00	8.30
Cedar	9.49	1.0056	40.4	8.53	0.04	222.30	9.21
Cedar	12.24	1.5001	39.6	10.98	0.03	330.15	11.15
Cedar	3.39	6.0025	239.5	2.06	0.05	38.89	8.1
Cedar	3.39	8.004	236	1.65	0.05	32.21	8.1
Cypress	0.68	0.0501	253.8	0.53	0.75	0.71	8.23
Cypress	20.33	0.1517	47.6	17.4	0.92	18.96	9.21
Cypress	0.68	0.1528	253.4	0.4	0.46	0.87	8.23
Cypress	0.68	0.2503	251.5	0.33	0.35	0.95	8.30
Cypress	9.49	0.3303	42	7.78	0.22	35.88	8.30
Cypress	3.39	0.5	252.3	2.63	0.38	6.88	8.1
Cypress	1.58	0.5008	252.4	0.99	0.30	3.32	7.26
Cypress	20.33	0.67	41.7	15.79	0.28	55.94	8.30
Cypress	9.49	0.6707	41.4	8.17	0.08	100.65	8.30
Cypress	1.58	0.7501	252.2	0.73	0.29	2.55	7.26
Cypress	3.39	1.0013	251.1	2.39	0.25	9.55	8.1
Cypress	1.58	1.0029	251.5	0.64	0.24	2.71	7.12
Cypress	12.24	1.5025	40.4	10.64	0.04	247.34	11.15
Cypress	15.00	1.503	39.7	13.05	0.05	253.36	11.15
Cypress	3.39	2	249.6	0.83	0.32	2.60	8.1
Cypress	1.58	2.0032	249.6	0.79	0.10	8.02	7.12
Cypress	3.39	4.0011	246.2	2.51	0.05	46.48	8.1
Cypress	1.58	4.0013	246.3	0.35	0.08	4.62	7.12
Cypress	1.58	4.0025	246.8	0.71	0.05	13.22	7.26
Cypress	1.58	5.0016	252.9	0.31	0.06	4.82	7.26

Table B6 Continued: Raw data for mono nitrate Freundlich isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cypress	3.39	6.0032	241.5	1.44	0.08	18.38	8.1
Cypress	1.58	7.0017	240.1	0.38	0.04	9.23	7.26
Cypress	3.39	8.001	237.4	2.46	0.03	89.39	8.1
Hardwood	0.68	0.0506	253.2	0.59	0.44	1.35	8.30
Hardwood	1.58	0.1514	254.1	1.3	0.47	2.76	7.26
Hardwood	9.49	0.1701	42.4	7.64	0.46	16.61	8.30
Hardwood	1.58	0.2508	253.4	1.26	0.32	3.89	7.26
Hardwood	9.49	0.2509	4.4	7.35	0.04	196.30	8.30
Hardwood	9.49	0.3312	41.7	7.89	0.20	39.29	8.30
Hardwood	1.58	0.5001	253.4	0.68	0.46	1.49	7.26
Hardwood	3.39	0.5011	251.7	2.51	0.44	5.69	8.1
Hardwood	20.33	0.6703	41.1	17.17	0.19	88.75	8.30
Hardwood	3.39	1.0007	251.7	2.33	0.27	8.76	8.1
Hardwood	15.00	1.5019	40.1	12.72	0.06	208.95	11.15
Hardwood	1.58	1.5022	251	0.51	0.18	2.85	7.26
Hardwood	3.39	4.0018	242.5	1.19	0.13	8.94	8.1
Hardwood	3.39	6.0022	241	2.31	0.04	53.39	8.1
Hardwood	3.39	8.0016	236.5	1.99	0.04	48.18	8.1

Table B7: Raw data for mono nitrate Langmuir isotherm (C_o<3.4 mg NO_3-N/L) after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	0.68	0.0512	253.8	0.48	0.98	0.49	8.30
Cedar	0.68	0.15	253.6	0.43	0.42	1.03	8.23
Cedar	1.58	0.15	252.3	1.19	0.66	1.81	7.26
Cedar	0.68	0.2506	253	0.25	0.43	0.58	8.23
Cedar	1.58	0.2519	254.6	1.04	0.55	1.90	7.26
Cedar	3.39	0.5009	253.7	2.54	0.43	5.92	8.1
Cedar	1.58	0.5014	253.5	0.72	0.44	1.65	7.26
Cedar	3.39	1.0012	251.5	2.26	0.28	7.98	8.1
Cedar	3.39	2.002	248.1	0.97	0.30	3.24	8.1
Cypress	0.68	0.0501	253.8	0.53	0.75	0.71	8.23
Cypress	0.68	0.1528	253.4	0.4	0.46	0.87	8.23
Cypress	0.68	0.2503	251.5	0.33	0.35	0.95	8.30
Cypress	1.58	0.5008	252.4	0.99	0.30	3.32	7.26
Cypress	0.68	0.5017	251.7	0.15	0.26	0.57	8.23
Cypress	1.58	0.7501	252.2	0.73	0.29	2.55	7.26
Cypress	0.68	0.7503	252.05	0.23	0.15	1.53	8.30
Cypress	1.58	1.0029	251.5	0.64	0.24	2.71	7.12
Cypress	3.39	2	249.6	0.83	0.32	2.60	8.1
Cypress	1.58	2.0019	250.8	0.41	0.15	2.80	7.26
Cypress	1.58	2.0032	249.6	0.79	0.10	8.02	7.12
Cypress	3.39	4.0011	246.2	2.51	0.05	46.48	8.1
Cypress	1.58	4.0013	246.3	0.35	0.08	4.62	7.12
Cypress	1.58	4.0025	246.8	0.71	0.05	13.22	7.26
Cypress	1.58	5.0016	252.9	0.31	0.06	4.82	7.26
Cypress	3.39	6.0032	241.5	1.44	0.08	18.38	8.1
Cypress	1.58	7.0017	240.1	0.38	0.04	9.23	7.26
Hardwood	0.68	0.0506	253.2	0.59	0.44	1.35	8.30
Hardwood	0.68	0.1504	253.4	0.45	0.38	1.17	8.30
Hardwood	1.58	0.2508	253.4	1.26	0.32	3.89	7.26
Hardwood	0.68	0.2523	253.4	0.33	0.35	0.95	8.23
Hardwood	1.58	0.5001	253.4	0.68	0.46	1.49	7.26
Hardwood	0.68	0.5018	252.2	0.14	0.27	0.52	8.23
Hardwood	1.58	0.7504	251.8	0.49	0.37	1.34	7.26

Table B7 Continued: Raw data for mono nitrate Langmuir isotherm (C_e<5 mg/L) after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Hardwood	1.58	1.0009	251.3	0.85	0.18	4.63	7.26
Hardwood	1.58	1.002	251.2	0.82	0.19	4.30	7.12
Hardwood	1.58	1.5022	251	0.51	0.18	2.85	7.26
Hardwood	3.39	2.0016	249	1.83	0.19	9.44	8.1
Hardwood	3.39	4.0018	242.5	1.19	0.13	8.94	8.1

Table B8: Raw data for mono nitrate Langmuir isotherm (C_o<3.4 mg NO_3-N/L) removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	*g/L*	
Cedar	3.39	6.0025	239.5	2.06	0.05	38.89	8.1
Cedar	3.39	8.004	236	1.65	0.05	32.21	8.1
Cypress	3.39	0.5	252.3	2.63	0.38	6.88	8.1
Cypress	3.39	1.0013	251.1	2.39	0.25	9.55	8.1
Cypress	3.39	8.001	237.4	2.46	0.03	89.39	8.1
Hardwood	1.58	0.1514	254.1	1.3	0.47	2.76	7.26
Hardwood	3.39	0.5011	251.7	2.51	0.44	5.69	8.1
Hardwood	3.39	1.0007	251.7	2.33	0.27	8.76	8.1
Hardwood	3.39	6.0022	241	2.31	0.04	53.39	8.1
Hardwood	3.39	8.0016	236.5	1.99	0.04	48.18	8.1

Table B9: Raw data for mono nitrate Freundlich isotherm (C_o<3.4 mg NO_3-N/L) after statistical analysis

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	
Cedar	0.68	0.0512	253.8	0.48	0.98	8.30
Cedar	0.68	0.15	253.6	0.43	0.42	8.23
Cedar	0.68	0.2506	253	0.25	0.43	8.23
Cedar	3.39	0.5009	253.7	2.54	0.43	8.1
Cedar	1.58	0.5014	253.5	0.72	0.44	7.26
Cedar	3.39	1.0012	251.5	2.26	0.28	8.1
Cedar	3.39	2.002	248.1	0.97	0.30	8.1
Cypress	0.68	0.5017	251.7	0.15	0.26	8.23
Cypress	0.68	0.7503	252.05	0.23	0.15	8.30
Cypress	3.39	1.0013	251.1	2.39	0.25	8.1
Cypress	1.58	1.0029	251.5	0.64	0.24	7.12
Cypress	1.58	2.0019	250.8	0.41	0.15	7.26
Cypress	1.58	2.0032	249.6	0.79	0.10	7.12
Cypress	3.39	4.0011	246.2	2.51	0.05	8.1
Cypress	3.39	6.0032	241.5	1.44	0.08	8.1
Hardwood	0.68	0.1504	253.4	0.45	0.38	8.30
Hardwood	0.68	0.2523	253.4	0.33	0.35	8.23
Hardwood	0.68	0.5018	252.2	0.14	0.27	8.23
Hardwood	1.58	0.7504	251.8	0.49	0.37	7.26
Hardwood	3.39	1.0007	251.7	2.33	0.27	8.1
Hardwood	1.58	1.0009	251.3	0.85	0.18	7.26
Hardwood	1.58	1.002	251.2	0.82	0.19	7.12
Hardwood	1.58	1.5022	251	0.51	0.18	7.26
Hardwood	3.39	2.0016	249	1.83	0.19	8.1

Table B10: Raw data for mono nitrate Freundlich isotherm (C_o<3.4 mg NO_3-N/L) removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg NO_3-N /L	*grams*	*mL*	*mg NO_3-N /L*	*mg/g*	
Cedar	1.58	0.15	252.3	1.19	0.66	7.26
Cedar	1.58	0.2519	254.6	1.04	0.55	7.26
Cedar	3.39	6.0025	239.5	2.06	0.05	8.1
Cedar	3.39	8.004	236	1.65	0.05	8.1
Cypress	0.68	0.0501	253.8	0.53	0.75	8.23
Cypress	0.68	0.1528	253.4	0.4	0.46	8.23
Cypress	0.68	0.2503	251.5	0.33	0.35	8.30
Cypress	3.39	0.5	252.3	2.63	0.38	8.1
Cypress	1.58	0.5008	252.4	0.99	0.30	7.26
Cypress	1.58	0.7501	252.2	0.73	0.29	7.26
Cypress	3.39	2	249.6	0.83	0.32	8.1
Cypress	1.58	4.0013	246.3	0.35	0.08	7.12
Cypress	1.58	4.0025	246.8	0.71	0.05	7.26
Cypress	1.58	5.0016	252.9	0.31	0.06	7.26
Cypress	1.58	7.0017	240.1	0.38	0.04	7.26
Cypress	3.39	8.001	237.4	2.46	0.03	8.1
Hardwood	0.68	0.0506	253.2	0.59	0.44	8.30
Hardwood	1.58	0.1514	254.1	1.3	0.47	7.26
Hardwood	1.58	0.2508	253.4	1.26	0.32	7.26
Hardwood	1.58	0.5001	253.4	0.68	0.46	7.26
Hardwood	3.39	0.5011	251.7	2.51	0.44	8.1
Hardwood	3.39	4.0018	242.5	1.19	0.13	8.1
Hardwood	3.39	6.0022	241	2.31	0.04	8.1
Hardwood	3.39	8.0016	236.5	1.99	0.04	8.1

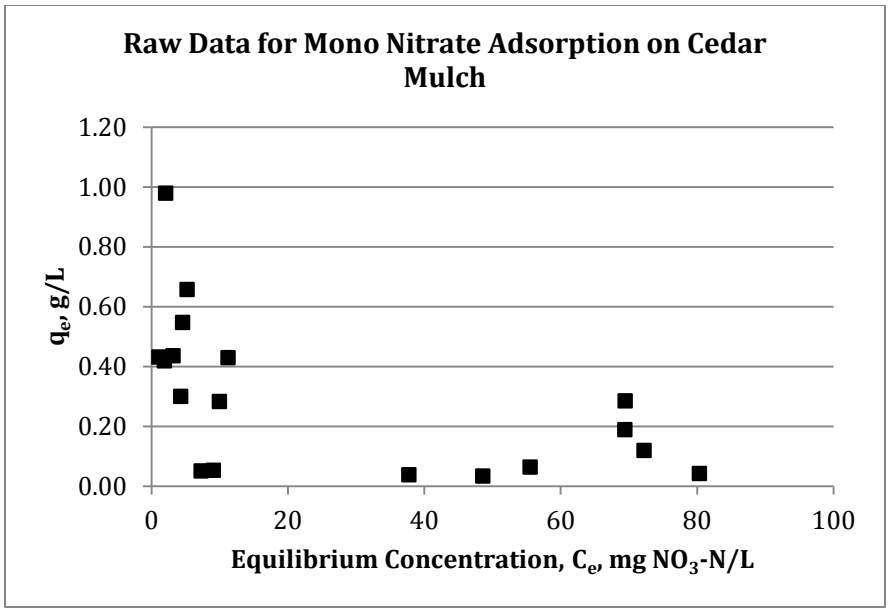

Figure B1: Raw data for mono atrazine isotherm on cedar mulch

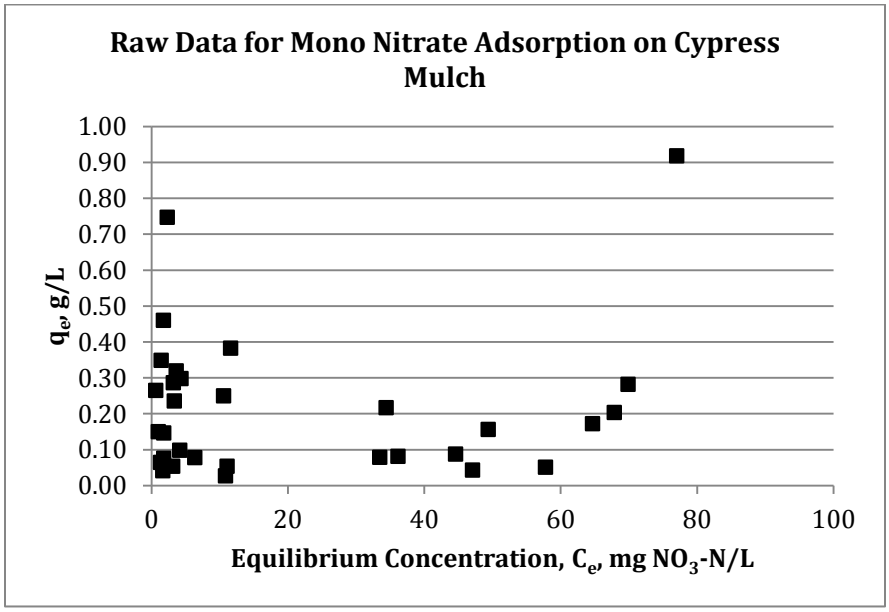

Figure B2: Raw data for mono atrazine isotherm on cypress mulch

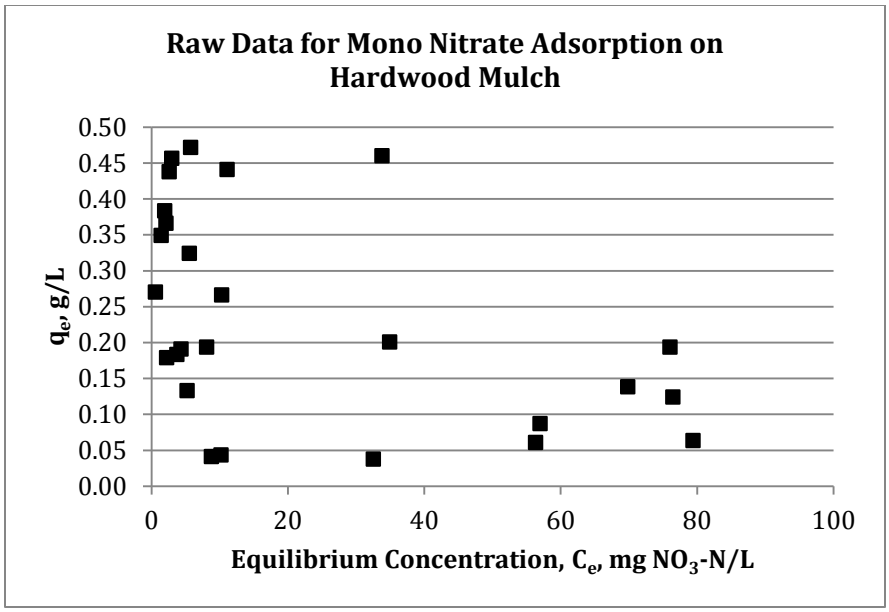

Figure B3: Raw data for mono atrazine isotherm on hardwood mulch

Appendix C: Graphs for Mono Atrazine System

The following figures show the graphs for the mono isotherms for atrazine using a Langmuir isotherm (Figures C1-C3) and Freundlich isotherm (Figures C4-C6).

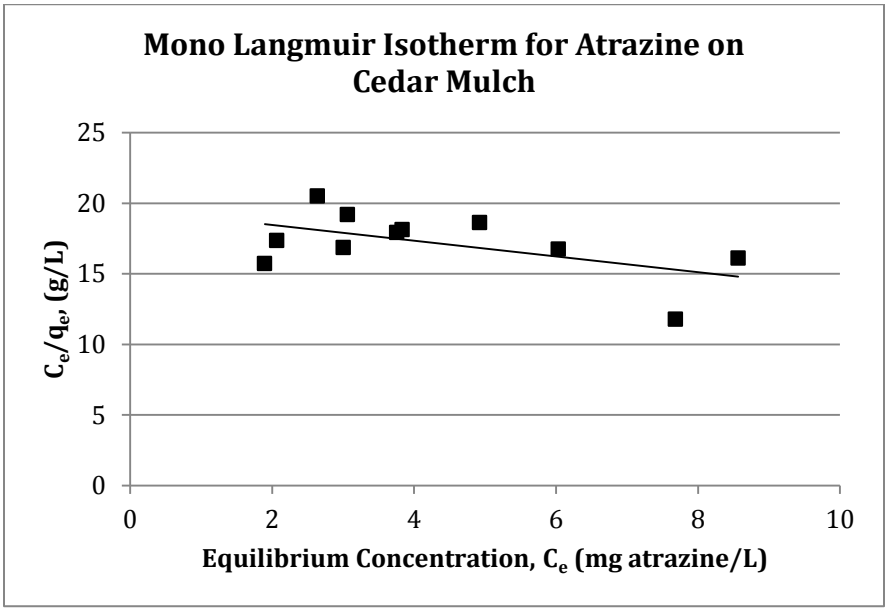

Figure C1: Langmuir adsorption isotherm for atrazine in the mono system on cedar mulch

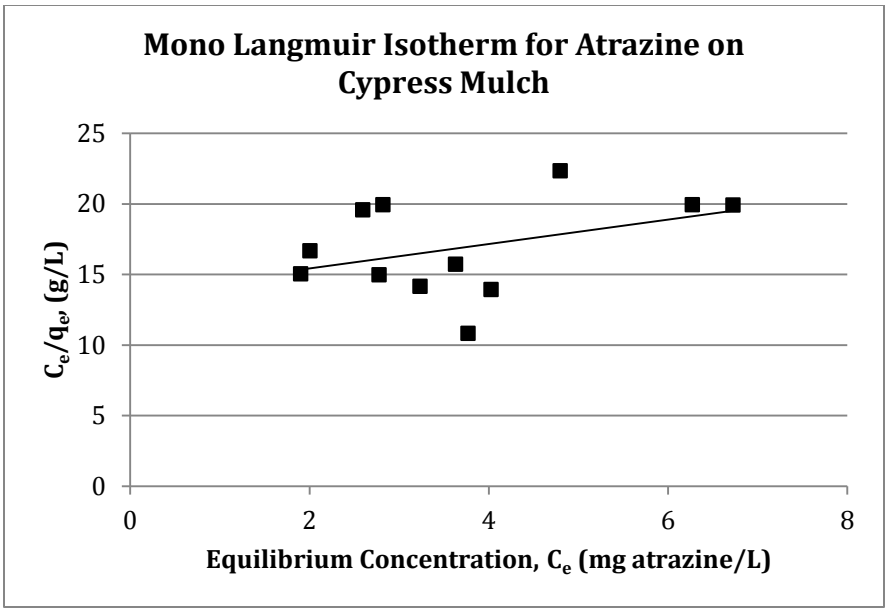

Figure C2: Langmuir adsorption isotherm for atrazine in the mono system on cypress mulch

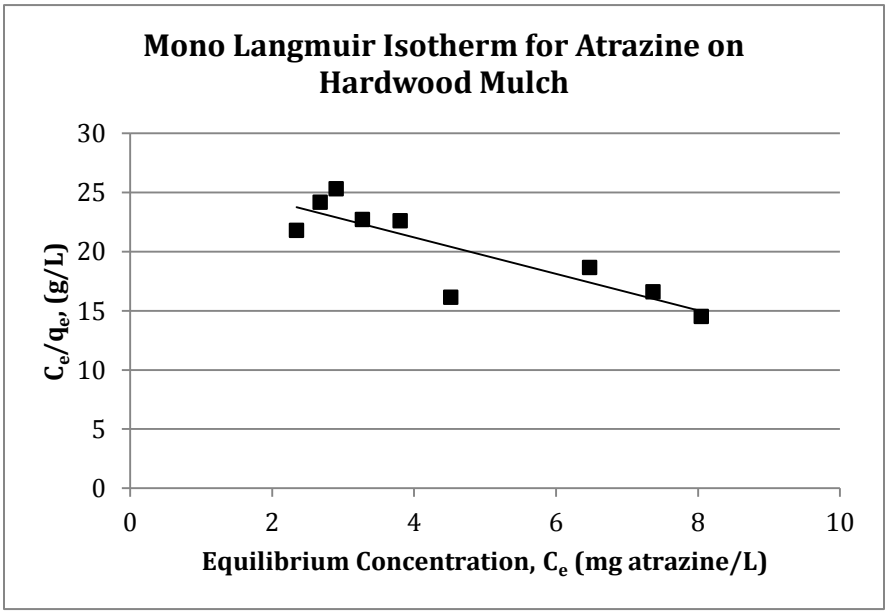

Figure C3: Langmuir adsorption isotherm for atrazine in the mono system on hardwood mulch

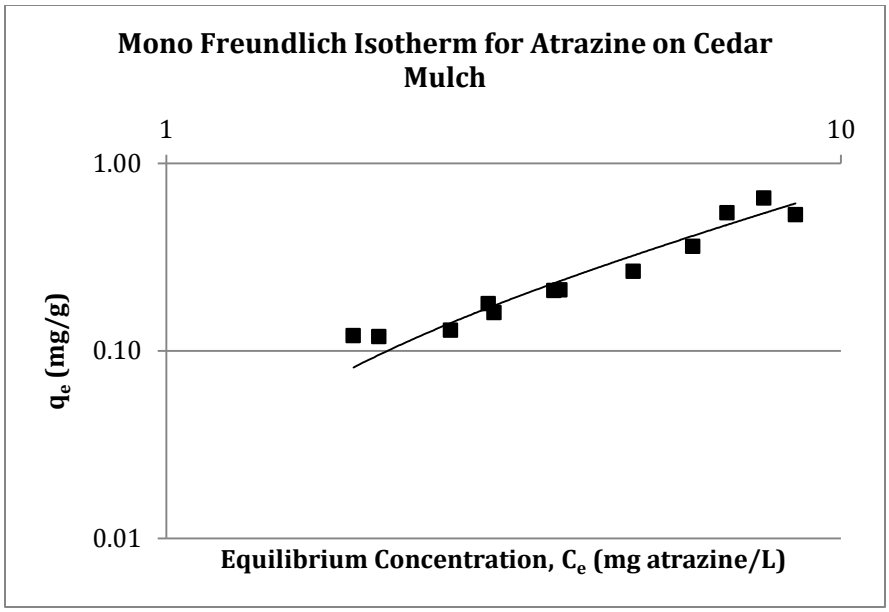

Figure C4: Freundlich adsorption isotherm for atrazine in the mono system on cedar mulch

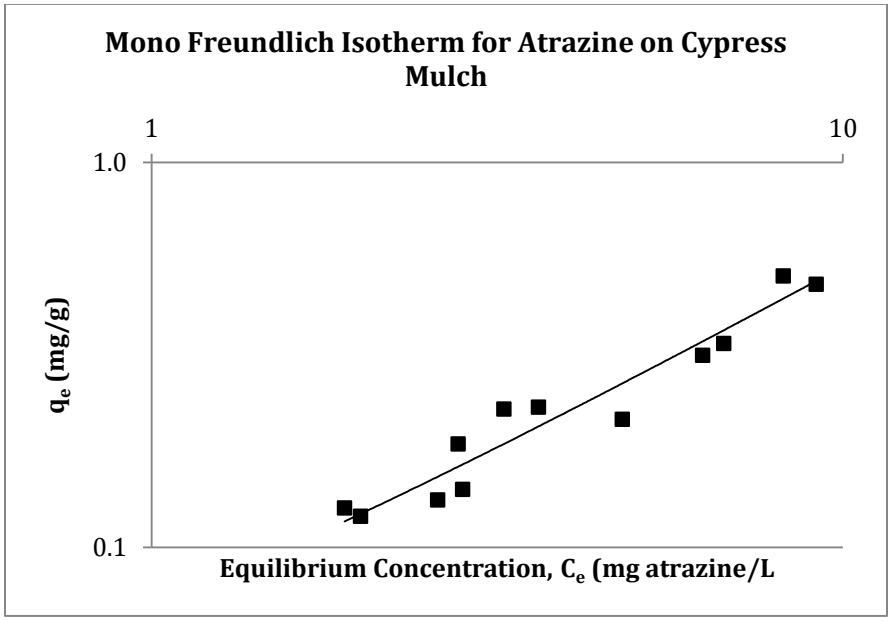

Figure C5: Freundlich adsorption isotherm for atrazine in the mono system on cypress mulch

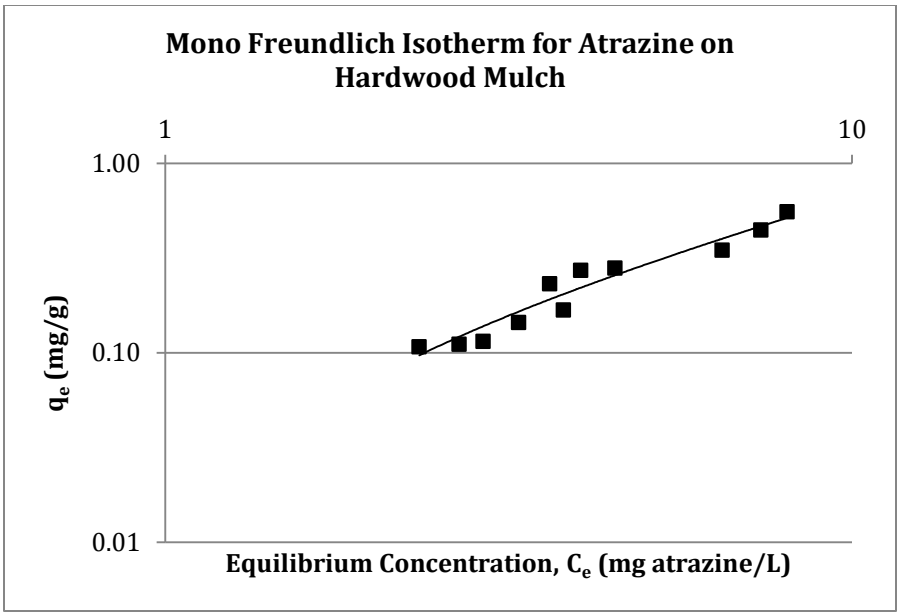

Figure C6: Freundlich adsorption isotherm for atrazine in the mono system on hardwood mulch

Appendix D: Graphs for Mono Nitrate System

The following figures show the graphs for the mono isotherms for nitrate using a Langmuir isotherm (Figures D1-D3) and Freundlich isotherm (Figures D4-D6).

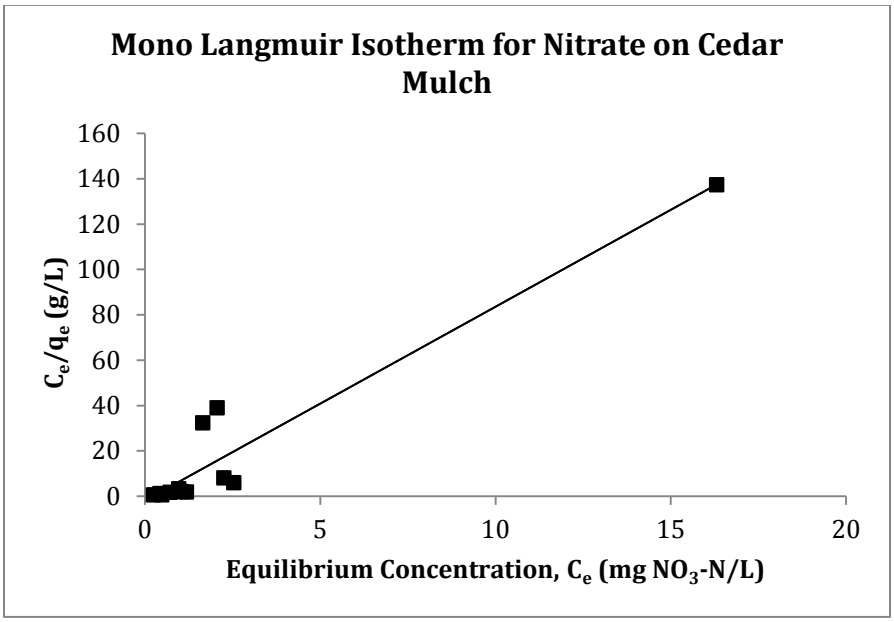

Figure D1: Langmuir adsorption isotherm for nitrate in the mono system on cedar mulch

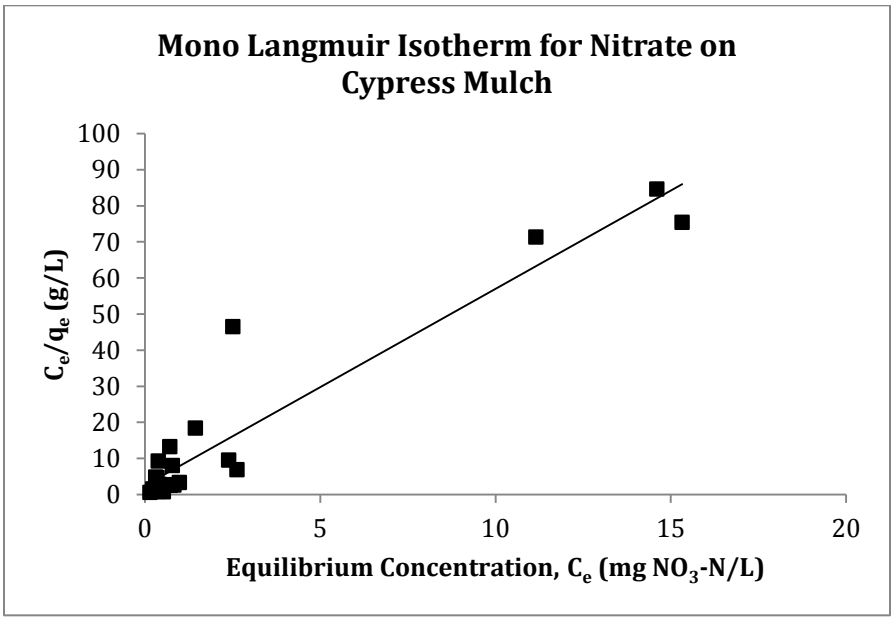

Figure D2: Langmuir adsorption isotherm for nitrate in the mono system on cypress mulch

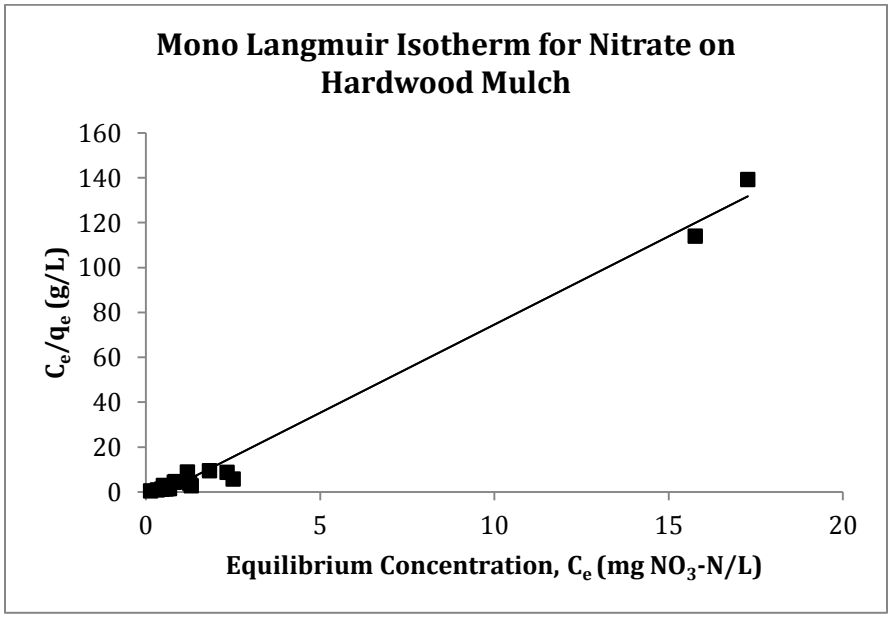

Figure D3: Langmuir adsorption isotherm for nitrate in the mono system on hardwood mulch

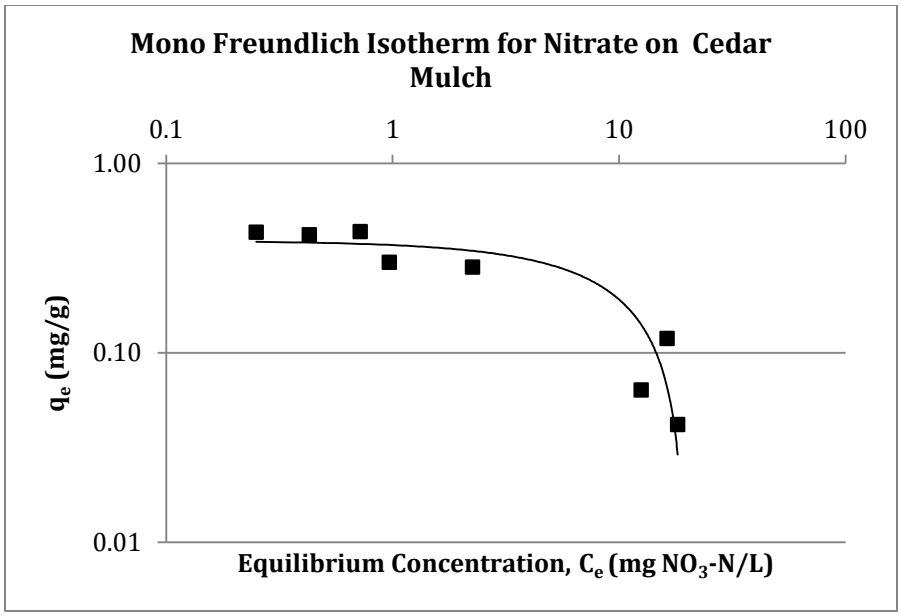

Figure D4: Freundlich adsorption isotherm for nitrate in the mono system on cedar mulch

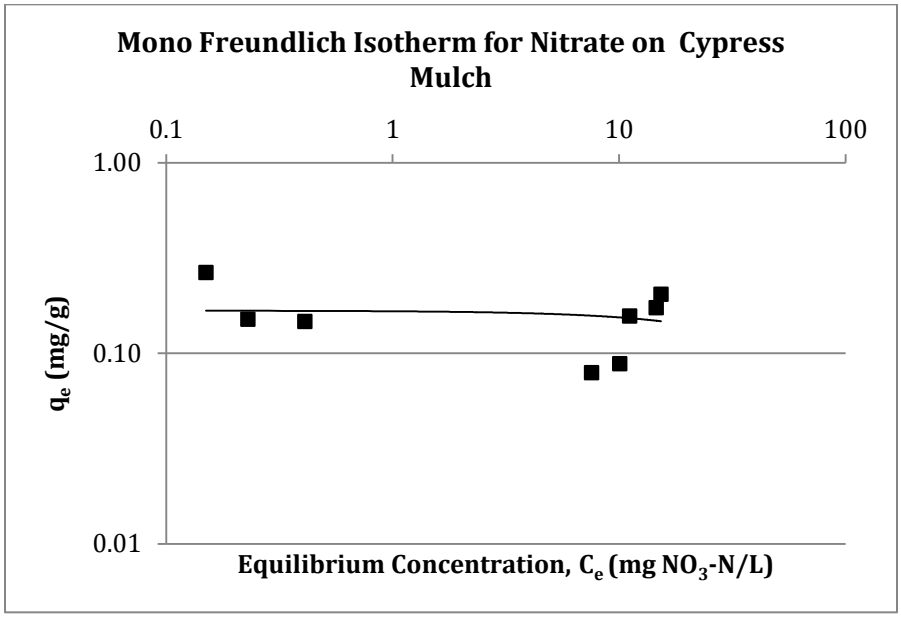

Figure D5: Freundlich adsorption isotherm for nitrate in the mono system on cypress mulch

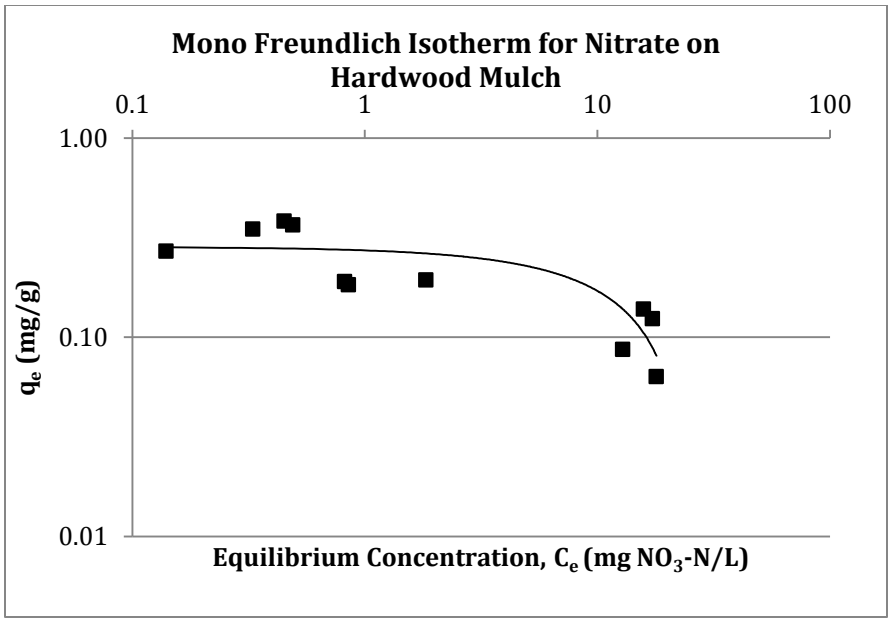

Figure D6: Freundlich adsorption isotherm for nitrate in the mono system on hardwood mulch

Appendix E: Graphs for Mono Nitrate System for C_o<3.4 mg NO_3-N/L

The following figures show the graphs for the mono isotherms for nitrate (C_o<3.4 mg NO_3-N/L) using a Langmuir isotherm (Figures E1-E3) and Freundlich isotherm (Figures E4-E6).

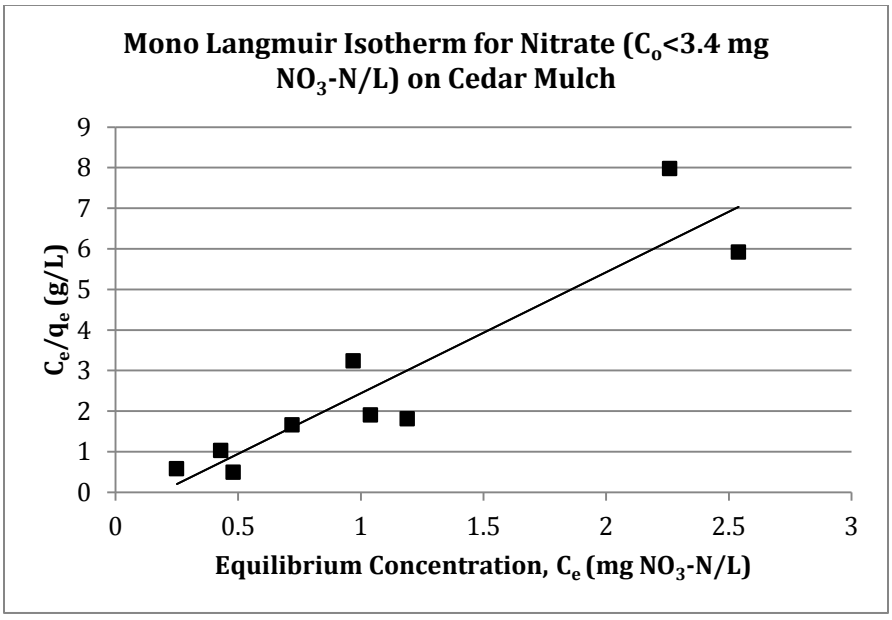

Figure E1: Langmuir adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on cedar mulch

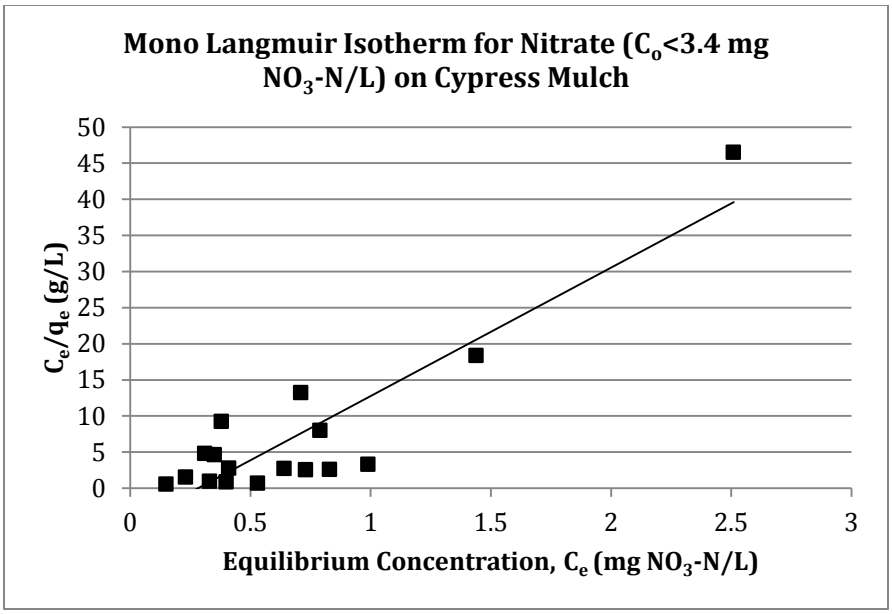

Figure E2: Langmuir adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on cypress mulch

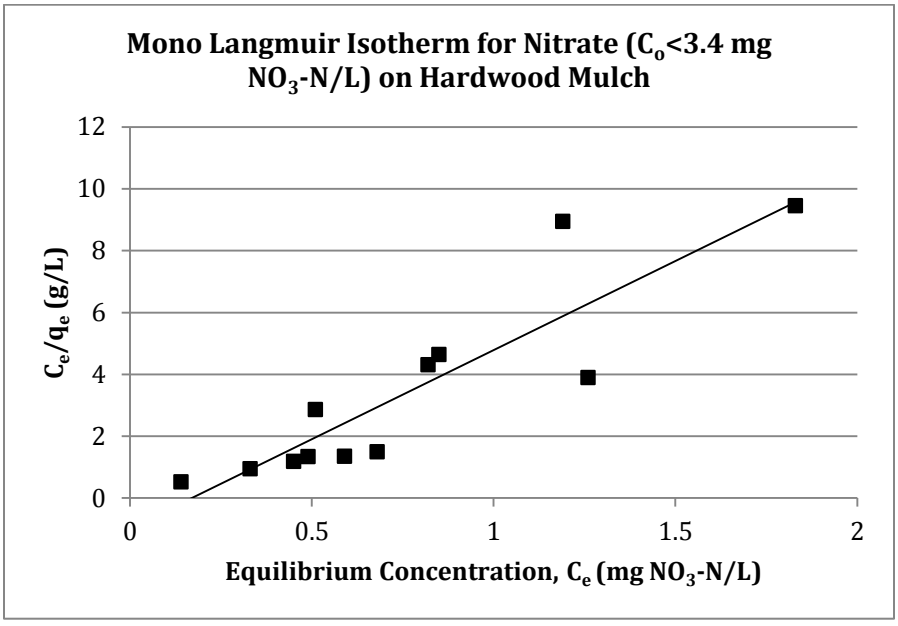

Figure E3: Langmuir adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on hardwood mulch

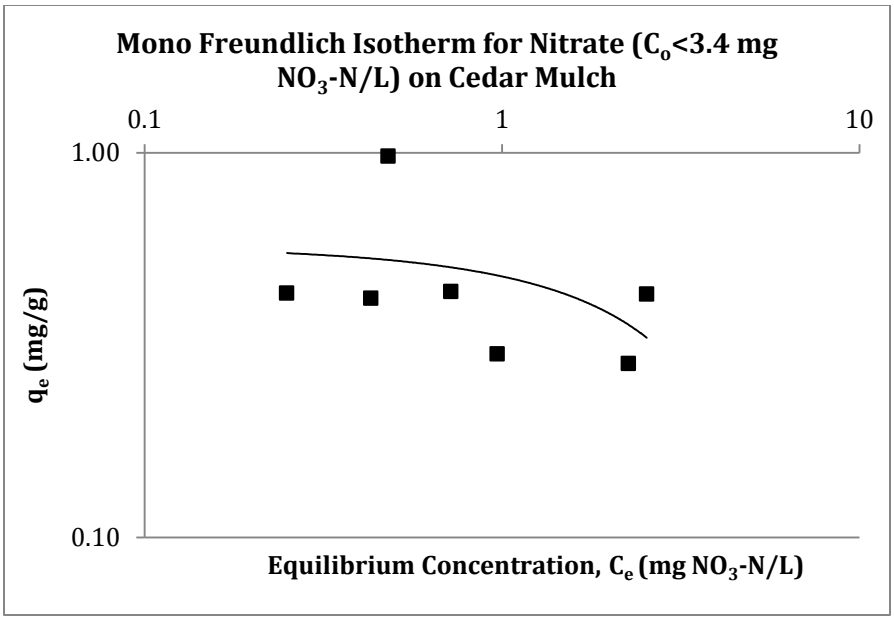

Figure E4: Freundlich adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on cedar mulch

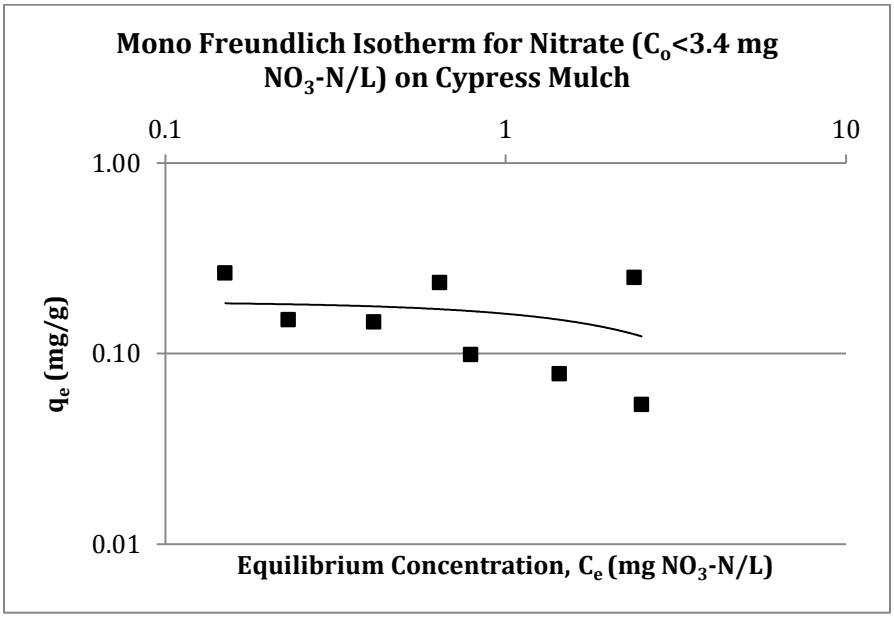

Figure E5: Freundlich adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on cypress mulch

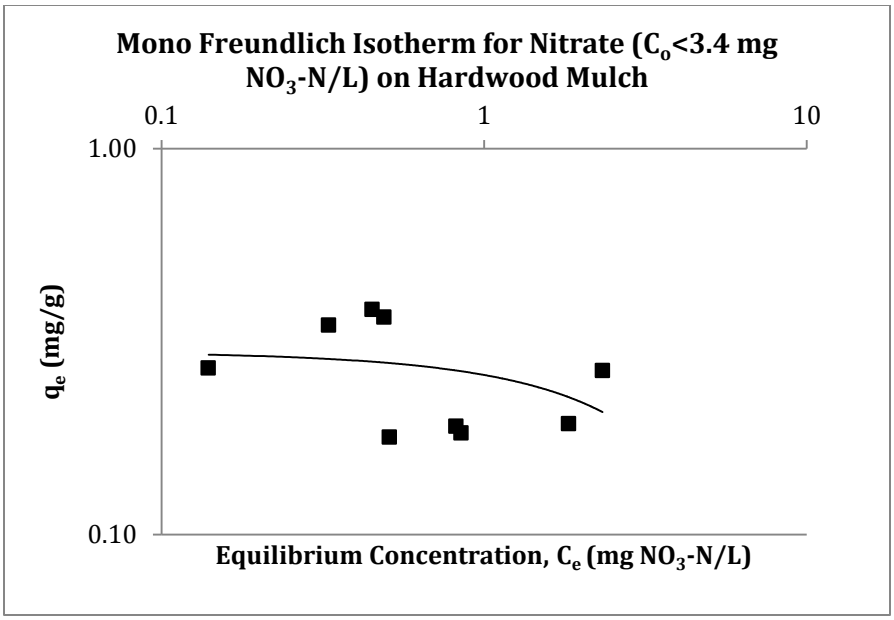

Figure E6: Freundlich adsorption isotherm for nitrate (C_o<3.4 mg NO_3-N/L) in the mono system on hardwood mulch

Appendix F: Raw Data for Binary Atrazine System

The following tables include the raw data for the binary Langmuir and Freundlich isotherms for atrazine. Tables F1 and F3 shows the Langmuir and Freundlich isotherm data, respectively, after a statistical analysis removing data points that had a final solution concentration either greater than 90% or less than 10% of the initial solution concentration and that fell outside a 95% confidence interval. The removed data points based pm the 95% confidence interval can be seen in Tables F2 and F5 for the Langmuir and Freundlich isotherms, respectively. Control tests were performed to ensure the atrazine or nitrate were not adhering to the glass containers. The data from the control tests can be seen in Table F4. The raw data is plotted in Figures F1-F3.

Table F1: Raw data for binary atrazine Langmuir isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc.(C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg atrazine/L*	*mg/g*	*g/L*	
Cedar	5	7	0.5014	41.6	2.851	0.178	15.99	10.26
Cedar	5	7	1.5009	39.4	1.285	0.098	13.18	10.26
Cedar	5	7	0.7513	41.2	2.041	0.162	12.57	11.15
Cedar	5	7	1.0045	40.47	1.639	0.135	12.10	11.15
Cedar	2.5	3.5	0.7506	41	1.075	0.078	13.82	10.26
Cedar	2.5	3.5	1.0033	40.6	0.642	0.075	8.55	11.15
Cedar	2.5	3.5	1.2506	40.4	0.672	0.059	11.39	11.15
Cypress	5	7	0.503	41.4	2.595	0.198	13.11	10.26
Cypress	5	7	0.75	41.6	2.289	0.150	15.22	10.26
Cypress	5	7	1.0036	40.7	1.677	0.135	12.44	10.26
Cypress	5	7	1.25	39.4	1.555	0.109	14.32	10.26
Cypress	5	7	1.5008	40	1.230	0.100	12.24	10.26
Cypress	5	7	1.0038	40.9	2.012	0.122	16.52	11.15
Cypress	2.5	3.5	0.5032	42	1.302	0.100	13.02	10.26
Cypress	2.5	3.5	0.7516	41.9	0.820	0.094	8.75	10.26
Cypress	2.5	3.5	1.0028	40.8	0.701	0.073	9.57	10.26
Cypress	2.5	3.5	0.7503	41.6	0.942	0.086	10.91	11.15
Cypress	2.5	3.5	1.0022	40.8	0.671	0.074	9.01	11.15
Hardwood	5	7	0.2506	42.1	3.457	0.259	13.33	10.26
Hardwood	5	7	0.5	41.6	2.797	0.183	15.27	10.26
Hardwood	5	7	0.7508	41.3	2.376	0.144	16.47	10.26
Hardwood	5	7	0.7514	41.3	2.414	0.142	16.99	11.15
Hardwood	5	7	1.0015	40.9	1.936	0.125	15.47	11.15
Hardwood	5	7	1.2508	40.1	1.715	0.105	16.29	11.15
Hardwood	2.5	3.5	0.2507	42.3	1.790	0.120	14.95	10.26
Hardwood	2.5	3.5	0.7505	41.5	1.013	0.082	12.31	11.15
Hardwood	2.5	3.5	1.0015	40.5	0.864	0.066	13.06	11.15
Hardwood	2.5	3.5	1.2517	40.6	0.771	0.056	13.74	11.15

Table F2: Raw data for binary atrazine Langmuir isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg atrazine/L*	*mg/g*	*g/L*	
Cedar	5	7	0.7503	40.7	2.178	0.153	14.22	10.26
Cedar	5	7	1.0017	40.1	1.574	0.137	11.48	10.26
Cedar	5	7	1.2508	39.9	1.498	0.112	13.41	10.26
Cedar	5	7	1.2525	39.6	1.381	0.114	12.07	11.15
Cedar	2.5	3.5	0.1506	42.9	1.891	0.173	10.91	10.26
Cedar	2.5	3.5	0.2506	42.2	1.661	0.141	11.75	10.26
Cedar	2.5	3.5	0.5013	41.6	1.339	0.096	13.89	10.26
Cedar	2.5	3.5	1.0016	40.5	0.789	0.069	11.40	10.26
Cedar	2.5	3.5	0.7517	40.7	0.757	0.094	8.02	11.15
Cypress	5	7	1.2527	40	1.809	0.102	17.76	11.15
Cypress	5	7	0.7514	41.4	1.083	0.216	5.02	11.15
Cypress	2.5	3.5	0.1508	42.8	1.676	0.234	7.17	10.26
Cypress	2.5	3.5	0.25	42.4	1.912	0.100	19.16	10.26
Cypress	2.5	3.5	1.251	40.8	0.783	0.056	13.97	11.15
Hardwood	5	7	1.003	40.3	2.315	0.108	21.47	10.26
Hardwood	5	7	1.2502	40.5	1.806	0.103	17.46	10.26
Hardwood	2.5	3.5	0.0501	42.8	2.158	0.292	7.40	10.26
Hardwood	2.5	3.5	0.1506	42.4	1.872	0.177	10.60	10.26
Hardwood	2.5	3.5	0.5007	41.9	1.007	0.125	8.06	10.26
Hardwood	2.5	3.5	0.7501	41.4	1.293	0.067	19.40	10.26

Table F3: Raw data for binary atrazine Freundlich isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg atrazine/L*	*mg/g*	
Cedar	5	7	0.5014	41.6	2.851	0.178	10.26
Cedar	5	7	0.7503	40.7	2.178	0.153	10.26
Cedar	5	7	1.0017	40.1	1.574	0.137	10.26
Cedar	5	7	1.2508	39.9	1.498	0.112	10.26
Cedar	5	7	0.7513	41.2	2.041	0.162	11.15
Cedar	5	7	1.0045	40.47	1.639	0.135	11.15
Cedar	5	7	1.2525	39.6	1.381	0.114	11.15
Cedar	2.5	3.5	0.2506	42.2	1.661	0.141	10.26
Cedar	2.5	3.5	1.0016	40.5	0.789	0.069	10.26
Cedar	2.5	3.5	1.0033	40.6	0.642	0.075	11.15
Cedar	2.5	3.5	1.2506	40.4	0.672	0.059	11.15
Cypress	5	7	0.503	41.4	2.595	0.198	10.26
Cypress	5	7	0.75	41.6	2.289	0.150	10.26
Cypress	5	7	1.0036	40.7	1.677	0.135	10.26
Cypress	5	7	1.5008	40	1.230	0.100	10.26
Cypress	5	7	1.0038	40.9	2.012	0.122	11.15
Cypress	5	7	1.2527	40	1.809	0.102	11.15
Cypress	2.5	3.5	0.5032	42	1.302	0.100	10.26
Cypress	2.5	3.5	0.7516	41.9	0.820	0.094	10.26
Cypress	2.5	3.5	1.0028	40.8	0.701	0.073	10.26
Cypress	2.5	3.5	0.7503	41.6	0.942	0.086	11.15
Cypress	2.5	3.5	1.0022	40.8	0.671	0.074	11.15
Hardwood	5	7	0.2506	42.1	3.457	0.259	10.26
Hardwood	5	7	0.5	41.6	2.797	0.183	10.26
Hardwood	5	7	0.7508	41.3	2.376	0.144	10.26
Hardwood	5	7	0.7514	41.3	2.414	0.142	11.15
Hardwood	5	7	1.0015	40.9	1.936	0.125	11.15
Hardwood	5	7	1.2508	40.1	1.715	0.105	11.15
Hardwood	2.5	3.5	0.2507	42.3	1.790	0.120	10.26
Hardwood	2.5	3.5	0.7505	41.5	1.013	0.082	11.15
Hardwood	2.5	3.5	1.0015	40.5	0.864	0.066	11.15
Hardwood	2.5	3.5	1.2517	40.6	0.771	0.056	11.15

Table F4: Control data for binary isotherm

Mulch Type	Initial Conc.	Initial Conc.	Mulch Weight	Liquid Volume	Final Conc. (C_e)	Conc. on Mulch (q_e)	Date
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg atrazine/L*	*mg/g*	
No Mulch	5	7	0	43	5.011	5.99	10.26
No Mulch	5	7	0	42.7	5.146	7.47	11.15

Table F5: Raw data for binary atrazine Freundlich isotherm removed based on a 95% confidence interval

Mulch Type	Initial Conc.	Initial Conc.	Mulch Weight	Liquid Volume	Final Conc. (C_e)	Conc. on Mulch (q_e)	Date
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg atrazine/L*	*mg/g*	
Cedar	5	7	1.5009	39.4	1.285	0.098	10.26
Cedar	2.5	3.5	0.1506	42.9	1.891	0.173	10.26
Cedar	2.5	3.5	0.5013	41.6	1.339	0.096	10.26
Cedar	2.5	3.5	0.7506	41	1.075	0.078	10.26
Cedar	2.5	3.5	0.7517	40.7	0.757	0.094	11.15
Cypress	5	7	1.25	39.4	1.555	0.109	10.26
Cypress	5	7	0.7514	41.4	1.083	0.216	11.15
Cypress	2.5	3.5	0.1508	42.8	1.676	0.234	10.26
Cypress	2.5	3.5	0.25	42.4	1.912	0.100	10.26
Cypress	2.5	3.5	1.251	40.8	0.783	0.056	11.15
Hardwood	5	7	1.003	40.3	2.315	0.108	10.26
Hardwood	5	7	1.2502	40.5	1.806	0.103	10.26
Hardwood	2.5	3.5	0.0501	42.8	2.158	0.292	10.26
Hardwood	2.5	3.5	0.1506	42.4	1.872	0.177	10.26
Hardwood	2.5	3.5	0.5007	41.9	1.007	0.125	10.26
Hardwood	2.5	3.5	0.7501	41.4	1.293	0.067	10.26

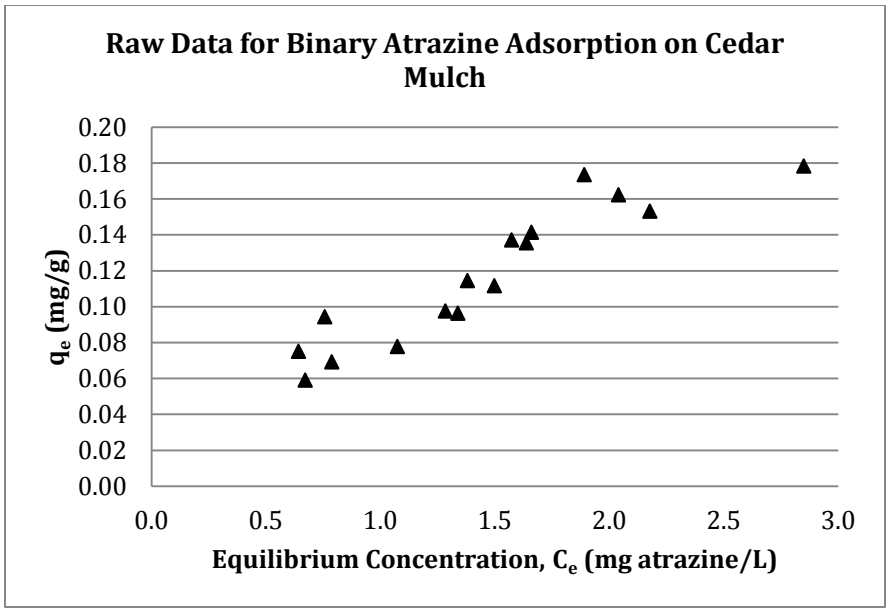

Figure F1: Raw data for binary atrazine isotherm on cedar mulch

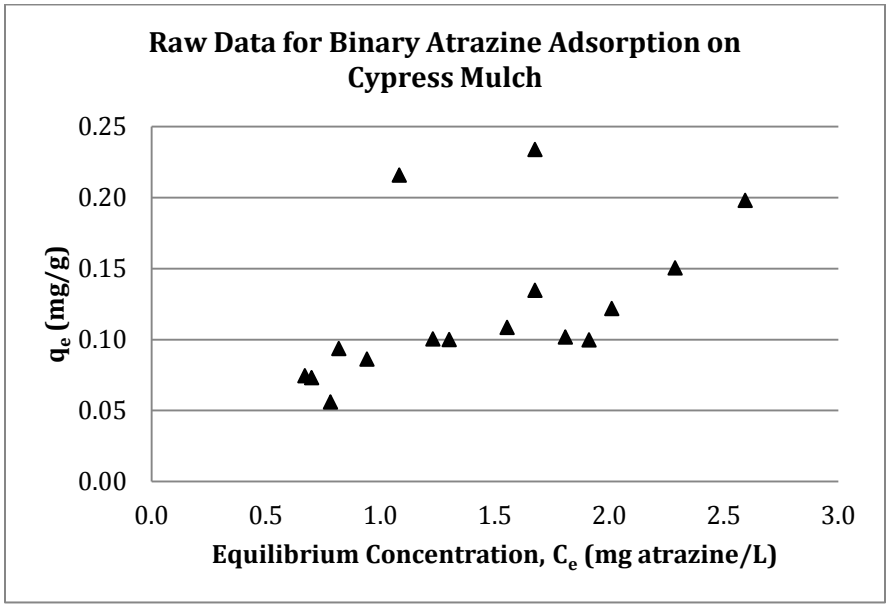

Figure F2: Raw data for binary atrazine isotherm on cypress mulch

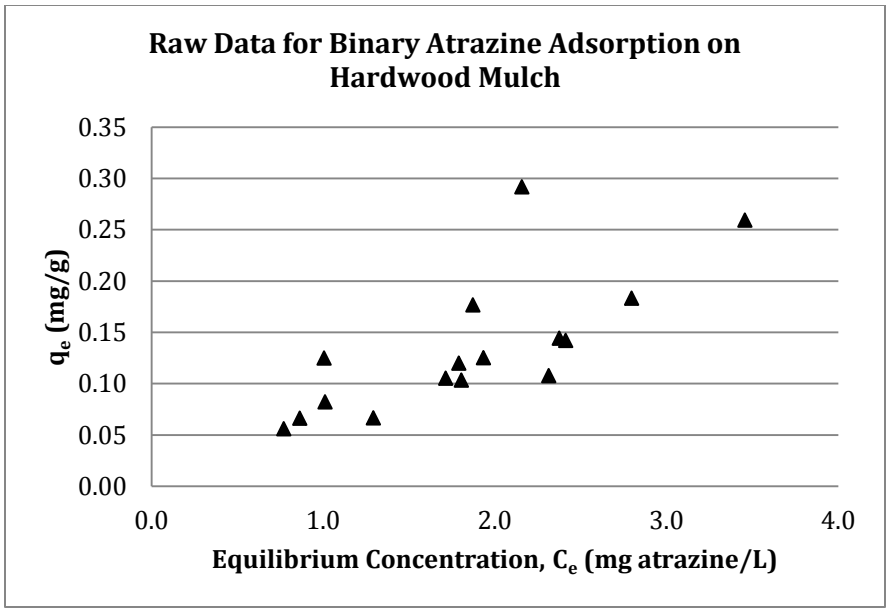

Figure F3: Raw data for binary atrazine isotherm on hardwood mulch

Appendix G: Raw Data for Binary Nitrate System

The following tables include the raw data for the binary isotherm for nitrate. Nitrate was analyzed using two isotherms, Langmuir and Freundlich. Tables G1 (Langmuir) and G4 (Freundlich) shows the data after a statistical analysis removing data points that had a final solution concentration either greater than 90% or less than 10% of the initial solution concentration and that fell outside a 95% confidence interval (90-10 Rule). The data points that were removed based on the 90-10 rule can be seen in Table G2. The data points that were removed based on a 95% confidence interval can be seen in Table G2 (Langmuir) and G5 (Freundlich). Control tests were performed to ensure the atrazine or nitrate were not adhering to the glass containers. The data from the control tests can be seen in Appendix F, Table F2. The raw data is plotted in Figures G1-G3.

Table G1: Raw data for binary nitrate Langmuir isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Vol.**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg NO_3-N/L*	*mg/g*	*g/L*	
Cedar	5	7	0.5014	41.6	5.21	0.15	35.08	10.26
Cedar	5	7	0.7503	40.7	5	0.11	46.09	10.26
Cedar	5	7	1.0017	40.1	4.9	0.08	58.29	10.26
Cedar	5	7	1.2508	39.9	5.07	0.06	82.35	10.26
Cedar	5	7	1.5009	39.4	5.16	0.05	106.83	10.26
Cedar	2.5	3.5	0.2506	42.2	2.13	0.23	9.23	10.26
Cedar	2.5	3.5	0.5013	41.6	2.54	0.08	31.88	10.26
Cedar	2.5	3.5	0.7506	41	2.65	0.05	57.08	10.26
Cedar	2.5	3.5	0.7517	40.7	2.7	0.04	62.33	11.15
Cedar	2.5	3.5	1.0016	40.5	2.38	0.05	52.55	10.26
Cedar	2.5	3.5	1.0033	40.6	2.85	0.03	108.35	11.15
Cedar	2.5	3.5	1.2506	40.4	2.81	0.02	126.06	11.15
Cypress	5	7	0.503	41.4	4.76	0.18	25.82	10.26
Cypress	5	7	0.75	41.6	5.09	0.11	48.05	10.26
Cypress	5	7	0.7514	41.4	6.2	0.04	140.66	11.15
Cypress	5	7	1.0036	40.7	4.96	0.08	59.95	10.26
Cypress	5	7	1.25	39.4	5.17	0.06	89.63	10.26
Cypress	2.5	3.5	0.5032	42	2.6	0.08	34.61	10.26
Cypress	2.5	3.5	0.7503	41.6	2.39	0.06	38.83	11.15
Cypress	2.5	3.5	0.7516	41.9	2.58	0.05	50.30	10.26
Cypress	2.5	3.5	1.0028	40.8	2.74	0.03	88.61	10.26
Cypress	2.5	3.5	1.251	40.8	1.44	0.07	21.43	11.15
Hardwood	5	7	0.2506	42.1	4.68	0.39	12.01	10.26
Hardwood	5	7	0.5	41.6	5.21	0.15	34.98	10.26
Hardwood	5	7	0.7508	41.3	5.49	0.08	66.10	10.26
Hardwood	5	7	1.003	40.3	5.51	0.06	92.04	10.26
Hardwood	5	7	1.2508	40.1	6.24	0.02	256.10	11.15
Hardwood	2.5	3.5	0.0501	42.8	2.46	0.89	2.77	10.26
Hardwood	2.5	3.5	0.1506	42.4	2.48	0.29	8.64	10.26
Hardwood	2.5	3.5	0.2507	42.3	2.53	0.16	15.46	10.26
Hardwood	2.5	3.5	0.7501	41.4	2.78	0.04	69.96	10.26
Hardwood	2.5	3.5	0.7505	41.5	2.78	0.04	69.83	11.15
Hardwood	2.5	3.5	1.0015	40.5	2.77	0.03	93.83	11.15

Table G2: Raw data for binary nitrate isotherm removed based on the 90-10 rule

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg NO_3-N/L*	*mg/g*	*g/L*	
Cedar	5	7	0.7513	41.2	6.63	0.02	326.76	11.15
Cedar	5	7	1.0045	40.47	6.32	0.03	230.69	11.15
Cedar	5	7	1.2525	39.6	6.33	0.02	298.82	11.15
Cypress	5	7	1.0038	40.9	6.68	0.01	512.33	11.15
Cypress	5	7	1.2527	40	6.56	0.01	466.92	11.15
Hardwood	5	7	0.7514	41.3	6.72	0.02	436.65	11.15
Hardwood	5	7	1.0015	40.9	6.57	0.02	374.13	11.15

Table G3: Raw data for mono binary Langmuir isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**C_e/q_e**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg NO_3-N/L*	*mg/g*	*g/L*	
Cedar	2.5	3.5	0.1506	42.9	2.58	0.26	9.84	10.26
Cypress	5	7	1.5008	40	5.16	0.05	105.22	10.26
Cypress	2.5	3.5	0.1508	42.8	2.37	0.32	7.39	10.26
Cypress	2.5	3.5	0.25	42.4	2.32	0.20	11.59	10.26
Cypress	2.5	3.5	1.0022	40.8	2.87	0.03	111.90	11.15
Hardwood	5	7	1.2502	40.5	5.24	0.06	91.91	10.26
Hardwood	2.5	3.5	0.5007	41.9	2.73	0.06	42.37	10.26
Hardwood	2.5	3.5	1.2517	40.6	2.82	0.02	127.85	11.15

Table G4: Raw data for binary nitrate Freundlich isotherm after statistical analysis

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg NO_3-N/L*	*mg/g*	
Cedar	2.5	3.5	0.2506	42.2	2.13	0.230702	10.26
Cedar	2.5	3.5	0.5013	41.6	2.54	0.079665	10.26
Cedar	2.5	3.5	0.7506	41	2.65	0.04643	10.26
Cedar	2.5	3.5	0.7517	40.7	2.7	0.043315	11.15
Cedar	2.5	3.5	1.0033	40.6	2.85	0.026303	11.15
Cedar	2.5	3.5	1.2506	40.4	2.81	0.02229	11.15
Cedar	5	7	0.5014	41.6	5.21	0.148512	10.26
Cedar	5	7	0.7503	40.7	5	0.10849	10.26
Cedar	5	7	1.0017	40.1	4.9	0.084067	10.26
Cedar	5	7	1.2508	39.9	5.07	0.061566	10.26
Cedar	5	7	1.5009	39.4	5.16	0.048302	10.26
Cypress	2.5	3.5	0.5032	42	2.6	0.075119	10.26
Cypress	2.5	3.5	0.7516	41.9	2.58	0.051288	10.26
Cypress	2.5	3.5	1.0028	40.8	2.74	0.030921	10.26
Cypress	2.5	3.5	0.7503	41.6	2.39	0.061543	11.15
Cypress	2.5	3.5	1.0022	40.8	2.87	0.025648	11.15
Cypress	2.5	3.5	1.251	40.8	1.44	0.067185	11.15
Cypress	5	7	0.503	41.4	4.76	0.184366	10.26
Cypress	5	7	0.75	41.6	5.09	0.105941	10.26
Cypress	5	7	1.0036	40.7	4.96	0.08273	10.26
Cypress	5	7	1.25	39.4	5.17	0.057682	10.26
Cypress	5	7	1.5008	40	5.16	0.049041	10.26
Hardwood	2.5	3.5	0.1506	42.4	2.48	0.287171	10.26
Hardwood	2.5	3.5	0.5007	41.9	2.73	0.064436	10.26
Hardwood	2.5	3.5	0.7501	41.4	2.78	0.039739	10.26
Hardwood	2.5	3.5	0.7505	41.5	2.78	0.039813	11.15
Hardwood	2.5	3.5	1.0015	40.5	2.77	0.029521	11.15
Hardwood	2.5	3.5	1.2517	40.6	2.82	0.022056	11.15
Hardwood	5	7	0.2506	42.1	4.68	0.389753	10.26
Hardwood	5	7	0.5	41.6	5.21	0.148928	10.26
Hardwood	5	7	0.7508	41.3	5.49	0.083062	10.26
Hardwood	5	7	1.003	40.3	5.51	0.059867	10.26
Hardwood	5	7	1.2508	40.1	6.24	0.024365	11.15

Table G5: Raw data for mono binary Freundlich isotherm removed based on a 95% confidence interval

Mulch Type	**Initial Conc.**	**Initial Conc.**	**Mulch Weight**	**Liquid Volume**	**Final Conc. (C_e)**	**Conc. on Mulch (q_e)**	**Date**
	mg atrazine/L	*mg NO_3-N/L*	*grams*	*mL*	*mg NO_3-N/L*	*mg/g*	
Cedar	2.5	3.5	0.1506	42.9	2.58	0.262072	10.26
Cedar	2.5	3.5	1.0016	40.5	2.38	0.045288	10.26
Cypress	2.5	3.5	0.1508	42.8	2.37	0.320716	10.26
Cypress	2.5	3.5	0.25	42.4	2.32	0.200128	10.26
Cypress	5	7	0.7514	41.4	6.2	0.044078	11.15
Hardwood	2.5	3.5	0.0501	42.8	2.46	0.888463	10.26
Hardwood	2.5	3.5	0.2507	42.3	2.53	0.163666	10.26
Hardwood	5	7	1.2502	40.5	5.24	0.057015	10.26

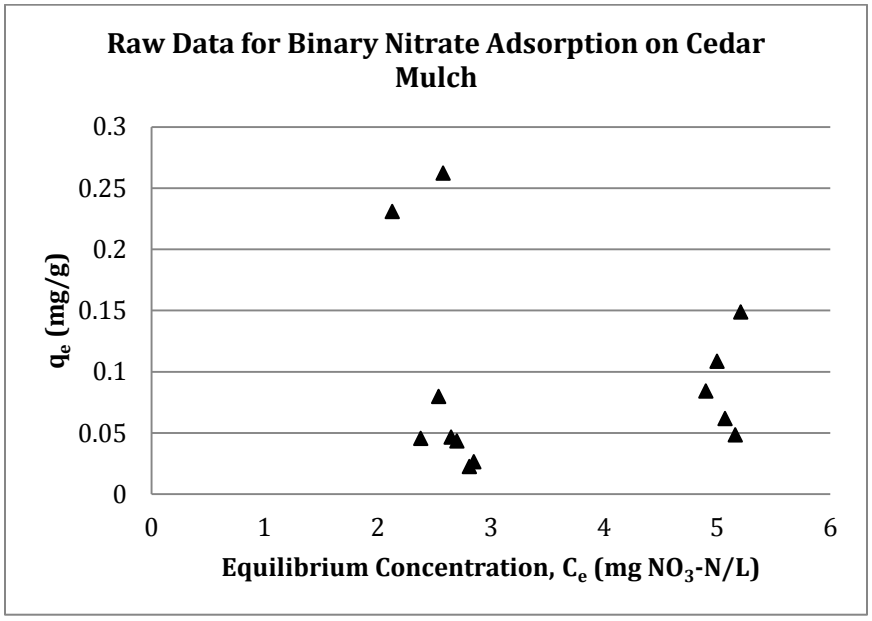

Figure G1: Raw data for binary nitrate isotherm on cedar mulch

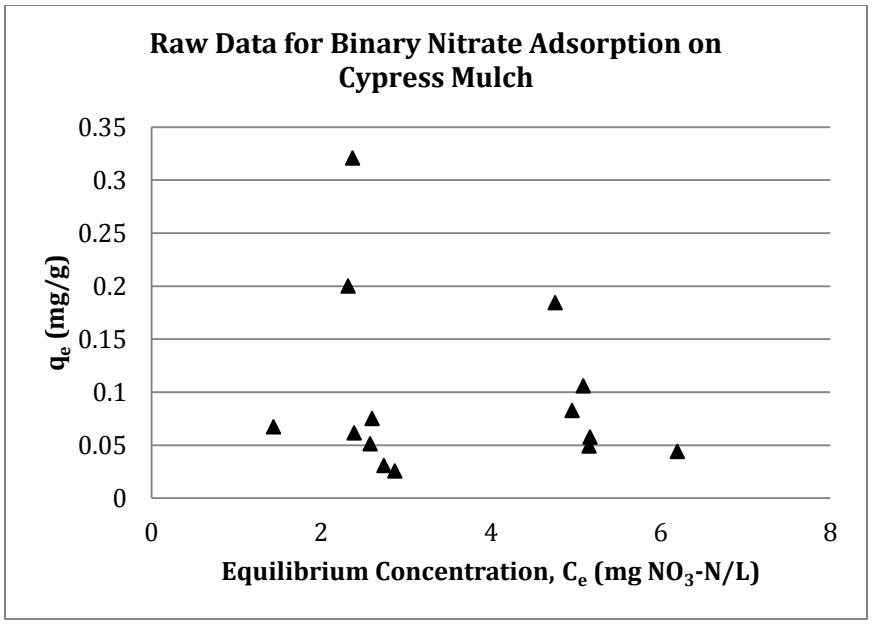

Figure G2: Raw data for binary nitrate isotherm on cypress mulch

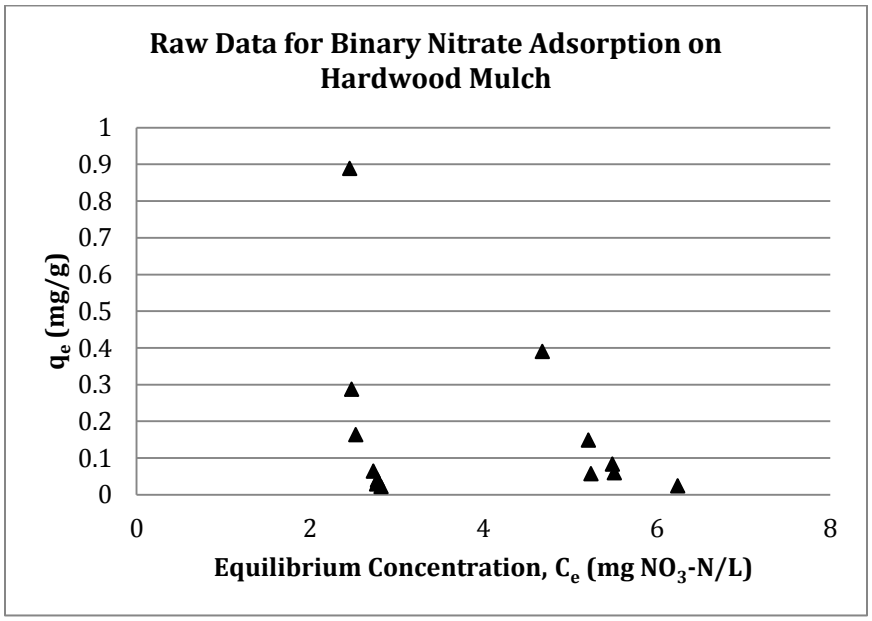

Figure G3: Raw data for binary nitrate isotherm on hardwood mulch

Appendix H: Graphs for Binary Atrazine System

The following figures show the graphs for atrazine in the binary system of atrazine-nitrate using Langmuir (Figures H1-H3) and Freundlich isotherms (Figures H4-H6).

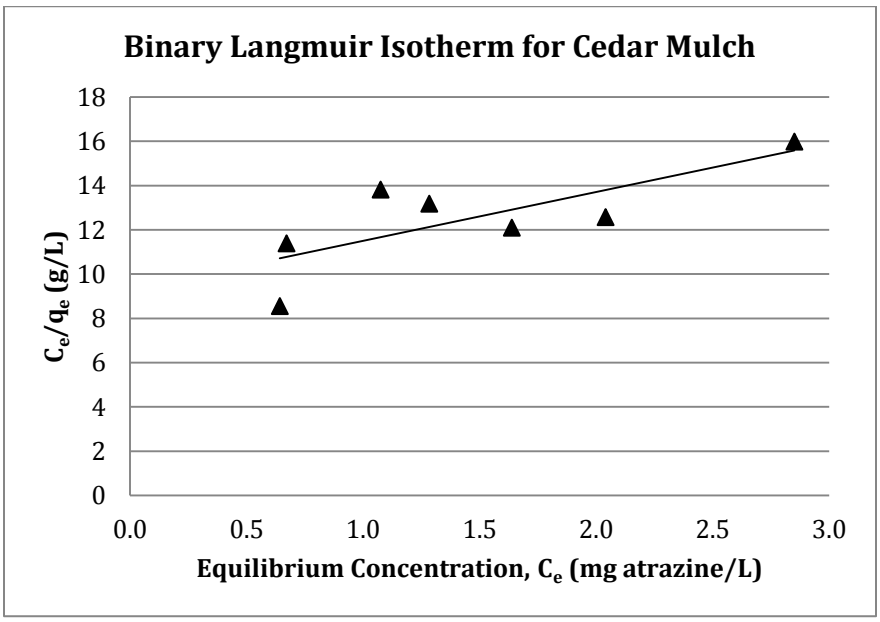

Figure H1: Langmuir adsorption isotherm for atrazine in the binary system on cedar mulch

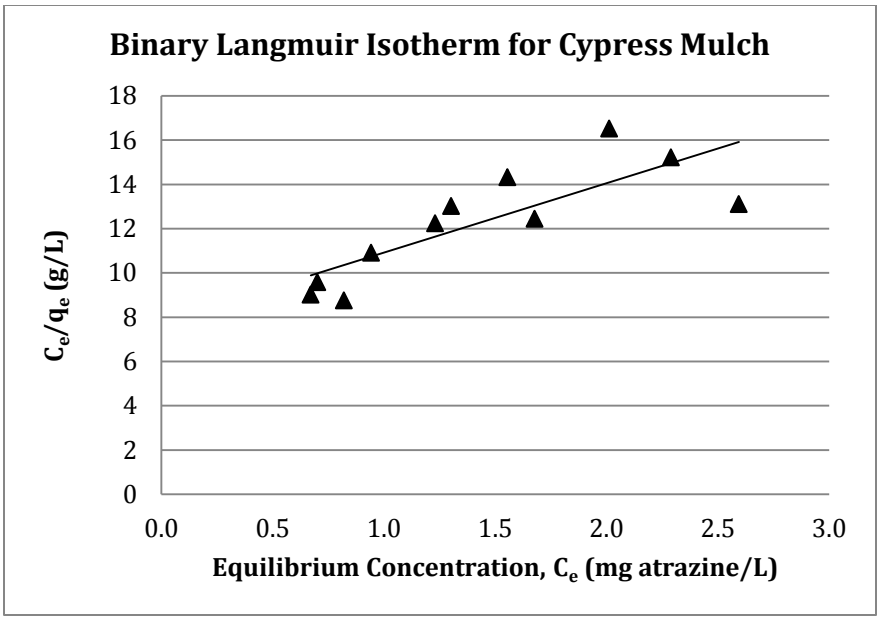

Figure H2: Langmuir adsorption isotherm for atrazine in the binary system on cypress mulch

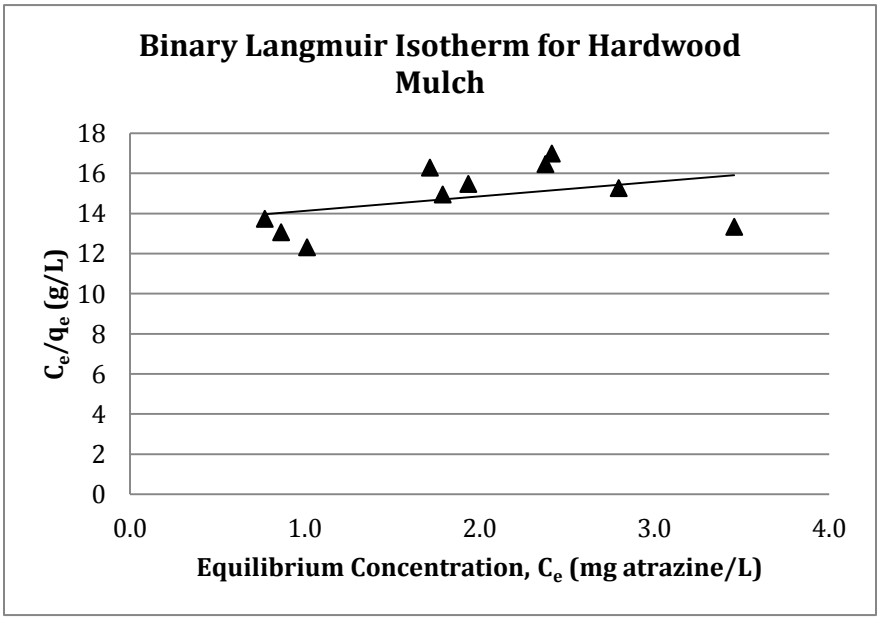

Figure H3: Langmuir adsorption isotherm for atrazine in the binary system on hardwood mulch

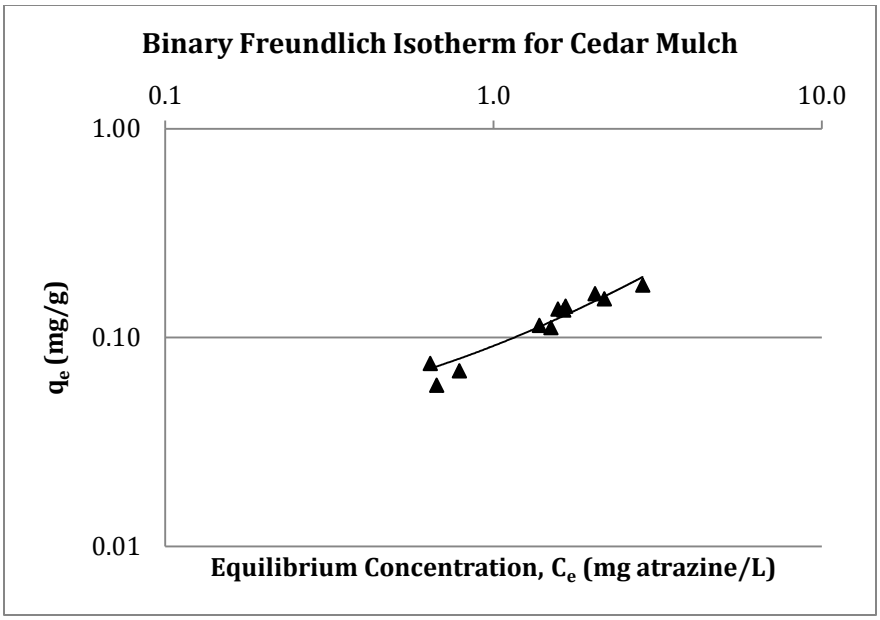

Figure H4: Freundlich adsorption isotherm for atrazine in the binary system on cedar mulch

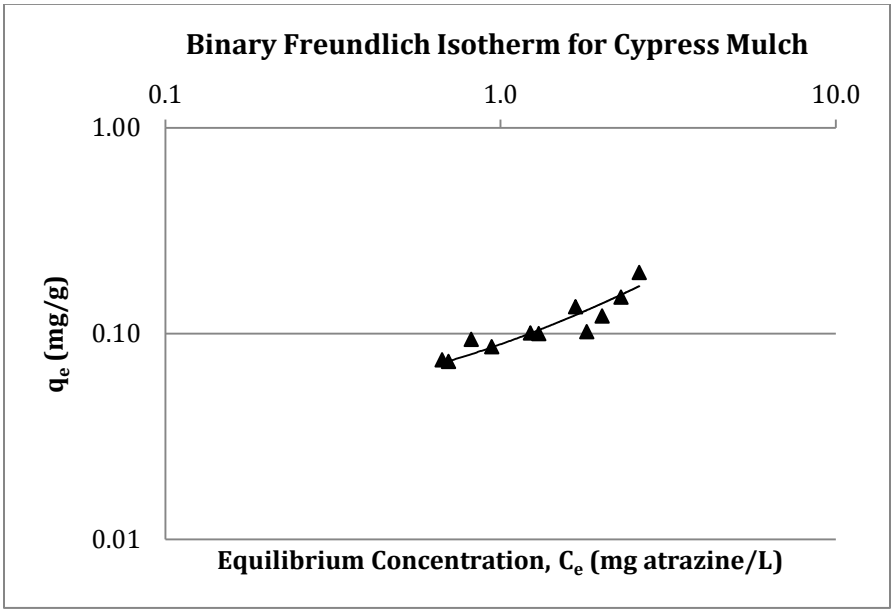

Figure H5: Freundlich adsorption isotherm for atrazine in the binary system on cypress mulch

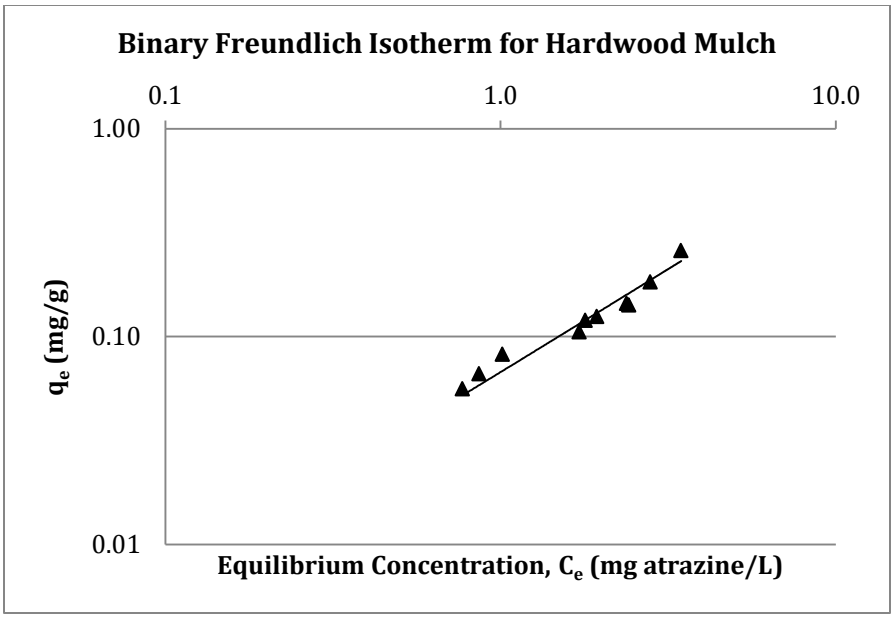

Figure H6: Freundlich adsorption isotherm for atrazine in the binary system on hardwood mulch

Appendix I: Graphs for Binary and Mono Atrazine System

The following figures show the graphs of the binary and mono Langmuir isotherms for atrazine (Figures I1-I3). The graphs for binary and mono Freundlich isotherms on cedar and hardwood mulch are also shown (Figures I4-I5).

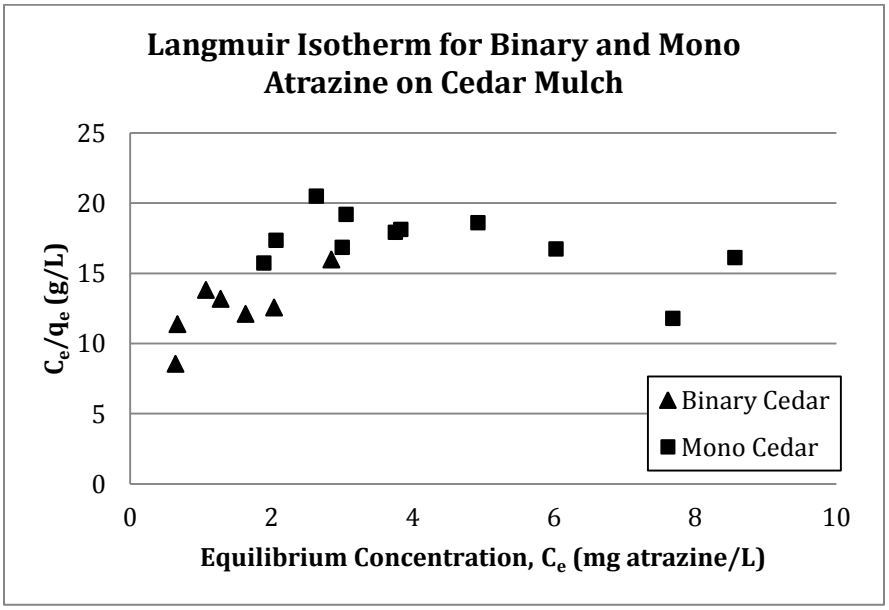

Figure I1: Langmuir adsorption isotherm for atrazine on cedar in the presence and absence of nitrate.

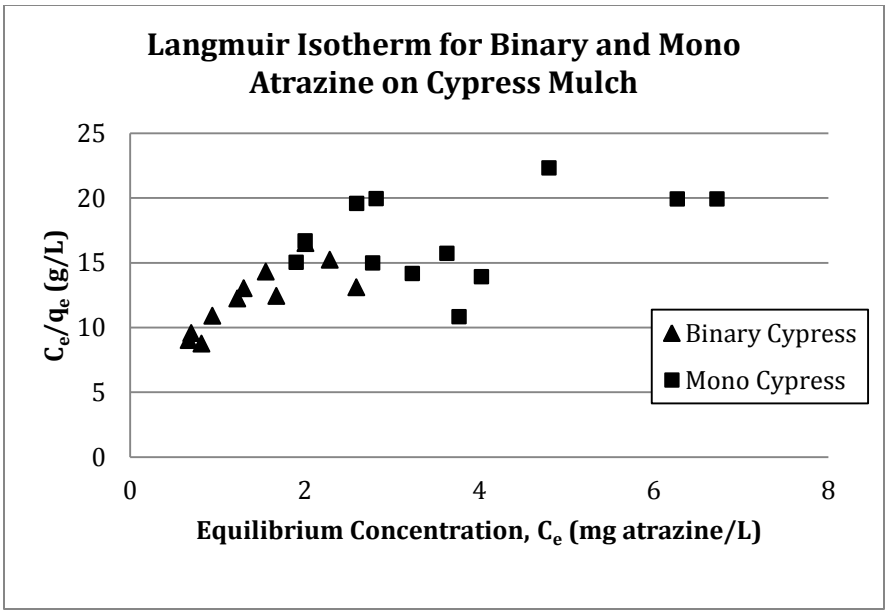

Figure I2: Langmuir adsorption isotherm for atrazine on cypress in the presence and absence of nitrate.

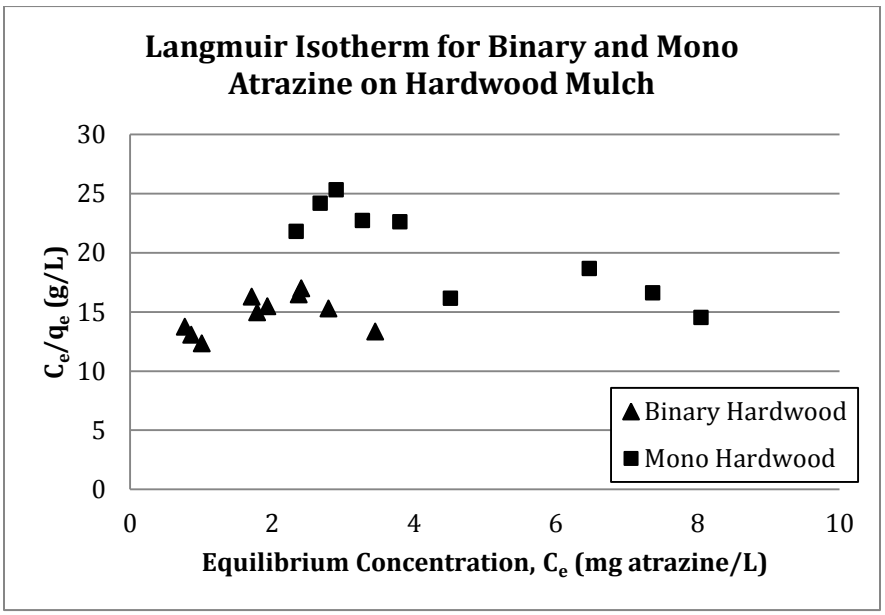

Figure I3: Langmuir adsorption isotherm for atrazine on hardwood in the presence and absence of nitrate.

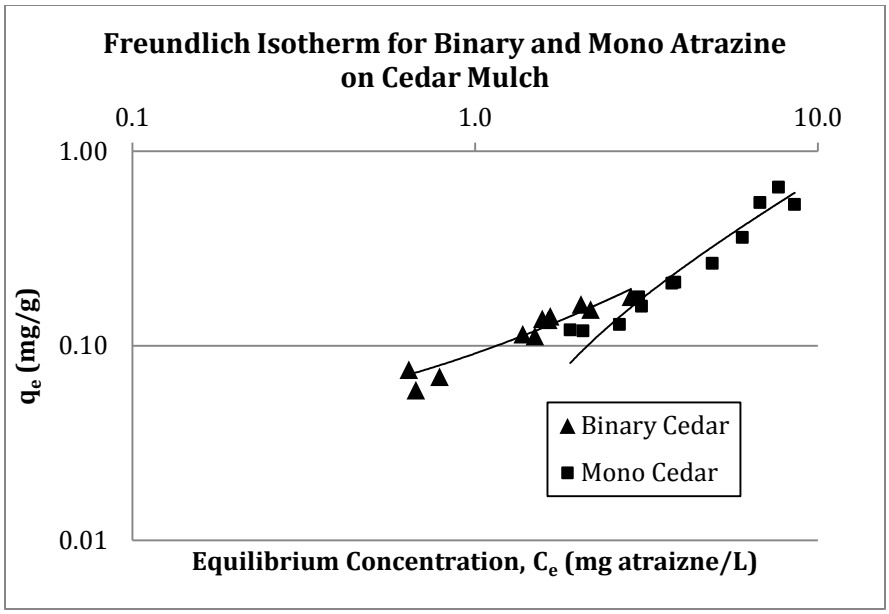

Figure I4: Freundlich adsorption isotherm for atrazine on cedar in the presence and absence of nitrate.

The equations for the best fit lines in Figure I4 are as follows:

In the mono cedar (absence of nitrate), the equation is:

$$\log(q_e) = 0.0794\log(C_e) - 0.069 \quad (1)$$

where q_e is the amount of atrazine adsorbed on the mulch at equilibrium (mg/g) and C_e is the concentration of atrazine in solution at equilibrium (mg/L). This equation has an R^2 value of 0.91.

In the binary cedar (presence of nitrate), the equation is:

$$\log(q_e) = 0.0563\log(C_e) + 0.035 \quad (2)$$

This equation has an R^2 of 0.92.

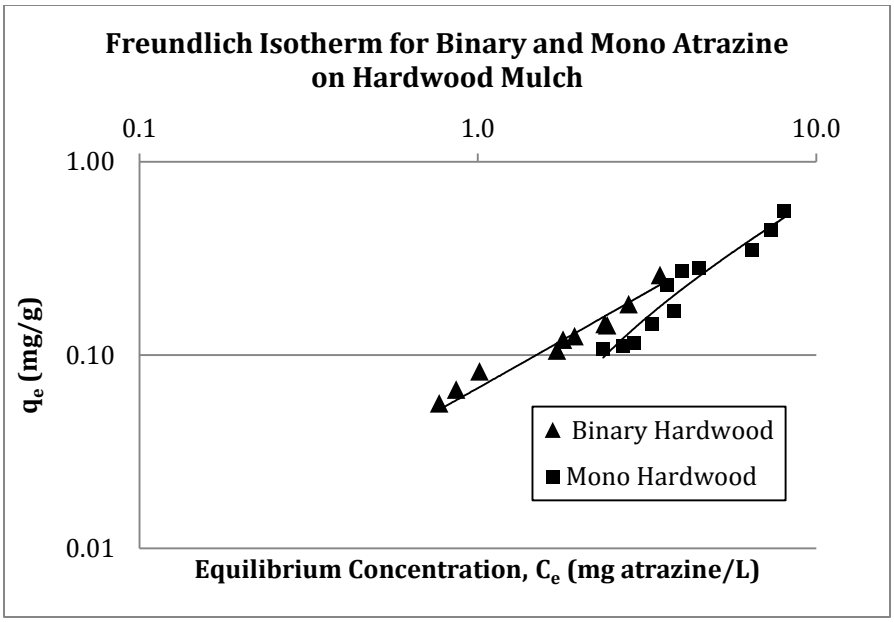

Figure I5: This figure shows the Freundlich adsorption isotherm for atrazine on hardwood in the presence and absence of nitrate.

The equations for the best fit lines in Figure I5 are as follows:

In the mono hardwood (absence of nitrate), the equation is:

$$\log(q_e) = 0.0733\log(C_e) - 0.0749 \quad (3)$$

where q_e is the amount of atrazine adsorbed on the mulch at equilibrium (mg/g) and C_e is the concentration of atrazine in solution at equilibrium (mg/L). This equation has an R^2 value of 0.94.

In the binary hardwood (presence of nitrate), the equation is:

$$\log(q_e) = 0.0666\log(C_e) + 0.0009 \quad (4)$$

This equation has an R^2 of 0.94.

Appendix J: Graphs for Binary Nitrate System, C_o=7 mg NO_3-N/L

The following figures show the graphs for nitrate in the binary system of atrazine-nitrate with an initial concentration of 7 mg NO_3-N/L. The entire nitrate binary isotherm was analyzed using both a Langmuir isotherm (Figure J1) and a Freundlich isotherm (Figure J5). Each type of mulch was analyzed using both the Langmuir isotherm (Figures J2-J4) and Freundlich isotherm (Figures J6-J8) for an initial concentration of 7 mg NO_3-N /L.

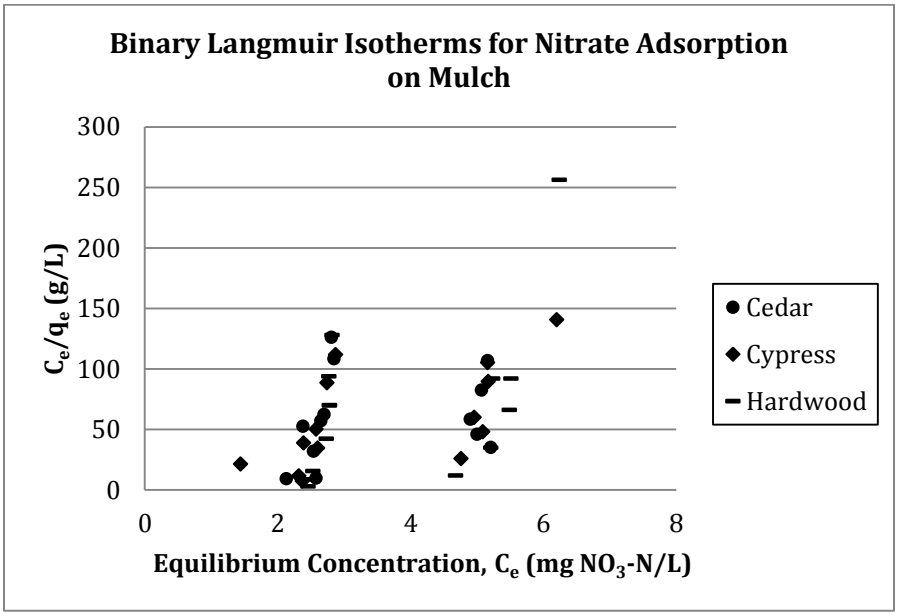

Figure J1: Langmuir adsorption isotherm for nitrate adsorbed on mulch in the binary system of atrazine-nitrate

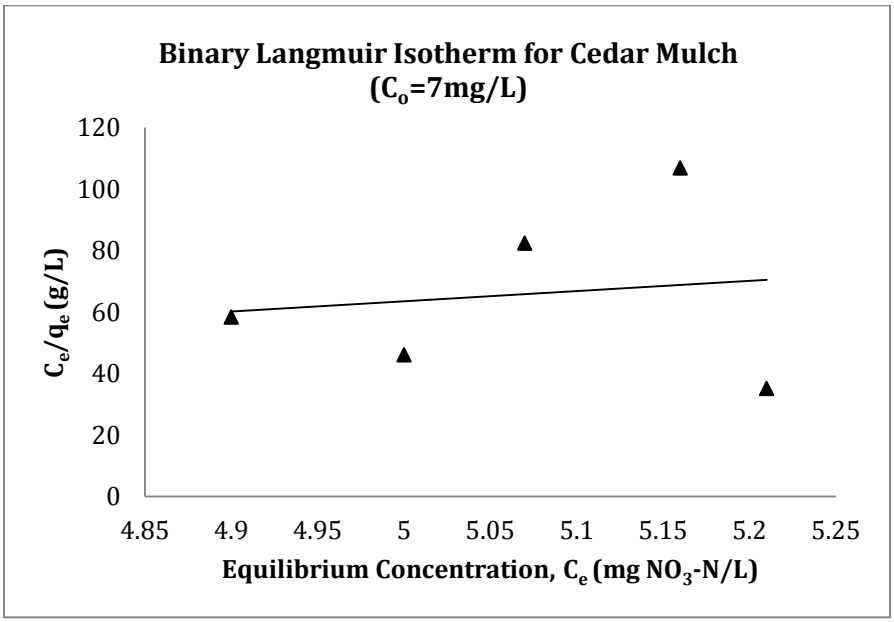

Figure J2: Langmuir adsorption isotherm for nitrate at an initial concentration of 7 mg NO_3-N/L in the binary system on cedar mulch

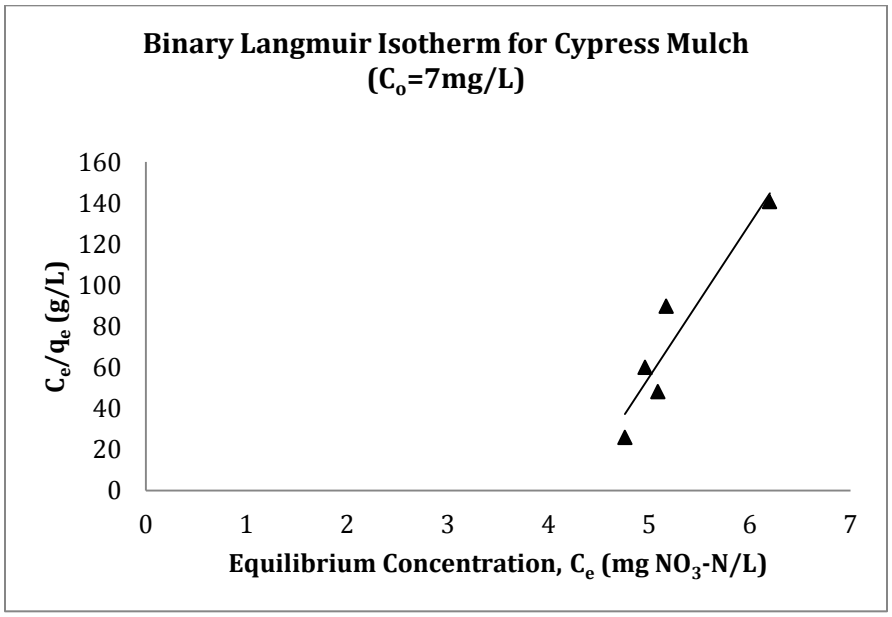

Figure J3: Langmuir adsorption isotherm for nitrate at an initial concentration of 7 mg NO_3-N/L in the binary system on cypress mulch

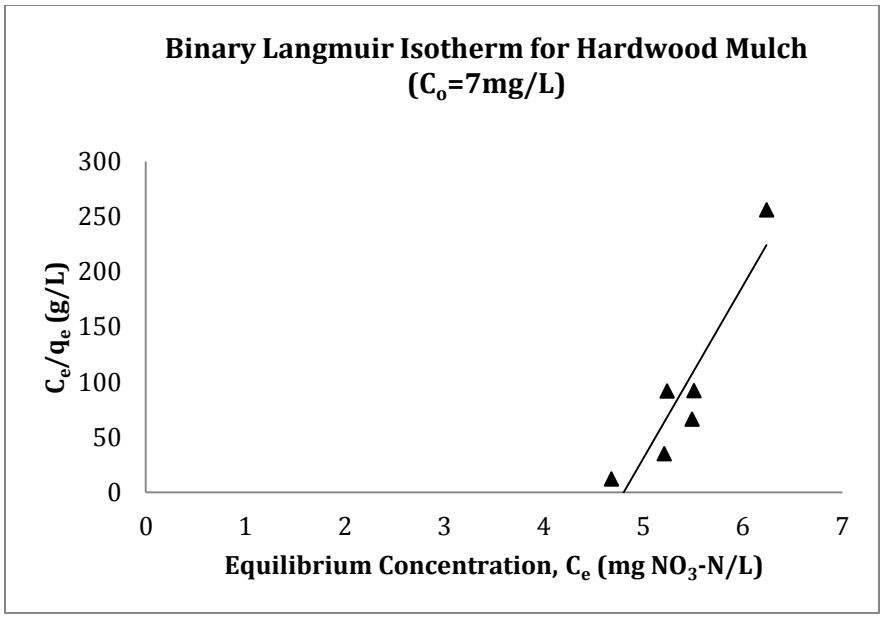

Figure J4: Langmuir adsorption isotherm for nitrate at an initial concentration of 7 mg NO_3-N/L in the binary system on hardwood mulch

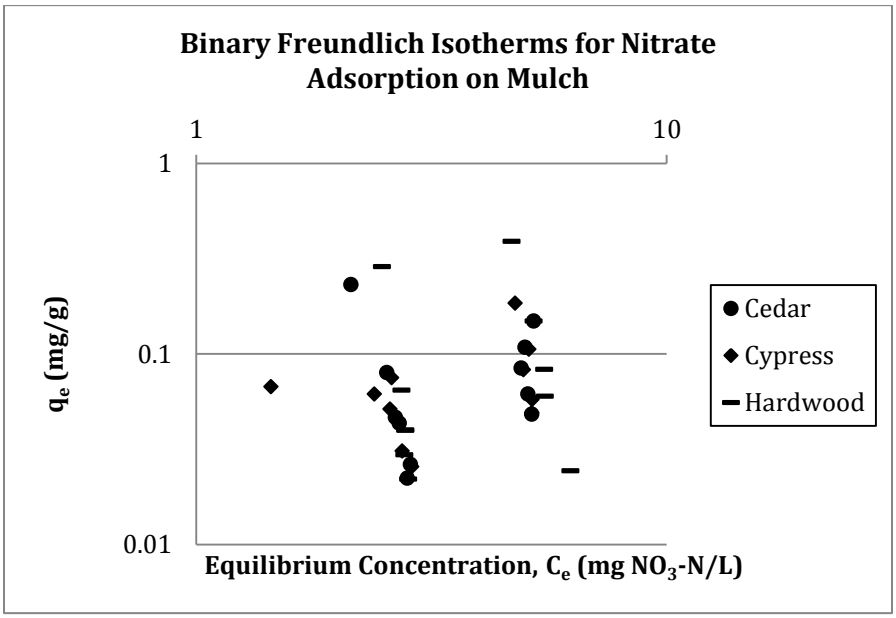

Figure J5: Freundlich adsorption isotherm for nitrate adsorption on mulch in the binary system of atrazine-nitrate

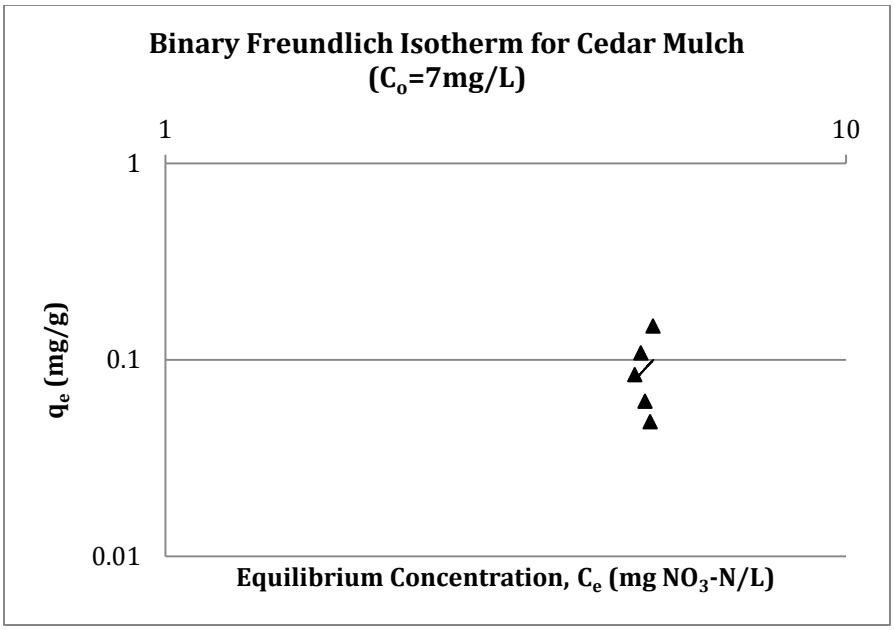

Figure J6: Freundlich adsorption isotherm for nitrate at an initial concentration of 7 mg NO3-N/L in the binary system on cedar mulch

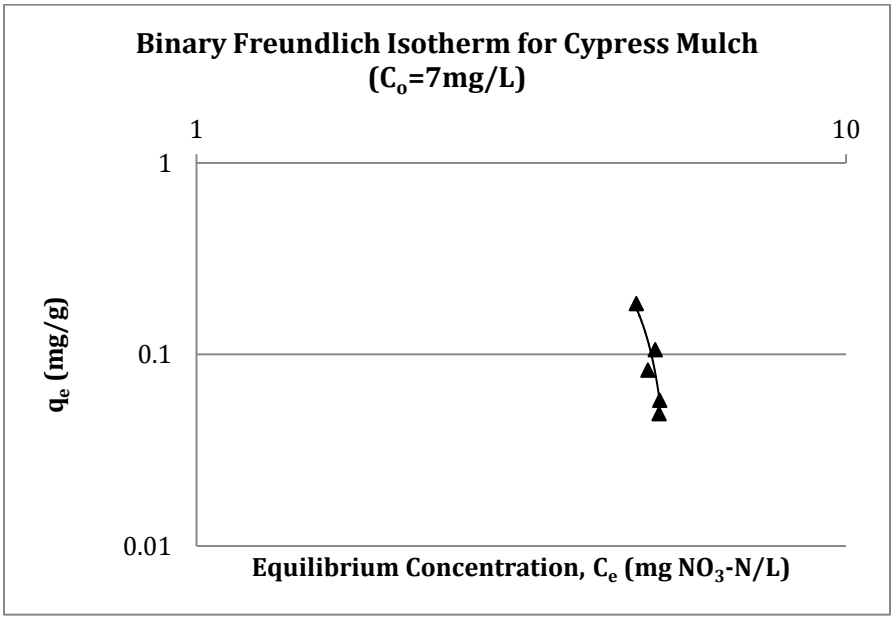

Figure J7: Freundlich adsorption isotherm for nitrate at an initial concentration of 7 mg NO_3-N/L in the binary system on cypress mulch

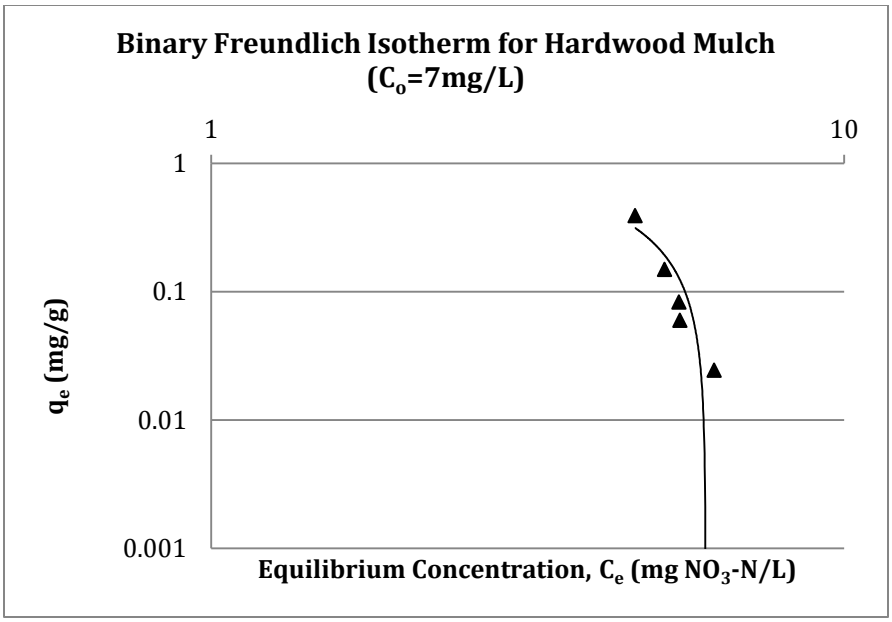

Figure J8: Freundlich adsorption isotherm for nitrate at an initial concentration of 7 mg NO_3-N/L in the binary system on hardwood mulch

Appendix K: Graphs for Binary Nitrate System C_o=3.5 mg NO_3-N/L

The following figures show the graphs for the binary isotherms for nitrate in the binary system of atrazine-nitrate with an initial concentration of 3.5 mg NO_3-N/L. The entire nitrate binary isotherm was analyzed using both a Langmuir isotherm (Figure K1) and a Freundlich isotherm (Figure K5). Each type of mulch was analyzed using both the Langmuir isotherm (Figures K2-K4) and Freundlich isotherm (Figures K6-K8) for an initial concentration of 3.5 mg NO_3-N /L.

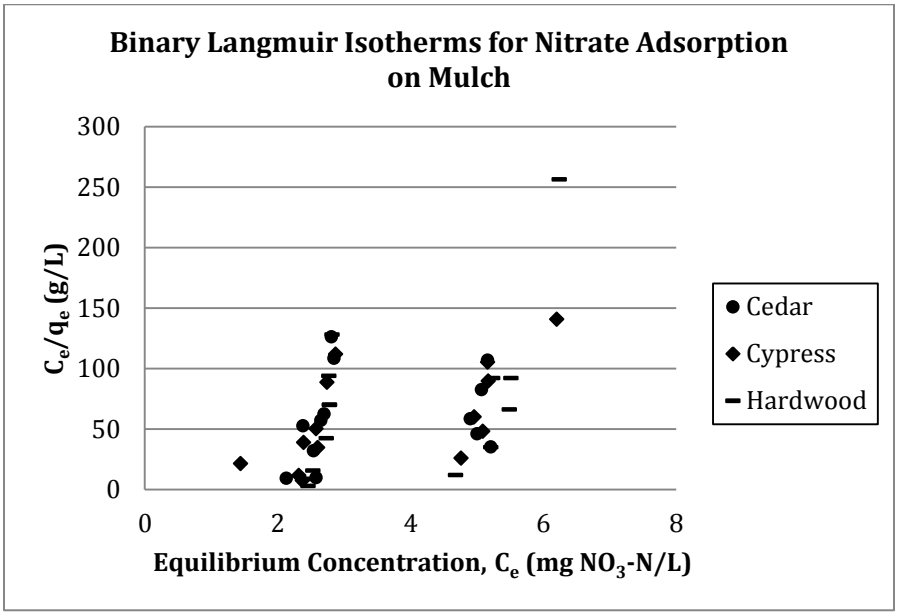

Figure K1: Langmuir adsorption isotherm for nitrate adsorbed on mulch in the binary system of atrazine-nitrate

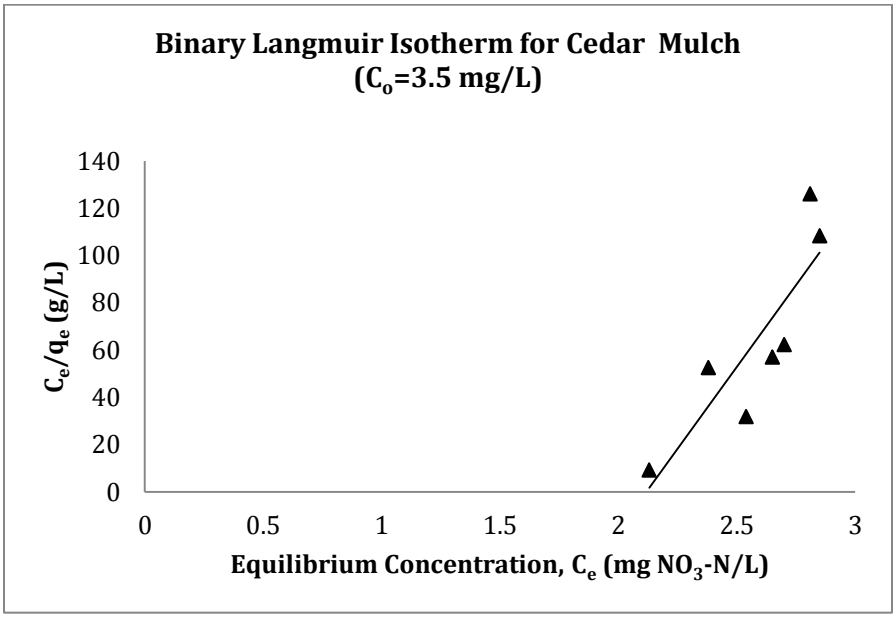

Figure K2: Langmuir adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on cedar mulch

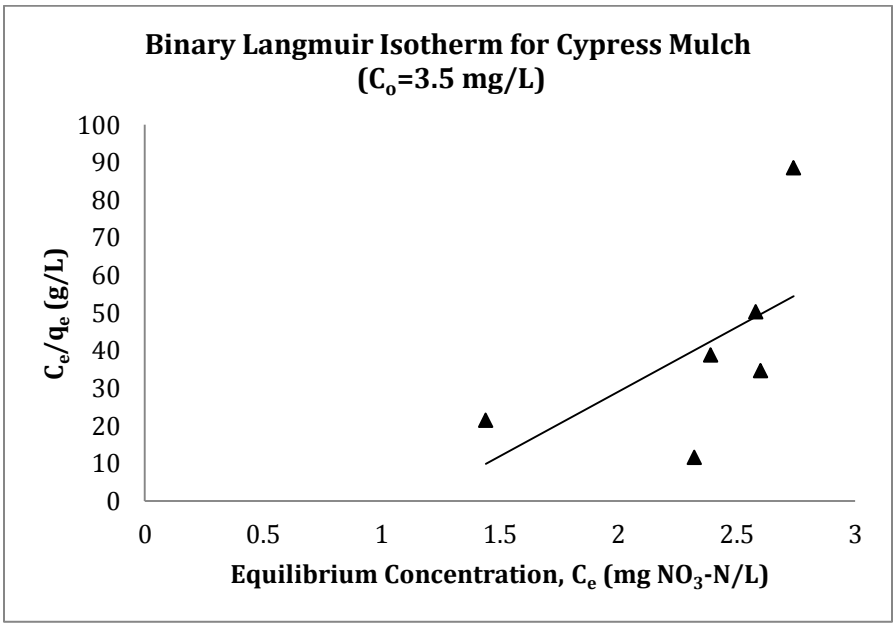

Figure K3: Langmuir adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on cypress mulch

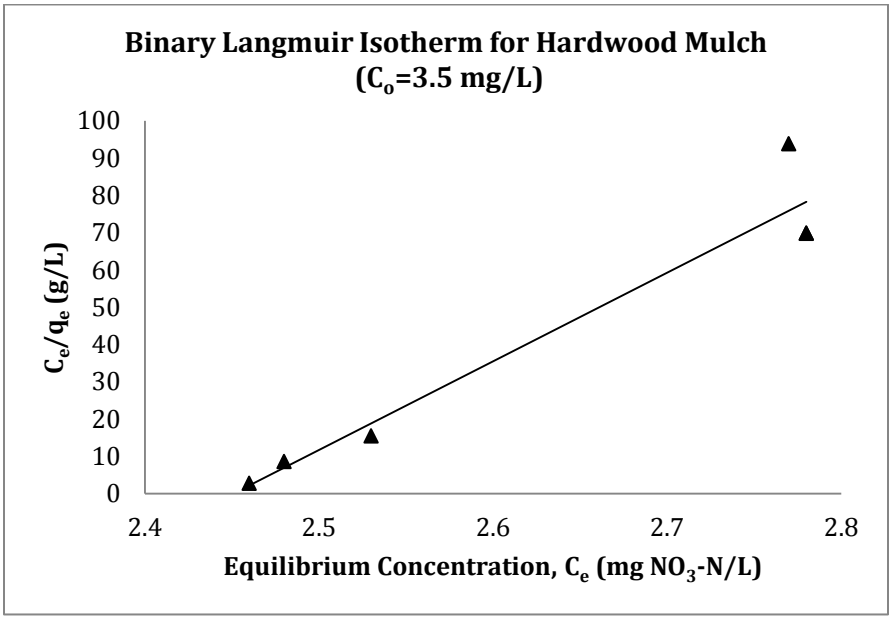

Figure K4: Langmuir adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on hardwood mulch

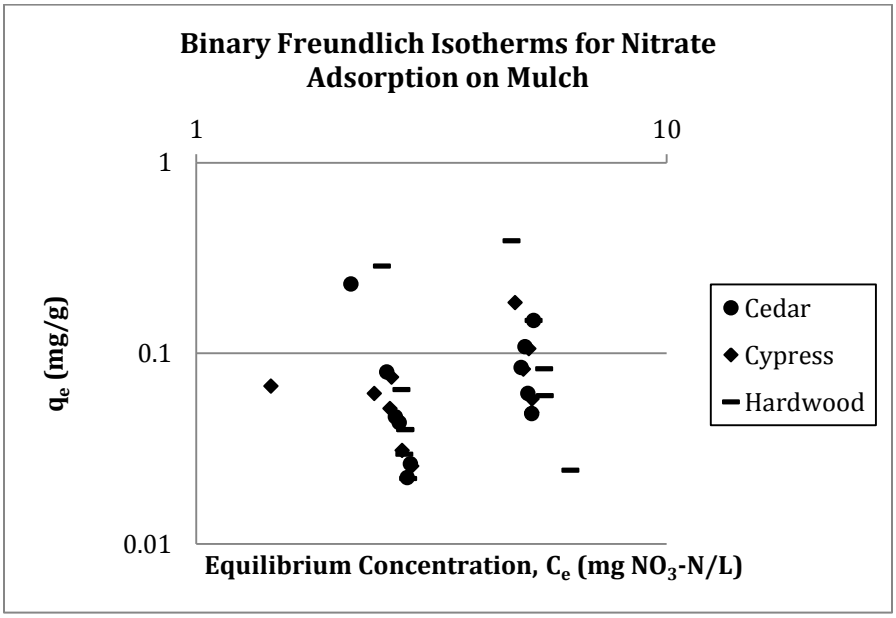

Figure K5: Freundlich adsorption isotherm for nitrate adsorption on mulch in the binary system of atrazine-nitrate

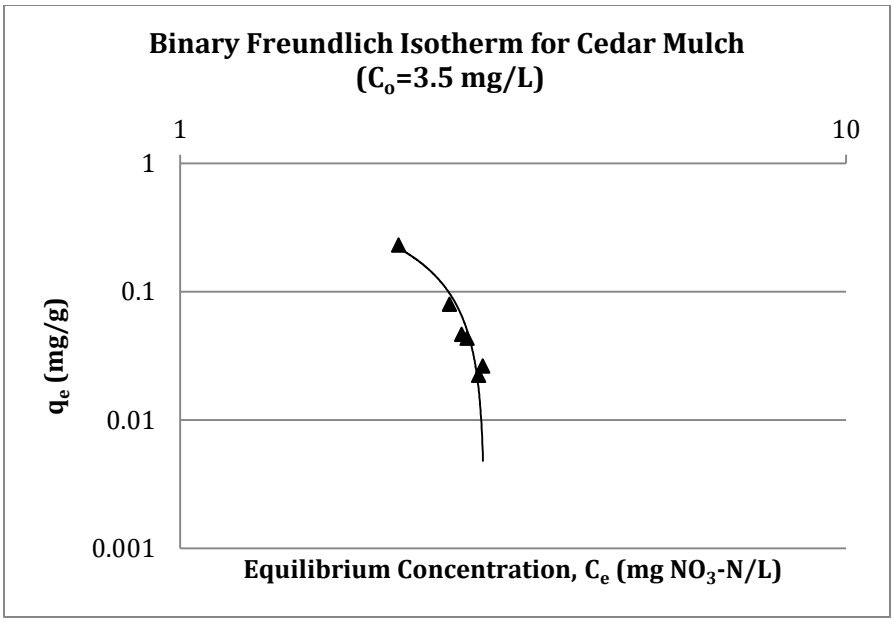

Figure K6: Freundlich adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on cedar mulch

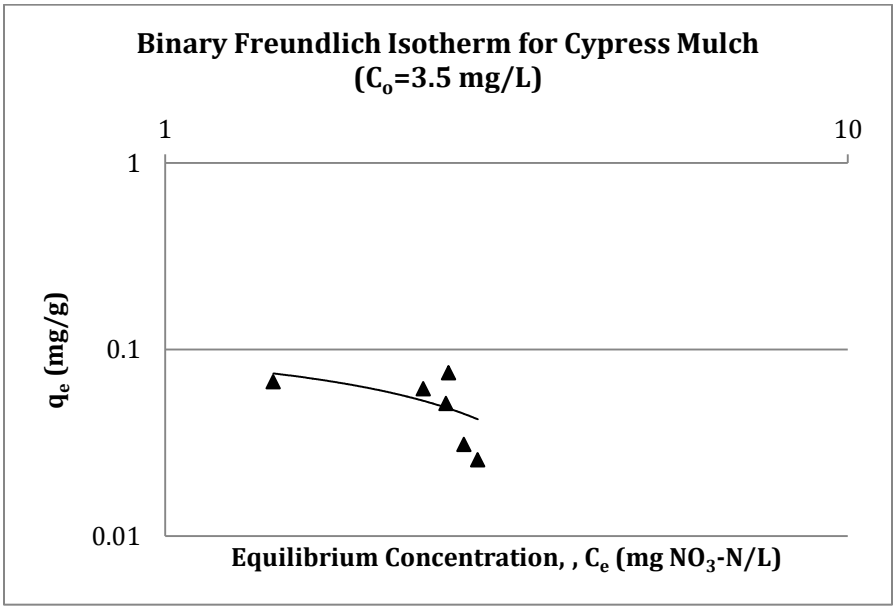

Figure K7: Freundlich adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on cypress mulch

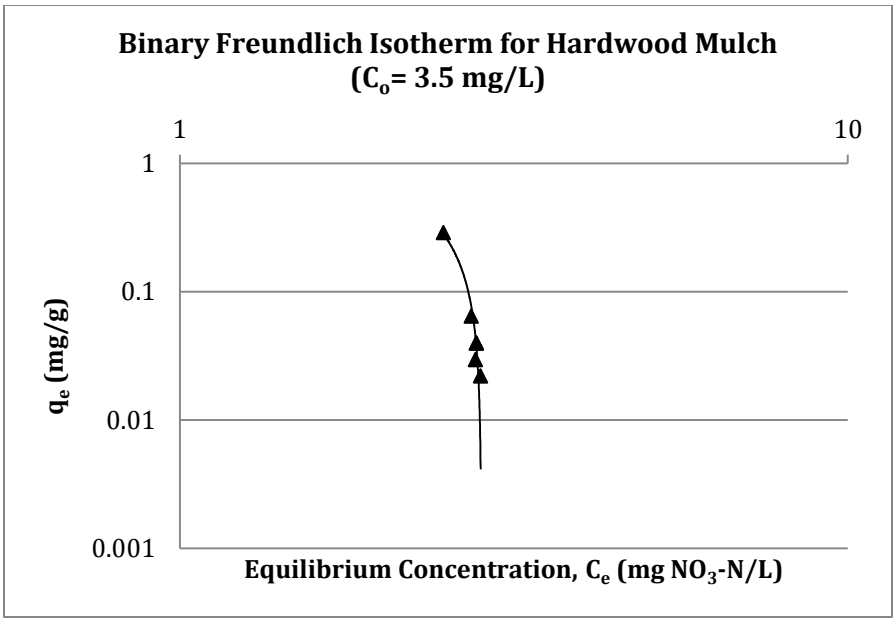

Figure K8: Freundlich adsorption isotherm for nitrate at an initial concentration of 3.5 mg NO_3-N/L in the binary system on hardwood mulch

Appendix L: Graphs for Binary and Mono Nitrate System

The following figures show the graphs of the binary (atrazine-nitrate) and mono isotherms for nitrate using a Langmuir isotherm (Figures L1-L3) and a Freundlich isotherm (Figures L4-L6).

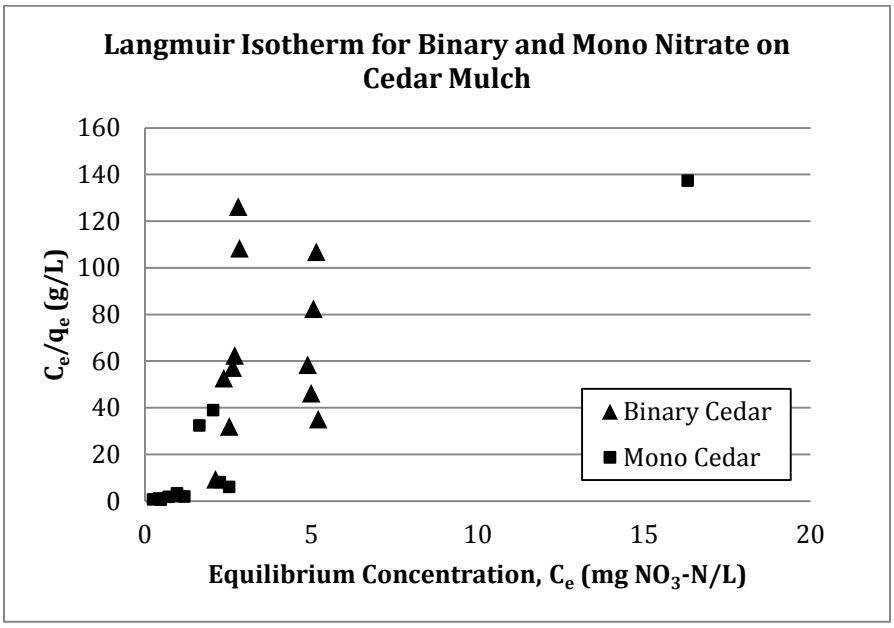

Figure L1: Langmuir adsorption isotherm for nitrate in the binary system on cedar mulch

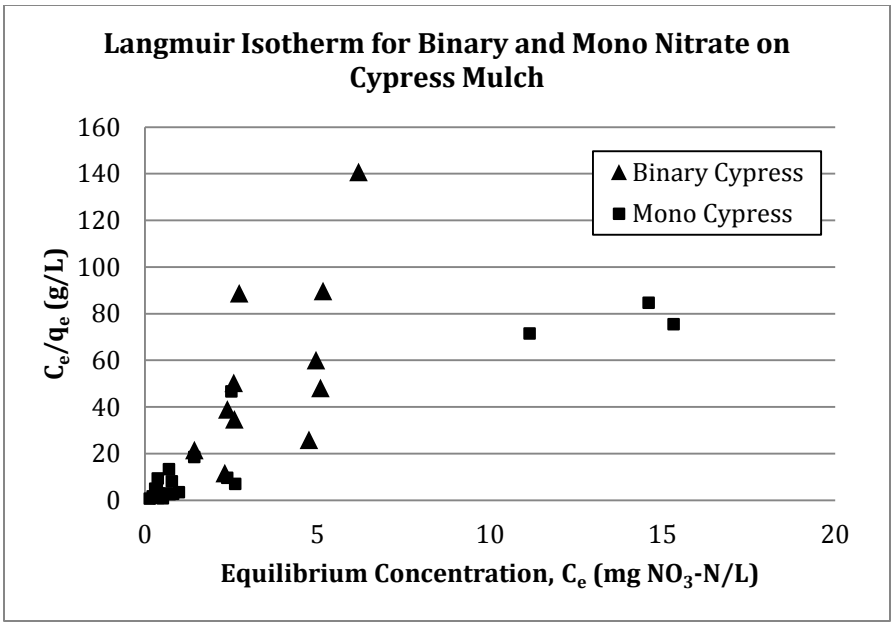

Figure L2: Langmuir adsorption isotherm for nitrate in the binary system on cypress mulch

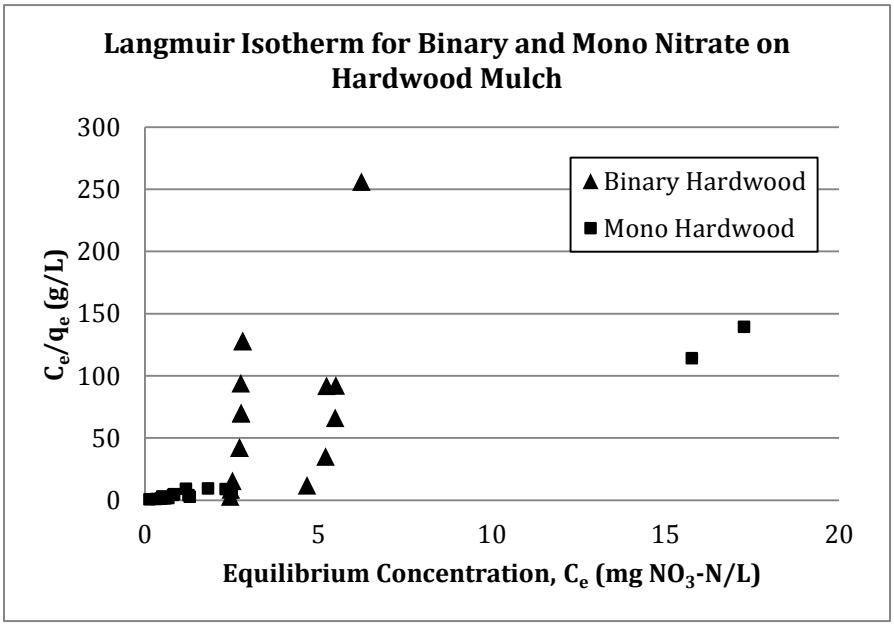

Figure L3: Langmuir adsorption isotherm for nitrate in the binary system on hardwood mulch

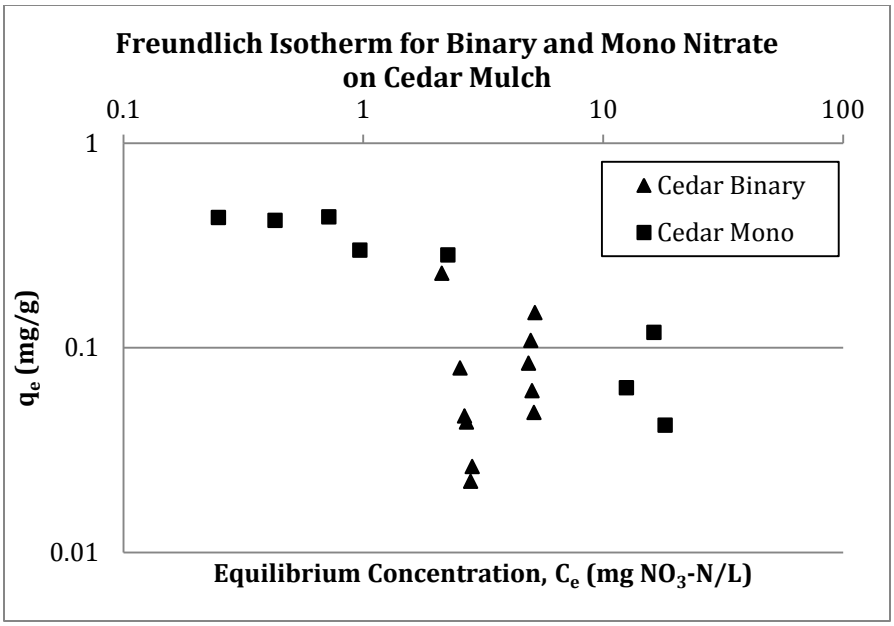

Figure L4: Freundlich adsorption isotherm for nitrate in the binary system on cedar mulch

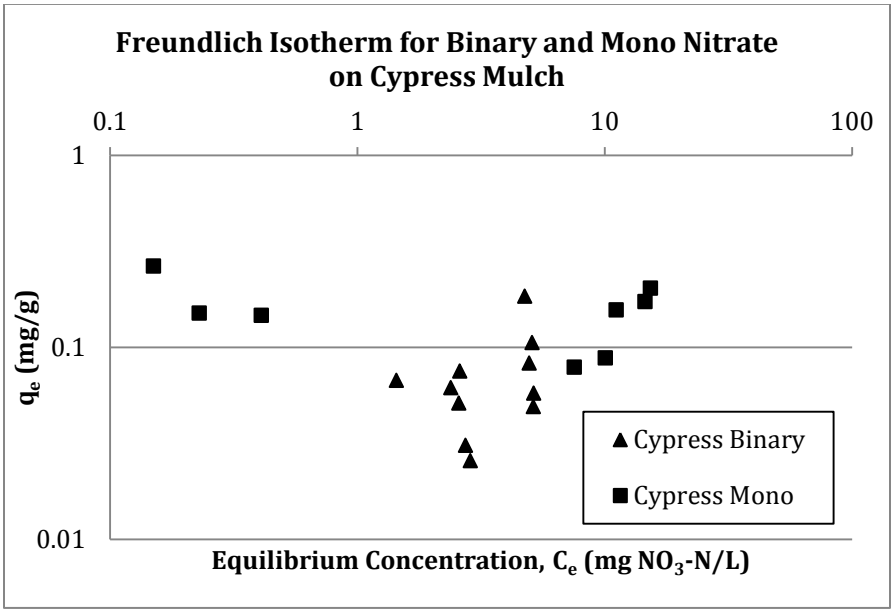

Figure L5: Freundlich adsorption isotherm for nitrate in the binary system on cypress mulch

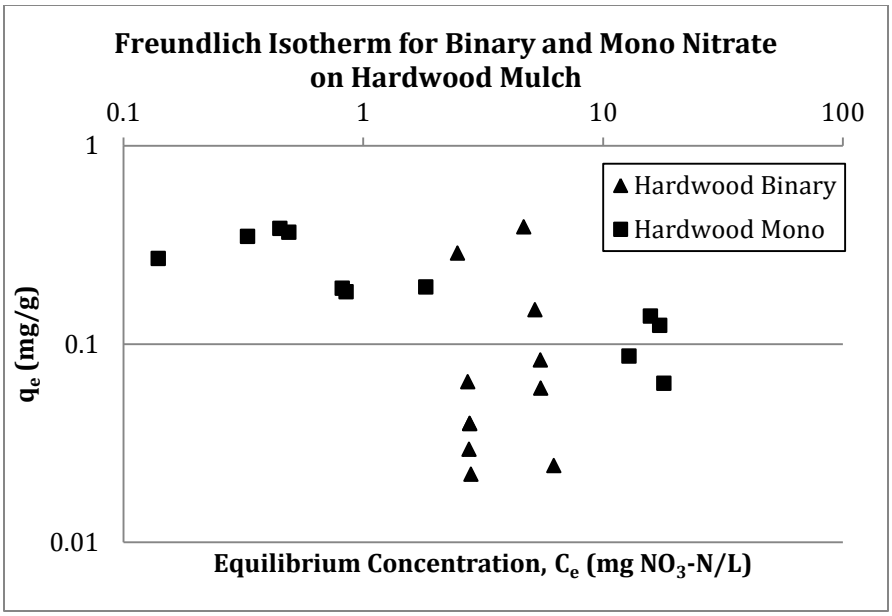

Figure L6: Freundlich adsorption isotherm for nitrate in the binary system on hardwood mulch

Appendix M: Raw Data for Biotic Column

The following tables include the raw data for the biotic cypress column. Table M1 lists the concentrations of atrazine and nitrate taken at the sample ports as well as notes about column operation and composition. Tables M2 and M3 list the measurements for dissolved oxygen and pH at the influent and effluent, respectively.

Table M1: Raw data for biotic cypress column

Date	Date #	Port	Calc. Initial Conc.	Final Conc. (C_e)	Calc. Initial Conc.	Final Conc. (C_e)
			mg atrazine /L	*mg atrazine/L*	*mg NO_3-N /L*	*mg NO_3-N /L*
3.6	*1*	*New Groundwater Solution*				
3.11	*6*	*New Groundwater Solution*125 mg/L sodium sulfite and 0.526 mg/L cobalt chloride added*				
3.15	*10*	*New Groundwater Solution*				
3.21	*16*	*New Groundwater Solution*				
3.23	18	1	1	0.73	7	5.33
3.23	18	3	1	0.73	7	5.22
3.23	18	5	1	0.74	7	5.1
3.23	18	6	1	0.61	7	5.29
3.25	*20*	*New Groundwater Solution*				
3.28	23	1	1	0.45	7	3.83
3.28	23	3	1	0.47	7	3.68
3.28	23	5	1	0.49	7	3.73
3.28	23	6	1	0.49	7	3.99
3.30	25	1	1	0.43	7	5.18
3.30	25	3	1	0.44	7	5.2
3.30	25	5	1	0.44	7	5.21
3.30	25	6	1	0.46	7	5.67
3.31	*26*	*New Groundwater Solution*				
4.3	29	1	1	0.56	7	3.47
4.3	29	3	1	0.68	7	3.53
4.3	29	5	1	0.78	7	3.56
4.3	29	6	1	0.61	7	1.88
4.5	*31*	*New Groundwater Solution*				
4.6	32	1	1	0.62	7	3.27
4.6	32	3	1	0.68	7	3.22
4.6	32	5	1	0.67	7	3.11
4.6	32	6	1	0.51	7	2.27
4.9	35	1	1	0.41	7	5.08
4.9	35	3	1	0.40	7	5.08
4.9	35	5	1	0.40	7	4.95
4.9	35	6	1	0.42	7	5.23
4.10	36	*New Groundwater Solution*250 mg/L sodium sulfite and 1.052 mg/L cobalt chloride (twice stoichiometric amount)*				

Table M1 Continued: Raw data for biotic cypress column

Date	Date #	Port	Calc. Initial Conc.	Final Conc. (C_e)	Calc. Initial Conc.	Final Conc. (C_e)
			mg atrazine /L	*mg atrazine/L*	*mg NO_3-N /L*	*mg NO_3-N /L*
4.16	42	*New Groundwater Solution*				
4.21	47	*New Groundwater Solution*				
4.30	56	*New Groundwater Solution*new sodium sulfite added*				
5.3	59	*New Groundwater Solution*				
5.4	60	*New Groundwater Solution* 24.3 mL/L acetic acid added*				
5.5	61	*New Groundwater Solution*24.3 mL/L acetic acid and 17,535 mg/L sodium hydroxide added*				
5.9	65	*Column Reseeded with Primary Effluent*				
5.10	66	*New Groundwater Solution*				
5.11	67	1	1	0.71	7	5.62
5.11	67	3	1	0.70	7	5.55
5.11	67	5	1	0.64	7	5.21
5.11	67	6	1	0.64	7	5.93
5.14	70	1	1	0.58	7	4.7
5.14	70	3	1	0.57	7	4.62
5.14	70	5	1	0.57	7	4.47
5.14	70	6	1	0.88	7	6.66
5.14	70	*New Groundwater Solution*				
5.17	73	1	1	0.65	7	8.13
5.17	73	3	1	0.74	7	6.48
5.17	73	5	1	0.73	7	5.76
5.17	73	6	1	0.82	7	5.74
5.17	73	*New Groundwater Solution*				
5.20	76	1	1	0.53	7	6.04
5.20	76	3	1	0.50	7	4.62
5.20	76	5	1	0.53	7	4.52
5.20	76	6	1	0.63	7	6.71
5.20	76	*New Groundwater Solution*				
5.22	78	1	1	0.42	7	4.73
5.22	78	3	1	0.43	7	4.51
5.22	78	5	1	0.42	7	4
5.22	78	6	1	0.59	7	5.79
5.23	79	*Column Stopped*				

Table M2: Influent pH and dissolved oxygen data for biotic cypress column

Date	**Date Number**	**pH**	**Dissolved Oxygen** *(mg O_2/L)*
3.12	7	*Sodium Sulfite and Cobalt Chloride Added*	
3.12	7	6.88	0.41
3.26	21	6.39	2.95
4.2	28	6.42	2.76
4.10	36	6.53	2.09
4.10	36	*Sodium Sulfite and Cobalt Chloride Added at Twice Stoichiometric Amount*	
4.18	44	6.48	1.49
4.19	45		2.36
4.20	46		2.68
4.21	47		4.03
4.21	47		1.43
4.23	49	*New Sodium Sulfite Added*	
4.23	49		2.75
4.30	56		2
4.30	56		1.71
4.30	56		1.39
5.1	57	7.19	2.3
5.3	59		3.13
5.3	59		2.91
5.3	59		0.72
5.3	59		2.81
5.4	60		2.44
5.5	61	*Acetic Acid Added*	
5.5	61	2.97	2.97
5.6	62	*Acetic Acid and Sodium Hydroxide Added*	
5.7	63	6.67	1.97
5.9	65	*Column Reseeded*	
5.10	66	6.63	2.36
5.14	70	6.63	1.38
5.14	70	6.64	1.13
5.22	78	6.58	1.15
Total Average		6.33	2.13
Average Since Acetic Acid Added		6.63	1.60

Table M3: Effluent pH and dissolved data for biotic cypress column

Date	**Date Number**	**pH**	**Dissolved Oxygen**
			mg O_2/L
5.7	63	6.28	0.89
5.14	70	6.61	1.71
5.22	78	6.57	1.65
Total Average		6.49	1.42

Appendix N: Graphs for the Biotic Column

The following figures show the measured concentrations of atrazine and nitrate in the third and fifth biotic cypress column ports. The measured concentrations of atrazine can be seen in Figure N1. The measured concentrations of nitrate can be seen in Figure N2. The influent concentrations of atrazine and nitrate were 1 mg atrazine/L and 7 mg NO_3-N/L, respectively. Note that on Day 61, acetic acid was added as a carbon source.

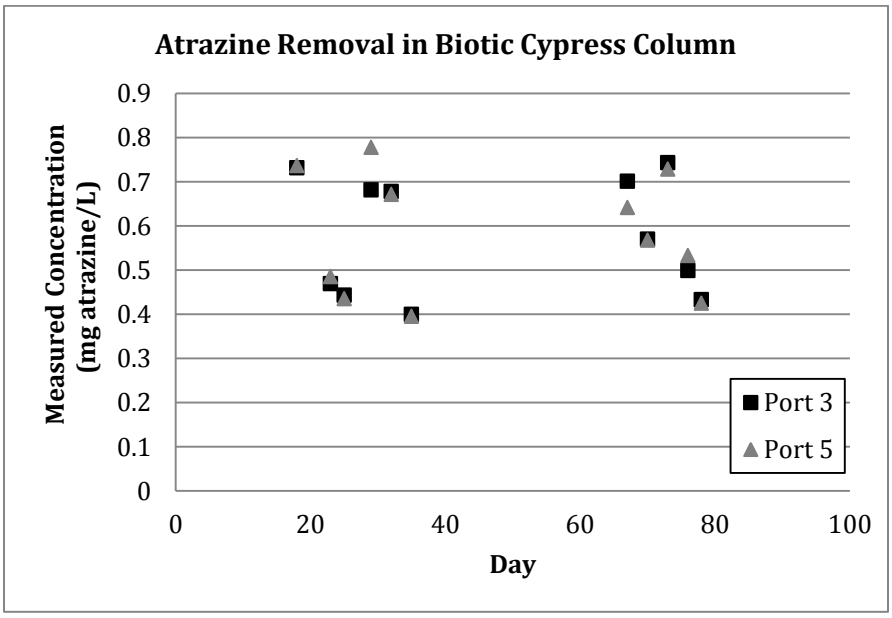

Figure N1: Measured atrazine concentrations in the third and fifth ports of a biotic cypress column.

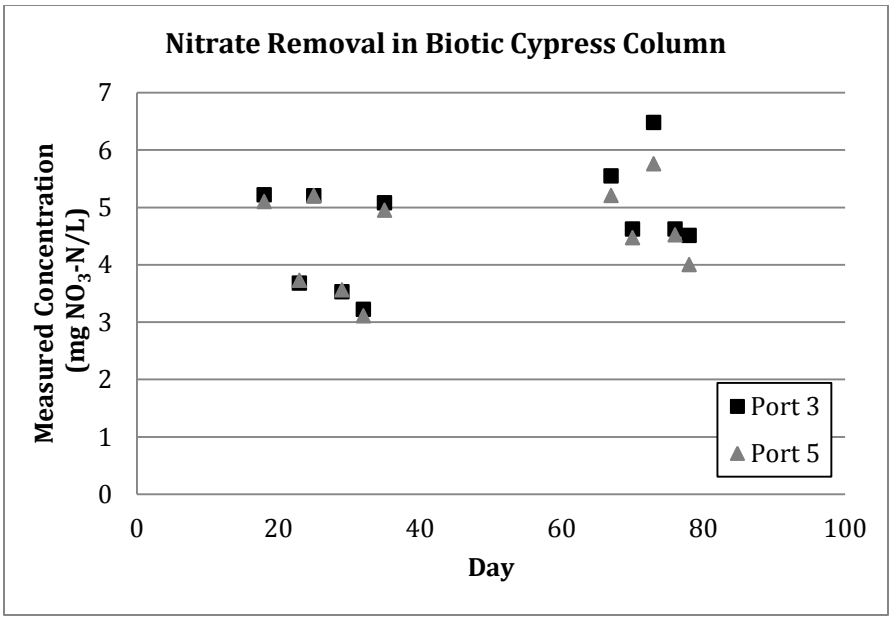

Figure N2: Measured nitrate concentrations in the third and fifth ports of a biotic cypress column.