ABSTRACT

ESLAMIAMIRABADI, MEHRNOOSH. Assessment of Alternative Herbicides for Root Intrusion Treatment and Their Effect on Wastewater Treatment Plant Processes. (Under the direction of Dr. Joel Ducoste.)

Roots intrusion into sewer lines poses significant problems in the conveyance of wastewater in sewer collection systems such as sewer blockages that produce sanitary sewer overflows (SSO), formation of septic pools that lead to the generation of hydrogen sulfide, structural pipe damage, and exfiltration of sewage into surrounding area of broken joints. The destruction of roots in sewer lines can be performed using non-chemical and chemical control methods. Chemical methods, which are mainly based on using herbicides, can significantly reduce the re-growth rate of the new roots but may impact the health of the above ground portion of the plants. In addition, these herbicides may lead to the contamination of downstream wastewater treatment plant unit processes. This research sought to evaluate alternative root control chemicals with high efficiency in killing intruded roots in sewer lines while minimizing adverse effect on the above ground portion of the plant and unit processes at wastewater treatment plants.

In this study, tests were performed with three alternative herbicides (Dithiopyr, Penoxsulam, and Triclopyr) to assess the mortality, root and shoot re-growth reduction, leaf damage, and overall health of exposed cottonwood tree roots. The results were compared with an existing commercial product based on Dichlobenil formula. Based on the Triphenyl Tetrazolium Chloride (TTC) results, Dichlobenil and Triclopyr displayed the highest root control (about 93% and 78%, respectively). However, the addition of these two herbicides caused the complete death of the upper portions of the plants. Dithiopyr, with the highest concentration (1% active ingredients) displayed only 76% root kill as well as root and shoot

re-growth after three weeks. Penoxsulam did not display any significant variation along the tested concentrations (i.e., the percentage root kill remained constant at 73%) and the above ground portion of the trees remained viable.

Additional tests were performed to assess the effect of herbicides on the nitrification process in the activated sludge system. Based on the results, all herbicides displayed nitrification inhibition using the same concentration at the point of application. None of the herbicides tested at diluted concentrations that will likely be measured at the entrance of the wastewater treatment plant displayed any nitrification inhibition. Based on these results, Penoxsulam (0.05% a.i.) was selected as the best performing herbicide based on cost and little impact on the health of the upper portion of the tree. However, in places where the health of the above ground portion of the trees is not an issue and based on a higher percent root kill, Triclopyr has been suggested as an alternative root control option.

Assessment of Alternative Herbicides for Root Intrusion Treatment and Their Effect on Wastewater Treatment Plant Processes

by
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DEDICATION

To my lovely parents,

My great sister, Forough,

And my awesome brother, Behrang.

BIOGRAPHY

Mehrnoosh was born and raised in Arak, Iran. She moved to Tehran, Iran to complete her undergraduate degree in Chemical Engineering at University of Tehran in August 2005. She worked on Moving Bed Biofilm Reactor (MBBR) for her Bachelors degree project and grew interest in environmental engineering. In August 2010, she enrolled in the Environmental Engineering program at North Carolina State University to pursue her Master of Science degree under her advisor Dr. Joel Ducoste. Mehrnoosh's research interests include water and wastewater treatment mainly focused on biological processes.

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1. INTRODUCTION

Intrusion of roots into sewer pipes is one of the most destructive problems encountered in a wastewater collection system. Root-related sewer problems include: (1) sewer stoppages and overflows; (2) structural pipe damage caused by growing roots; (3) formation of septic pools behind root masses that lead to the generation of hazardous atmospheres such as hydrogen sulfide, other gases, and odors; (4) reduction in hydraulic capacity and loss of self-scouring velocities; (5) infiltration in areas where pipes are seasonally under a water table; and (6) exfiltration of sewage into soils around cracked or separated joints (Duke et al., 2005). Approximately 25 percent of all collection systems contain roots (Hogan, 2001). Unfortunately, root intrusion problems are generally identified only after sewer stoppages and overflows have occurred (Duke et al., 2005). Therefore, an appropriate and preventive root control method must be performed to reduce the potential environmental and public health impact from a sanitary sewer overflow (SSO) event, capital cost, and damage to the sewer lines.

The destruction of root hair growth in pipes can be performed by non-chemical and chemical control methods. Planning and management during sewer line construction, physical control procedures, and mechanical root removal are examples of non-chemical methods (Duke et al., 2005). Mechanical removal of the roots does not prevent the recurrence of new growth while chemical methods, which can be performed alone or in combination with mechanical methods, can remove the roots and retard the root re-growth (Scholar et al., 2002). Repeated usages of chemical methods, which are mainly based on herbicides, may reduce the potential for further root intrusion in the pipes.

The addition of root control chemicals, although being an effective method for controlling root intrusions in sewer lines, may also affect wastewater treatment plant processes. The addition of some organic chemicals and metal ions has been found to inhibit the activity of microorganisms and disrupt their function (Lindeburg, 1975). However, no fundamental mechanism has been found to explain the inhibitory effect of these chemicals. Nitrification, the oxidation of ammonia and nitrite to nitrate in activated sludge system, is one of the processes that is affected most by the application of herbicides. Ake (1995) observed the instability in nitrification process a few hours after the application of 4 mg/L Metam Sodium, a concentration that may be significantly higher than the diluted level at the treatment plant influent. Nitrifying bacteria might take several days to several weeks to recover after exposure to herbicides due to their long generation time (Yeung et al., 2007). Therefore, careful consideration must be given to the application of root control chemicals as the concentration and type of herbicide may impact the wastewater treatment plant performance and in particular, nitrification if exposed to a significant concentration. This concentration is not yet known.

The purpose of this study was to explore alternative effective herbicides, in terms of root kill efficiency, reduced environmental impact on the above surface portion of the plant, and chemical cost for controlling root intrusion into sewer lines. Herbicides were selected among EPA registered chemicals and based on their characteristics such as mode of action and translocation within the plant. In addition, these root control chemicals were evaluated on any potential inhibition of the wastewater treatment plant nitrification process.

2. BACKGROUND

Tree roots are one of the most critical parts of the overall tree structure since a tree can recover with significant damage to a majority of the leaf and stem but die after losing its roots. Tree roots have three basic functions: 1) anchor the tree and keep it upright; 2) food storage; and 3) absorb the nutrients and water (Hogan, 2001). Most plants have one of two types of root systems: (a) the fibrous root system and (b) the tap-root system. Fibrous-root plant systems, such as garden plants, grasses, and weeds, do not normally cause sewer problems since they occupy the upper layers of the soil and radially extend outward from the base of the plant. Plants with tap-root system such as trees and woody shrubs do not grow outward like fibrous roots. Instead, they penetrate deeply into the soil and may potentially enter the pipe joints (Hogan, 2001).

2.1. PLANT STRUCTURE

Knowledge of the plant cell structure is important in understanding the potential function of herbicides. Each plant cell has a membrane that controls the transportation of chemicals into and out of the cell (Grimes, 1988). The membrane is made of a phospholipid bilayer with both hydrophobic (fatty acyl side chains facing the internal cell material) and hydrophilic (phosphor head group on the external side of the membrane) domains (Briskin, 1994). In addition, the membrane has different types of proteins that permit the traversing of water, ions, and metabolites (Grimes, 1988). Figure 2.1 displays a rendition of the cell membrane structure.

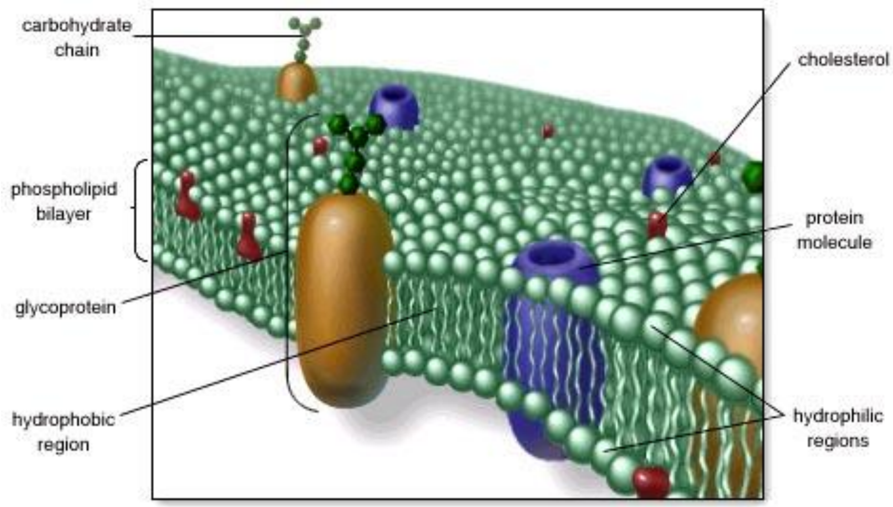

Figure 2.1. Cell Membrane Components (McGraw-Hill, 1999)

Xylem and phloem are two physical pathways that construct the plant's transport system (Figure 2.2a). The phloem is made of living cells, sugars, and amino acids (Bromilow et al., 1990). During spring, the phloem is used to move sugars to growing parts of the plant and help supply energy and metabolic materials for growing parts of the plant (Bromilow et al., 1990). The xylem consists of dead cells and can only transfer water and nutrients towards the leaf and stem (Colquhoun, 2001). Phloem and xylem structure are presented in Figure 2.2b. Environmental conditions such as air humidity and soil moisture are important factors that influence water transport (Colquhoun, 2001). If the chemical can pass the membrane system and enter the xylem or phloem, it can be transported to upper parts of the root system and reach the tree cells outside of the roots. Consequently, there could be a significant level of plant mortality that would extend beyond the intended target area if herbicides were able to utilize these physical pathways for transport beyond the root system.

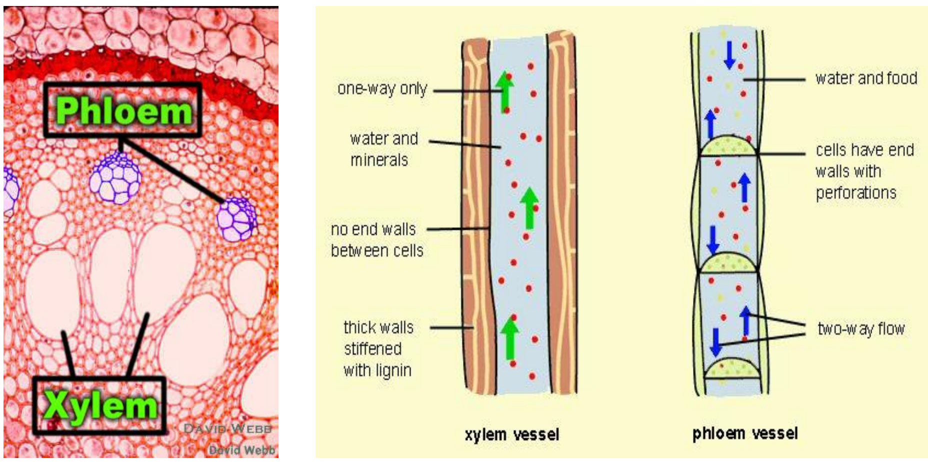

Figure 2.2.Vascular System of a Plant- Phloem and Xylem- a) Top View, (Webb, 2001) b) Front View (Kantharaj, 2000)

2.2. ROOTS IN SEWER LINE

Roots tend to grow in a direction where the environment is most favorable to its growth. The tips of the roots have the ability to detect the gradient in nutrient levels and moisture, which potentially allows them to find their way to sewer lines (Hogan, 2001). Condensation around sewer lines, due to temperature differences between sewer and surrounding environment, provides a significant source of moisture for roots. In addition, any cracks in pipes or joints can cause raw sewage to enter the soil and increase the surrounding nutrient content. Once the roots have determined the source of the nutrient and moisture from the sewer pipe, it only needs a very small opening to enter the pipe and achieve a significant increase in growth due to the abundance of water and nutrients.

Based on an EPA report (Duke et al., 2005), two main types of root structure that are found in sewer lines are veil type and tail type. Veil type is primarily found in pipes with steady and constant flows. They enter from the top and sides of the pipes and form curtain like surfaces. Veil type root systems can significantly block the wastewater flow and accumulate solids, grease, and other organic materials that form gases such as sulfuric acid and odors (Duke et al., 2005). Tail type root systems primarily occur in sewers with low flows and resemble horse tails; enter the sewer lines from the top, bottom, and sides and grow inside the pipes for distances in excess of 20 feet (Duke et al., 2005). Due to their formation in sewer systems that generally see a lower flow, tail type root systems do not generally lead to sanitary sewer overflows.

2.3. ROOT CONTROL METHODS

The methods for controlling root intrusion in sewer lines are mostly divided into two main approaches: non- chemical and chemical. The non-chemical methods are useful in cases where root intrusion has occurred in a short distance from wastewater treatment plants or where environmental safety limits are in place for the application of chemical methods

(Hogan, 2001). In addition, non-chemical methods are useful when rapid clean up of clogged sewer lines is necessary (Newton et al., 2001).

Non-chemical root control methods can be further categorized into cultural control, physical control, and mechanical control (Duke et al., 2005). Proper management and inspection during sewer line construction and careful selection of trees surrounding sewer pipes are defined as cultural methods, which must be applied before root intrusion becomes an issue for sewer lines (Scholar et al., 2002). Some examples of physical treatment are: tree removal, pipe replacement, and pipe lining (Hogan, 2001). Tree removal is useful when there is an intrusion of few tree roots in pipes. It is expensive and the roots remain alive for a while after removing the top portion of the tree (Hogan, 2001). Pipe replacement is a method of removing old and broken-joint lines and replacing them with new and properly installed pipes (Ralf et al., 1996). This method is expensive, could destroy the trees near sewer lines, and root intrusion could still occur from other existing pipes (Ralf et al., 1996). Pipe lining is a method for rehabilitating the sewer lines using acid-resistant polyester fiber impregnated with resin, which provides a new rigid cover inside the pipe (Ralf et al., 1996).). However, pipe lining suffer from the same drawbacks as pipe replacement (i.e., expensive and could destroy surrounding trees).

Mechanical control method is the most important non-chemical method, which utilizes devices such as drill, rodding machines, and winches to cut and remove the roots from sewers (Ralf et al., 1996). These mechanical approaches are rapid in solving an already blocked sewer line but are not a long term effective method. High efficiency can be obtained by using mechanical method followed by the application of chemical root control methods (Ralf et al., 1996, and Ducoste et al., 2008a).

Herbicides are chemicals that stop or deter the normal growth of the plant. Understanding how herbicides work and their mode of action is very important since inappropriate use of herbicides can cause serious environmental problems (Mosier et al., 2003). Herbicides can be classified based on selectivity (selective or non-selective), herbicide translocation (systemic or contact), and mechanism of action (Colquhoun, 2001).

The herbicide selectivity is the ability of a certain herbicide to kill a specific plant and depends on the environmental condition, amount of herbicide applied, and application time (Colquhoun, 2001). It also depends on the plant metabolism rate to break down the herbicide to smaller non-active compounds and reduce the toxic effect of herbicide (Goodman, 1988). These non-toxic compounds (herbicide metabolites) will bind to sugars and amino acids and move to vacuoles, which isolate the harmful compounds (Goodman, 1988).

Contact herbicides are not translocated in the plants and bind to the application site. Systemic herbicides move in plants and translocate using the plant's transport system (i.e., xylem and phloem). The herbicide can move from the point of application through one or both of these transport systems to the target site (Mosier et al., 2003).

The herbicide's mechanism of action can be classified based on the plant process they interfere, the sites they attack, and the damage they cause. These herbicides are grouped based on the plant system that is involved in the process and are described in Table 2.1 (Colquhoun, 2001). Except photosynthetic inhibitors and pigment inhibitors where their functions have an adverse impact on the above ground portion of the plants, the other five groups of herbicides have the potential for controlling the root intrusion in sewer lines by killing or inhibiting the growth and division of the plants root's cells.

Table 2.1. Herbicides Mechanism of Action

Mechanism of Action	**Function**	**Example**
Growth regulators	Interfere with the natural growth hormones balance, which are responsible for the regulation of cell division, elongation and protein synthesis in plants.	Triclopyr and MCPB
Meristematic (growth) inhibitors	Interfere with mitosis process, which is responsible for cell growth.	Dithiopyr and Dichlobenil
Photosynthetic inhibitors	Affect the plant during photosynthesis, which leads to blocking the production of the food in plant.	Uracil and Buctril
Amino acid synthesis inhibitors	Interfere with the synthesis of amino acids that leads to the reduction in the protein production and an increase in formation of toxic chemicals.	Penoxsulam and Arsenal
Lipid synthesis inhibitors	Block the production of new cells by inhibiting lipid synthesis (herbicide binds to acetyl-CoA carboxylase, an enzyme responsible for lipid production) which is an essential component of cell membrane.	Sethoxydim and Quizalofop
Cell membrane disrupter	Form highly reactive compounds that rupture the cell membrane and cause cell lysis and death.	Diquat, Paraquat and Carfentrazone
Pigment inhibitors	Inhibit the formation of carotenoids (photosynthetic pigments which capture energy and protect chlorophyll from excess light). Without carotenoids, plant photosynthesis is disrupted and bleaching of the plant and formation of toxic by-products will occur.	Norflurazon and Clomazone

Low xylem translocation is another criterion that must be considered in selecting the appropriate herbicide for controlling the root intrusion in sewer lines in addition to factors discussed earlier. Bromilow et al. (1990) represented polarity or lipophilicity and acid strength (pKa), as two main herbicide characteristics that help in understanding the transport pattern in plants. Octanol-water partition coefficient (K_{OW}) is one of the parameters normally used for measuring the polarity. Figure 2.3 represents the relation between log K_{OW} and permeation rate of nonionized compounds through the cell membrane (Bromilow et al., 1990). As the chemicals become more polar and more lipophilic, the permeation rate decreases.

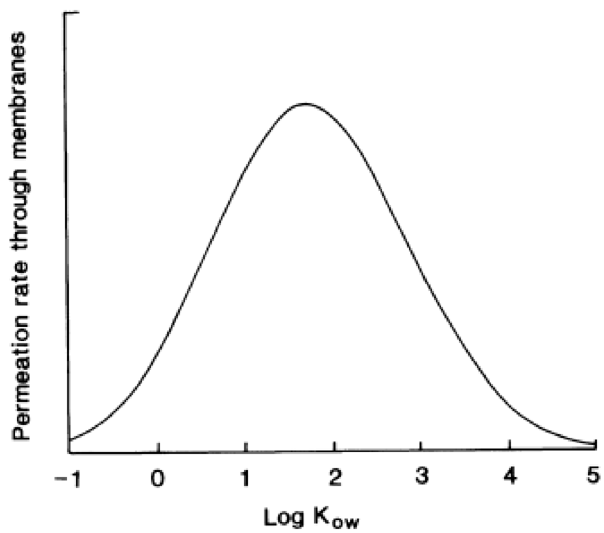

Figure 2.3. Relationship between Lipophilicity and Permeation Rates Through Membrane (Bromilow et al., 1990)

The lipophilic compounds, with high $K_{OW,}$ partition strongly to the plant tissues and as such, no translocation will occur inside the plant from roots to shoots. Based on different log K_{OW} of the metabolism products inside each of the plants, different translocation patterns might occur. Based on the pH, acids start to ionize when they enter the cells. Since ions are more polar than the acid form, they escape the cell slowly and translocate at a slower rate. Weak acids mostly translocate in phloem. For example, Dithiopyr has a Log K_{OW} value of 4.75 meaning that Dithiopyr is a non-systemic herbicide and the translocation within the plant must be low. Triclopyr, which is a weak acid herbicide with the Log K_{OW} value of 2.68, will likely display phloem translocation. Figure 2.4 illustrates the physicochemical properties required of weak acids and nonionized compounds for various types of systemic behavior (Bromilow et al., 1990).

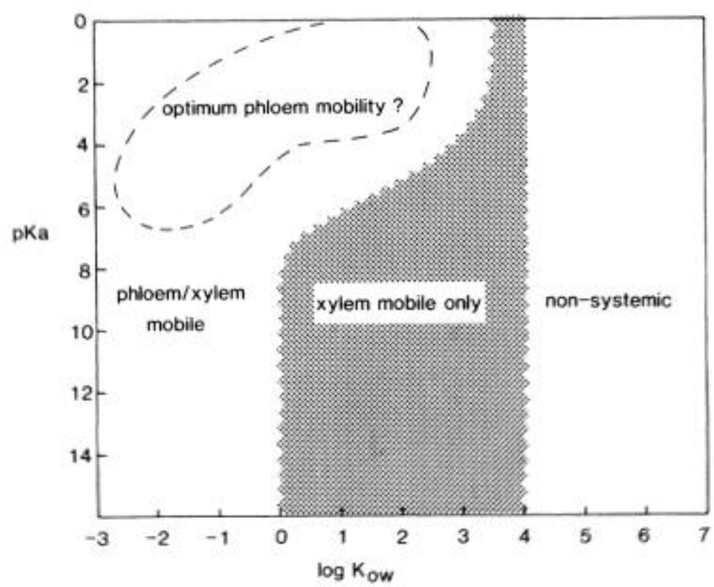

Figure 2.4. Translocation Pattern based on Polarity and Acid Strength (Bromilow et al., 1990)

2.4. PREVIOUS STUDIES WITH ROOT CONTROL METHODS

In the study performed by Ahrens et al. (1970), an attempt was made to develop a method to control tree roots intrusion in sewer lines in Sacramento County, California. The rooted cuttings of the willow, grape, peach, and red gum eucalyptus were used in Ahrens et al.'s research. The cuttings were grown in plastic pots containing fertilized mixture of sand and peat and then placed on top of second pots filled with sand or vermiculite. After the growing period, the roots were exposed to selected herbicides: Bensulide, Dichlobenil, Dinseb Endothall, Metam, Paraquat, Trifluralin, 2,4-D, 2,4,5-T, Copper sulfate, and Chlorthiamid. The effects of treatment were evaluated weekly by measuring the extent of browning of the roots tissue. Among these herbicides, Dichlobenil and Metam displayed better results but higher transportation of herbicide and foliage injury were observed in plants treated with Metam. The problem with Metam and Dichlobenil, as stated in Ahrens et al. (1970), was the foliage damage followed by plant death in using selected concentration of the herbicide.

Groninger et al. (1997) evaluated the effect of eight herbicides (Asulam, DCPA, EPTC, MSMA, Glufosinate, Glyphosate, Sodium chlorate, and Triclopyr) on four tree species and evaluated the cost of treatment compared to the results with Metam application. The herbicides were used at rates of 1 to 10 times the cost of Metam. The species used in their study were water tupelo, wax myrtle, water oak, and chinaberry. The saplings were placed in 164ml tubes in contact with dilute nutrient solution. After the growing period, the roots were exposed to the herbicides for 20 minutes. The shoot health was determined using visual assessment and the root mortality was determined based on the percentage of the weight of dead root biomass to total exposed root biomass. Although Groninger et al. (1997) showed that Metam had the highest root mortality rate, the plant death occurred only several days after treatment. After Metam, Glufosinate was the only herbicide, which provided significant root mortality without causing foliar damage; but the cost was 10 times that of Metam providing the same mortality rate. Groninger et al. (1997) concluded that the same plant death will not happen in actual sewer line since only small portions of the roots enter the pipes and the tree can still survive after losing some portion of the roots.

Groninger et al. (2000) further investigated the effect of using Diquat for controlling Black Willow root intrusion in sewer lines. The cuttings were placed in tubes containing a mixture of peat moss and sand. After a growing period of 6 months, the roots were exposed to 2 different concentrations of Diquat (0.48 and 0.96 a.i./L) for 20 minutes. Groninger et al. (2000) found complete mortality at the high concentration of Diquat with some live roots observed in plants at the lower concentration. Groninger et al. noted a significant reduction in re-growth at the higher concentration. No foliar damage, based on low translocation, was observed for both concentrations. Therefore, Groninger concluded that Diquat could be an acceptable choice for use in places where aboveground damage is not acceptable.

In the study performed by Ducoste et al. (2008a), the effects of chemical and mechanical methods were investigated in controlling root intrusion in sewer lines. A pilot scale sewer system was built to simulate the sewer line environment. Four types of trees were used in their study: black willow, river birch, silver maple, and turkey fig. Saplings of these trees with sufficient roots were placed on top of the pipe so that the roots of the saplings were inserted into the pipe. The addition of fresh water and nutrient was performed weekly. After a growing period of 3 months, the mechanical removal (i.e., shearing the intruded roots and manually removing them from the pipes) was conducted at two shearing levels (85% and complete removal). Root re-growth within a month was observed with the 85% removal level. Although no re-growth was observed in the complete removal, the authors concluded that mechanical treatment alone cannot prevent root re-growth. After mechanical removal, Ducoste et al. investigated the efficiency of two commercially available herbicide products, Diquat and Dichlobenil. Their results showed that both of the chemical products stopped the root growth. Dichlobenil caused more damage to the overall health of the tree and re-growth was observed in plants using this treatment. Diquat had little to no damage to the aboveground part of the plant, did not allow any root re-growth, and had long-lasting control effect.

As presented above, most of the studies resulted in the application of Metam, Diquat or Dichlobenil as a root control chemical in sewer lines. Although high percent root kill is a desirable result, the potential injury that might occur to the above ground portion of the plants as a consequence of herbicide exposure is an important factor, which decreased the demand for using the product. An appropriate concentration of the herbicide must be selected to provide a significant percent root kill while keeping a viable upper portion of the tree. For example, lower concentration of Dichlobenil must be tested and the result must be compared to the existing data. Furthermore, the impact of root control chemicals on the wastewater treatment plants downstream from the herbicide application point is an important issue that needs to be considered.

2.5. IMPACT OF HERBICIDES ON WASTEWATER TREATMENT PLANT UNIT PROCESS PERFORMANCE

Nitrogen in the form of ammonia or organic nitrogen is one of the toxic components in a wastewater, which can be removed biologically in a nitrification-denitrification process. Nitrogen compounds in wastewater have a variety of effects such as oxygen depleting, toxicity to fish, and eutrophication. Therefore, nitrifying bacteria play an important role in wastewater treatment plants in removing nitrogen from wastewater. Nitrification is a two-step process under aerobic condition: oxidation of ammonia to nitrite by ammonia oxidizing bacteria (AOB) such as *Nitrosomonas* followed by the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB) such as *Nitrobacter*. Nitrifying bacteria are sensitive to some chemicals and metal ions (Richardson, 1985). Richardson (1985) presented more than 200 chemicals that could inhibit nitrification process at different levels. Nitrifying bacteria have long growth periods so they need significant time to recover from inhibition caused by addition of root control chemicals (Ake, 1995). Consequently, wastewater treatment plants are cautious about chemicals in sewers that could affect their nitrification process.

In a study performed by California Environmental Protection Agency on Metam Sodium characterization (Rubin, 2004), the author concluded that Metam is harmful to human, livestock, and the environment and have adverse impact on wastewater treatment plants. Metam and its byproduct (toxic gas Methyl Isothiocyanate) are also labeled as Toxic Air Contaminant. The volatility and toxicity of Metam can put exposed workers at risk of cancer (Malavenda et al., 1999). In another study performed by Randall et al. (2003) on the effect of Diquat Bromide on wastewater treatment plant, the results indicate low effect on nitrifying growth rate at low concentration of herbicide but inhibition of nitrification process and the prevention of ammonia oxidation recovery at concentrations higher than 10 mg/L.

Consequently, there is a pressing need to determine potential alternative herbicides that have a high performance in killing intruded roots inside the sewer lines, little to no re-growth rate, low chemical cost, little to no above ground plant mortality, and no impact on unit processes in wastewater treatment plants.

3. MATERIALS AND METHODS

3.1. HERBICIDE TESTING ON ROOTS

Greenhouse Set-Up

Black cottonwood (P. trichocarpa) was used as the test tree roots species that were exposed to selected herbicides. Black cottonwood has an aggressive growth, establishes large root masses, and has been found as a tree species that leads to sewer line blockages (Ducoste et al., 2008b).

Approximately 55 shoots were propagated from black cottonwood stem cuttings to prepare the trees for exposure to herbicides (Figure 3.1.a). These cottonwood shoots were grown at the Department of Forestry and Environmental Resources at North Carolina State University. Shoots were trimmed on the top and bottom to achieve consistent shoot size for all cuttings. These shoots were placed in 2 pots of water and then placed in a greenhouse to maximize access to direct sun light. The water level was checked daily. After 2 weeks, small white roots were observed at the bottom of the shoots (Figure 3.1.b). After 1 more week, the shoots were ready for transplantation in the soil.

Figure 3.1. a) Black Cottonwood, b) Roots after 2 Weeks in Water

Eleven 18 L boxes were purchased to conduct the herbicide exposure experiments. Two holes were drilled in the bottom of these boxes for drainage of excess water and nutrients (Figure 3.2.a) and six holes were drilled in the top cover for placement of the saplings (Figure 3.2.b).

Figure 3.2. Pictures of Box a) Drainage Holes, b) Top Holes

Deepots with a volume of 444 ml were purchased from Stuewe and Sons Company for holding the shoots. The deepots have 4 holes at the bottom to provide enough space for the roots growth beyond the soil matrix (Figure 3.3).

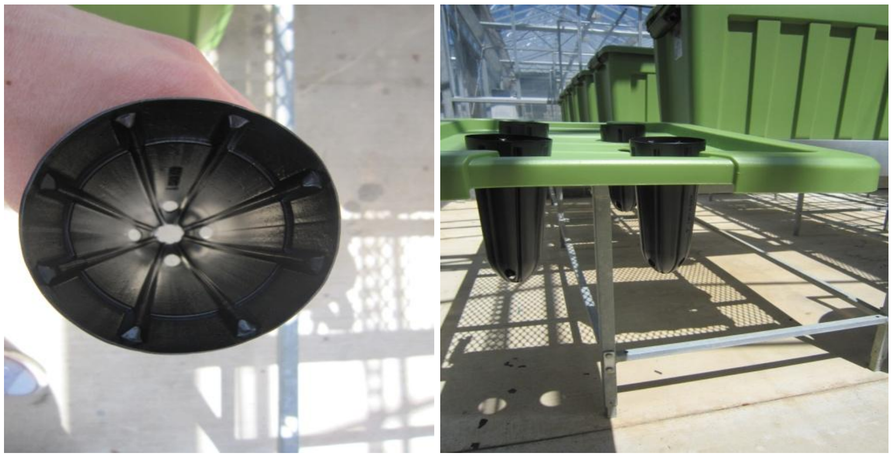

Figure 3.3. Pictures of Deepots

Cuttings were moved to Method Road greenhouse to grow under greenhouse conditions in 18 gallon boxes containing 4 tubes, each with one cutting per tube (Figure 3.4).

Figure 3.4. Picture of Boxes and Deepots in Greenhouse

A tray was placed in the boxes such that the base of each deepot was suspended in an aqueous nutrient solution (Miracle Growth) to facilitate root growth outside each tube. Root growth was further facilitated by maintaining the rooting zone in darkness by covering the tubes and the tray with aluminum foil. The two additional holes shown in Figure 3.5 were used to monitor the root growth and water level in the boxes and minimize sun light exposure to the tree roots. These holes were covered when roots were not inspected.

Figure 3.5. Picture of the Set Up in Greenhouse

Manual separation of the roots was performed weekly to prevent the mixing of roots among adjacent cuttings. After three weeks, the shoots were planted in the aforementioned deepots. The planting process included the following steps: Metro Mix and peat moss were mixed together and the soil was kept moist with the daily addition of water. Osmocote slow release fertilizer was added to the potting soil. Unfortunately, eleven trees died in a month after transplantation into the soil matrix even though the plants were being watered daily and the water level in the tray below the plants was also checked daily. The deaths were likely caused by the lack of sufficient roots and high temperature of the greenhouse a few days after the planting process. In order to keep a moderate temperature in the greenhouse, two fans were continuously operated and a shield was installed under the roof of the greenhouse to prevent overheating due to sunlight.

After two weeks, some damage (i.e., holes not originally planned) was observed on the trays underneath the deepots. These holes were likely the result of a reaction between nutrient solution (Miracle Growth) and the aluminum. Consequently, the trays were replaced by two attached cups, which were taped back to back. Figure 3.6 displays the cup arrangement in the revised setup. The water was replaced weekly and more nutrients were added as needed.

Figure 3.6. Pictures of Cups Arrangement a) Side View, b) Top View

Figure 3.7. Picture of Planting Set Up in Geenhouse

Figure 3.7 displays the greenhouse setup after 10 weeks of tree growth with the average height of 5ft for each tree. Each plant’s root achieved strong growth inside the cup under its deepot. The individual cups minimized any mixing of roots among adjacent cuttings. Almost all of the roots display a whitish color and appeared viable. Figure 3.8 displays the roots after 10 weeks of growth.

Figure 3.8. Pictures of Roots after 10 Weeks

Some aphids (white insects) were observed at the bottom of 3 plants leaves. These plants were exposed to volck oil on the top and the bottom of all the leaves to kill the aphids. This product is non-toxic and its insecticide effect can last for 2-3 weeks. In addition, straight bamboo sticks were added to prevent plants from bending over from their own weight at this initial stage of growth.

Test Procedure

44 cottonwood plants with sufficient root density were exposed to the herbicides after seven months of growing period. Four plants were used for each herbicide and four plants served as a control. Each of the four plants was selected randomly using Randomized Complete Block Design (RCBD) with a SAS code presented in Appendix C- SAS Program I (Statistical Software, 2002). Figure 3.9 displays the selection pattern of the plants.

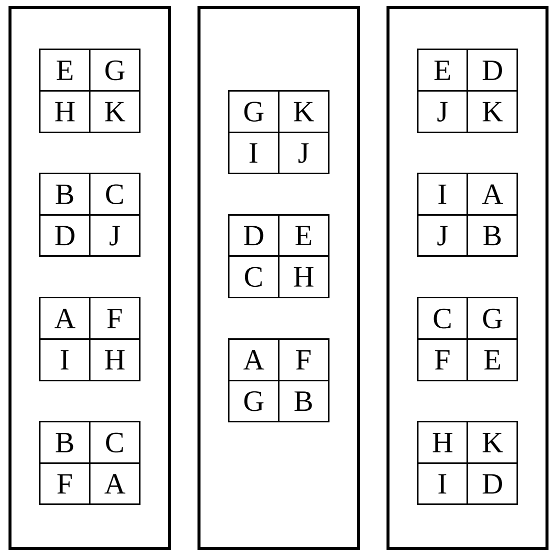

Figure 3.9. Selection Pattern of Trees for Herbicide Testing: A= Dithiopyr 1%, B= Dithiopyr 0.25%, C= Dithiopyr 0.1%, D=Triclopyr 1%, E= Triclopyr 0.25%, F= Triclopyr 0.1%, G= Penoxsulam 0.5%, H= Penoxsulam 0.15%, I= Penoxsulam 0.05%, J= Dichlobenil, K= Control

Each herbicide was prepared with 500 ml water in 18 oz. plastic cups that were placed at the bottom of each deepot (Herbicides characteristics are presented in Appendix A). Each tree root was placed inside each cup to expose them to the herbicide solution. The rates tested for each herbicide are summarized in Table 3.1. The selections are based on the typical commercially available concentrations of the herbicides used for root intrusion problem (Raffensperger, 2012). The roots were washed with water and placed in cups filled with the herbicides for an hour. After one hour, the herbicide was discarded and the plants were placed back into the boxes (Figure 3.10).

Table 3.1. Treatments and Rates

Treatment #	Treatment	Rate (% Active Ingredients)	Rate (mg/500ml)
1	Dithiopyr	1	21.04
2	Dithiopyr	0.25	5.22
3	Dithiopyr	0.1	2.08
4	Triclopyr	1	8.38
5	Triclopyr	0.25	2.08
6	Triclopyr	0.1	0.83
7	Penoxsulam	0.5	11.57
8	Penoxsulam	0.15	3.46
9	Penoxsulam	0.05	1.15
10	Dichlobenil	0.55	24
11	Control	-	-

Based on the number of available boxes in the greenhouse and the number of herbicides to be tested, individual boxes were assigned to one herbicide concentration. Daily watering was performed to maintain the same growing conditions prior to the herbicide exposure.

Figure 3.10. Pictures of Roots a) Before Applying Herbicide, b) After Applying Herbicide

After 3 weeks of daily evaluation, the Triphenyl Tetrazolium Chloride (TTC) test was performed to assess the viability of the roots. TTC test is an alternative method for measuring the viability of plant tissue (Ruf et al., 2003). TTC is colorless until the dehydrogenase enzymes in living cells reduce it to a bright red pigment called formazan (Briskin, 2000). Formazan can be easily extracted and measured by spectrophotometer (Steponkus et al., 1967). The bacterial effect was not taken into consideration since Steponkus et al. (1967) found that their reduction of TTC was lower than 2% of the total reduction. Roots were washed and six replicate samples were performed per plant. 0.4 grams were collected for each sample to perform the TTC test. Samples were obtained from different parts of the roots (top, middle, and bottom portion). In the TTC method, the roots were placed in 15ml test tubes and 3 ml of 0.6% TTC in 0.05M phosphate buffer plus 0.05% wetting agent was added to each test tube (Steponkus et al., 1967). All the samples were incubated for 18 hours at room temperature, rinsed with 10ml distilled water, and extracted with 10 ml of 95% ethanol in a boiling water bath (Steponkus et al., 1967). The extracts were cooled (Figure 3.11) and the absorbance of the solution was recorded at 530 nm (Steponkus et al., 1967). The data was compared to the absorbance of untreated roots (control) and boiled roots (boiled roots are the dead tissue that are killed by the boiling process for 20 minutes).

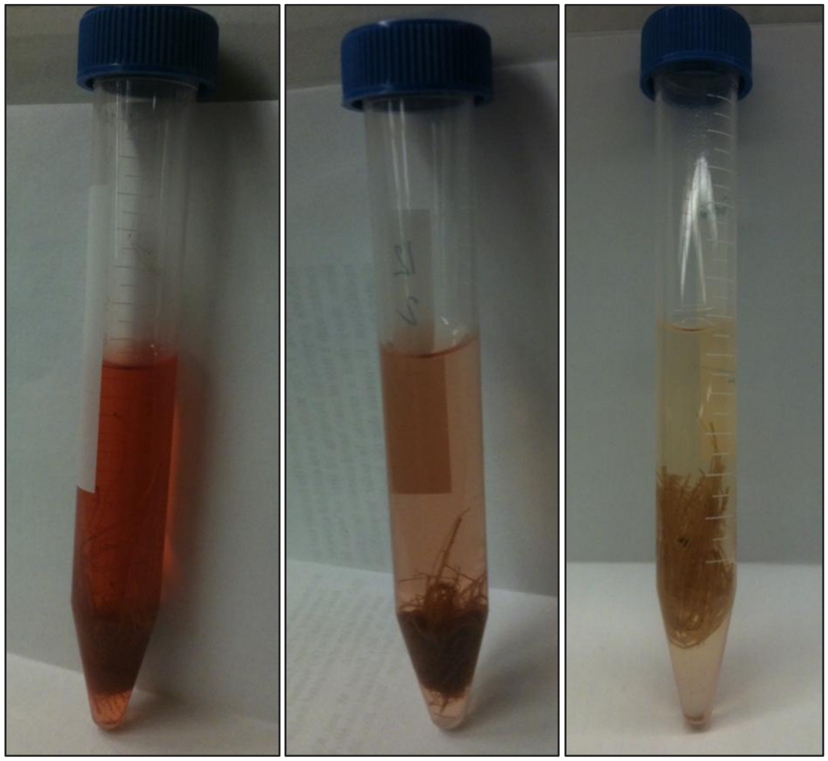

Figure 3.11. Pictures of TTC Results. a) Control, b) Herbicide, c) Boiled

3.2. HERBICIDE TESTING ON NITRIFICATION PROCESS

The effect of herbicides on wastewater treatment plant nitrification process in activated sludge system was investigated in two levels of herbicide concentration as part of this study. In the first set of tests, four premade batch reactors made of polyethylene were used for this portion of the study to test the same concentration of herbicides used at the point of application in sewer lines. In order to remove the supernatant and feed the reactors, two outlets for effluent and influent were designed at 2 and 4 liters of the reactors with the diameter of 3cm. Influent and effluent was delivered by peristaltic pumps (Cole-Parmer) at a rate of 400 ml/min using flexible plastic tubes. All reactors were operated at room temperature (25 °C).

The flexible spiral diffusers were placed at the bottom of each reactor to distribute the air evenly within the reactor at a flow rate of 2.2 $Lmin^{-1}$ to maintain oxygen above 2 mg L^{-1} and provide mixing for the system. The diffuser was connected to an air pump with a flexible plastic tube connected to a hard plastic tube. The diagram of reactors, pumps, feed tank, and effluent tank is presented in Figure 3.12.

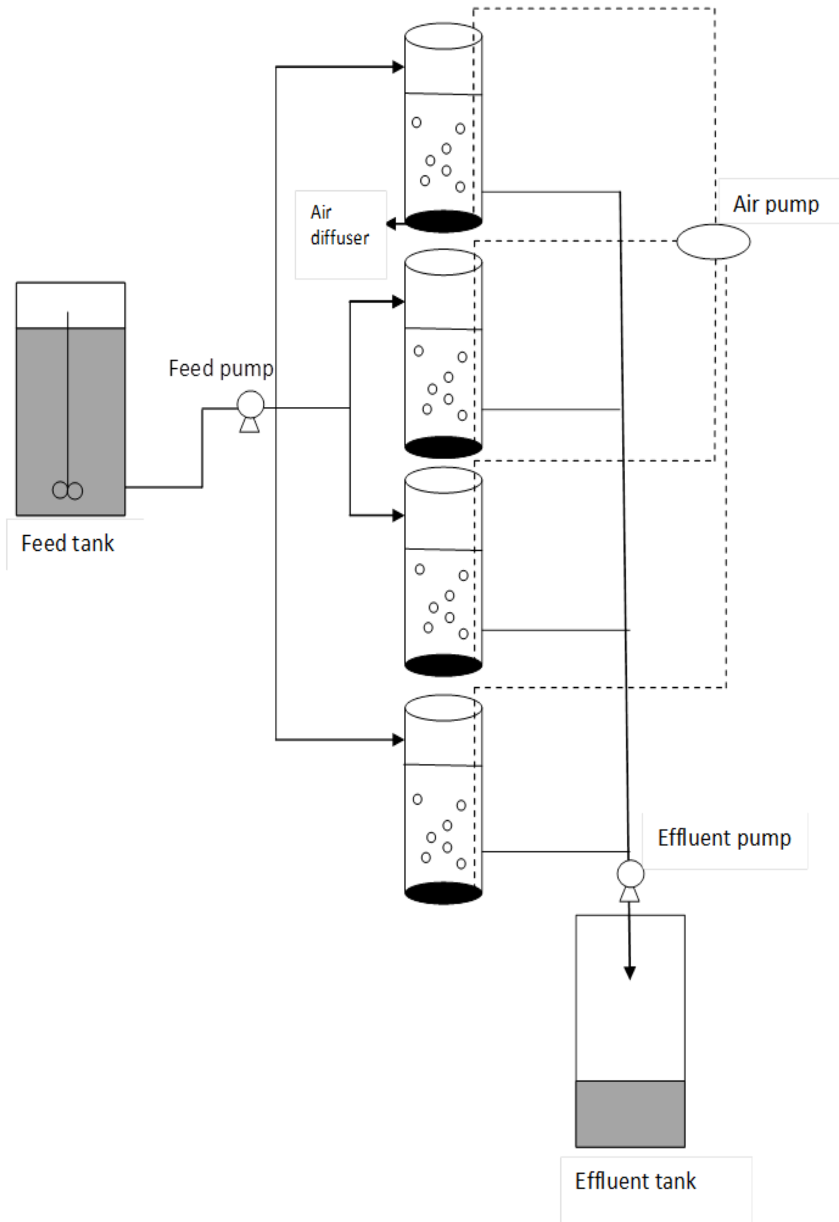

Figure 3.12. Reactors Set Up for Exposure of Nitrification Process to Herbicide Concentration at Point of Application

The reactors were fed with synthetic wastewater with the composition presented in Table 3.2. The feed composition has been obtained from S. Rhee et al. (1997).

Table 3.2. Synthetic Wastewater Composition

Chemical	**mg/L**	**Chemical**	**mg/L**
Glucose	280	$CaCl_2$.2H2O	3.75
Acetate	485	KH_2PO_4	44
$(NH_4)_2SO_4$	235.96	Na_2CO_3	66
$MgSO_4.7H_2O$	50	$NaHCO_3$	105
$FeCl_3$	0.25	$MnSO_4.H_2O$	5

All reactors were inoculated with 4 L of same activated sludge from North Cary wastewater treatment plant. Before starting the feeding process, the activated sludge in all 4 reactors was aerated for 1 hour with the following 0.5 hr settling time. 2 L of supernatant was removed from each reactor to a separate plastic container. The reactors were fed with 2 L of synthetic wastewater followed by 4 hours of aeration, 0.5 hr settling time and removal of 2 L supernatant. 2 L Synthetic wastewater was added to each reactor and aeration was started with maintaining the oxygen concentration at 2-4 mg/L. After 15 minutes of aeration, herbicides with the concentrations presented in Table 3.3 were added to each reactor.

The herbicide concentrations in Table 3.3 were based on the percent root kill results which presented a minimum of 75 % root mortality. Immediately after the addition of herbicides, samples were taken directly from reactors using a 10 ml glass pipette entering from the top of each reactor during aeration period at a frequency of 1-1.5 hour for a period of 4 hours and were taken for analysis of COD, ammonia, nitrite, and nitrate concentrations using Standards Methods (APHA, 1995).

Table 3.3. Herbicides and Rates (Full Concentration Test)

Treatment #	**Treatment**	**Rate (% AI)**	**Rate (mg/2L)**
1	Dithiopyr	1	84.16
2	Triclopyr	0.25	8.32
3	Penoxsulam	0.05	4.6
4	Control	-	-

Since the actual concentration of herbicide entering the wastewater treatment plant is much lower than the concentration used at the point of treatment, another set of tests with 100X and 1000X dilution factor were performed to assess the impact of these lower herbicide concentrations. The change in concentration is based on the dilution effect of herbicides in joining the other sewer lines, adsorption of the herbicides to the surface of the pipes, and removal of herbicide in previous steps before entering the activated sludge system such as grit chamber or primary clarifier. Eight 1000 ml beakers were used for this portion of the study; three reactors for three herbicides (Dithiopyr, Penoxsulam, and Triclopyr) using 100X dilution factor, three reactors for three herbicides using 1000X dilution factor, one as live microbial control, and one as dead microbial control. For preparing the dead control, 1 L activated sludge was autoclaved for 20 minutes at 250 F. All reactors were operated at room temperature (25 °C).

An air stone was placed at the bottom of each reactor to distribute the air evenly within the reactor and provide mixing for the system. Each air stone was connected to a manifold with eight outlets. Each out let had a valve to control the air flow rate at 2.2 $Lmin^{-1}$ to maintain oxygen at 2-4 mg L^{-1} in the reactors. The manifold was connected to an air pump with a hard plastic tube.

The reactors were fed with the same synthetic wastewater composition presented in Table 3.2. All reactors were inoculated with 1 L of activated sludge from North Cary wastewater treatment plant. The activated sludge for dead control was autoclaved during the aeration period of other reactors. The activated sludge in 7 reactors was aerated for 1 hour followed by a 0.5 hr settling time. 500 ml of supernatant was removed from each reactor to a separate plastic container. All 8 reactors were fed with 500 ml of synthetic wastewater and aeration was started to maintain the oxygen concentration at 2-4 mg/L for 4 hours followed by a 0.5 hour settling time. After the settling period, the removal of 500 ml supernatant and the addition of 500 ml synthetic wastewater were performed, the aeration was started, and the herbicides with the concentrations presented in Table 3.4 were added to each reactor. As will be discussed in the results section 4.3, the herbicide concentrations in Table 3.4 were based on the level needed to achieve a minimum of 75 % root mortality with 100X and 1000X dilution factors to consider the potential reduction in the herbicide concentration prior to entering the activated sludge system.

Table 3.4. Herbicides and Rates (Diluted Test)

Treatment #	Treatment (D.F)	Rate (% AI)	Rate (mg/500 ml)
1	Dithiopyr (100X)	0.025	0.0522
2	Dithiopyr (1000X)	0.0025	0.00522
3	Triclopyr (100X)	0.001	0.0083
4	Triclopyr (1000X)	0.0001	0.00083
5	Penoxsulam (100X)	0.0005	0.0115
6	Penoxsulam (1000X)	0.00005	0.00115
7	Live Control	-	-
8	Dead Control	-	-

Immediately after the addition of herbicides, samples were taken directly from reactors using a 10 ml glass pipette entering from the top of each reactor during aeration period and were taken for analysis of COD, ammonia, nitrite, and nitrate concentrations using Standards Methods (APHA, 1995). Sampling continued at a frequency of 1-1.5 hour for a period of 4 hours. All samples were taken in triplicates.

4. JOURNAL ARTICLE

Title: Assessment of Alternative Herbicides for Root Intrusion Treatment and Their Effect on Wastewater Treatment Nitrification Process.

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ABSTRACT

Roots intrusion into sewer lines poses significant problems in the conveyance of wastewater in sewer collection systems and can be treated by non-chemical and chemical control methods. In this study, a chemical method was used with three proposed herbicides (Dithiopyr, Penoxsulam, and Triclopyr) to assess the mortality, root and shoot re-growth reduction, and overall health of exposed cottonwood tree roots. The results were compared with an existing commercial product based on Dichlobenil formula. The results showed that Dichlobenil and Triclopyr achieved the highest root control (about 93% and 78%) but killed the upper portions of the plants. Dithiopyr with the highest concentration (1% a.i.) displayed 76% root kill but root re-growth was observed after three weeks. Penoxsulam displayed 73% root kill without any damage to the above ground portion of the plant.

Additional tests were performed to assess the effect of herbicides on the nitrification process in activated sludge system. Based on the results, all herbicides displayed nitrification inhibition using the same concentration at the point of application in the sewer lines and no inhibition using diluted concentrations at the point of entry into the activated sludge system. Overall, Penoxsulam (0.05% a.i.) was selected as the best performing herbicide that displayed desirable root kill, a favorable environmental impact, and low cost. In locations where the health of the above ground portion of the trees is not an issue, Triclopyr was recommended as an alternative herbicide based on higher percent root kill and lowest cost for the rate of application.

4.1. INTRODUCTION

Intrusion of roots into sewer pipelines is among the most destructive problems encountered in a wastewater collection system. Root-related sewer problems include: (1) sewer stoppages and overflows; (2) structural damage caused by growing roots; (3) formation of septic pools behind root masses that lead to potential generation of hazardous atmospheres such as hydrogen sulfide, other gases, and odors; (4) reduction in hydraulic capacity and loss of self-scouring velocities; (5) infiltration in areas where pipes are seasonally under a water table; and (6) exfiltration of sewage into soils around cracked or separated joints (Duke et al., 2005). Approximately 25 percent of all collection systems contain some fraction of roots intrusion (Hogan, 2001).

The removal of intruded root hairs in sewer pipes can be accomplished by non-chemical and chemical control methods. Planning and management during sewer line construction, physical control procedures, and mechanical root removal are examples of non-chemical methods (Duke et al., 2005). Non-chemical methods for the removal of roots do not prevent the recurrence of new growth. However, chemical methods, which can be performed alone or in combination with mechanical methods, have been shown to remove the roots and retard the root re-growth (Newton et al., 2001; Scholar et al., 2002; Ducoste et al., 2008a).

Several chemical and non-chemical methods have been studied for controlling root intrusion in pipes. Ahrens et al. (1970), Groninger et al (1997), and Groninger et al. (2000) tested the effect of a wide range of herbicides such as Metam, Dichlobenil, and Glufosinate with varying concentrations of active ingredient and modes of action responsible for killing intruded roots in sewer lines. Ducoste et al. (2008a) investigated the effect of both mechanical (cutting and shearing) and chemical methods (Diquat and Dichlobenil) for controlling the tree roots in sewer lines. The authors concluded that mechanical treatment alone cannot prevent root re-growth but chemical methods using Diquat and Dichlobenil resulted in high percent root kill. The results of these prior experiments concluded that Metam, Diquat or Dichlobenil were the most effective chemical treatment of intruded roots in sewer lines based on the high percent root kill achieved by these chemicals. However, the problem with existing chemicals such as Metam and Diquat is that the herbicide has significant translocation through the plant's xylem and phloem leading to foliage damage and plant death when using typical commercially available concentrations of the herbicides (Yeung et al., 2007). This potential transport of herbicides to the upper portion of the plant is a concern since the aboveground portion of the trees is not an intended target of treatment. In addition, some herbicides have been reported to have negative effects on treatment facilities by inhibiting the nitrification process (Rubin, 2004; Randall, 2003).

Nitrification, the oxidation of ammonia and nitrite to nitrate in activated sludge system, is one of the processes, which is affected most by the application of herbicides. The addition of a range of organic chemicals and metal ions have been shown to inhibit the activity of microorganisms and disrupt their function (Richardson, 1985). However no fundamental mechanism has been reported to explain the impact of these chemicals on microbial physiology. A study was performed by Stanford University (Yeung et al., 2007) on the effect of Metam Sodium on nitrification inhibition in activated sludge system. In their study, seven concentrations of Metam Sodium ranging from 0-1000 mg/L were applied in batch assays. The Stanford study revealed ammonia and nitrite oxidation inhibition with long recovery times (2 weeks) at concentrations higher than 100 mg/L of Metam Sodium. In another study performed by Randall et al. (2003) on the effect of Diquat Bromide on wastewater treatment plant performance, the results indicated minimal impact on nitrifying growth rate at low herbicide concentrations but inhibition of nitrification process and the prevention of ammonia oxidation recovery at concentrations higher than 10 mg/L. These prior results suggest that complete recovery of these microorganisms exposed to higher Metam Sodium concentrations may take several days to weeks. Consequently, ammonia concentration in the wastewater treatment plant effluent may exceed regulatory limits during this period of exposure to herbicides. Therefore, careful consideration must be given to the application of root control chemicals as the concentration and type of herbicide may impact the wastewater treatment plant performance and in particular, nitrification if exposed to a significant concentration. This concentration is not yet known.

The objective of this study was to explore alternative effective herbicide, in terms of root kill efficiency, limited environmental impact on the above plant survival, and chemical cost. In this study, Dithiopyr, Triclopyr, and Penoxsulam were the alternative herbicides tested for controlling root intrusion into sewer lines and compared against an existing commercially available product that utilized Dichlobenil. Since no published studies have been performed on the effect of these alternative herbicides on wastewater treatment processes, this study also investigated the impact of these three herbicides on the nitrification process.

4.2. MATERIALS AND METHODS

Herbicide Testing on Roots

Greenhouse studies. Black cottonwood (*P. trichocarpa*) saplings were propagated at the Department of Forestry and Environmental Resources of North Carolina State University. The shoots were rooted in 444ml deepots, which had four holes at the bottom to provide enough space for the roots' growth beyond the soil matrix. The deepots contain a 1:1 mixture of MetroMix and peat moss. Eleven boxes, each containing six holes at the box cover, were used to hold four deepots, each with one cutting. The other two holes were covered with plastic cups and used as viewing ports to check the condition of the roots and reduce the amount of light exposure. In addition, two attached cups, which were taped back to back containing nutrient solution, were placed inside the box, below the deepots, to provide nutrient and damp conditions (as in sewer lines) for the plants and facilitate root growth outside each tube. These attached cups were covered with aluminum foil to further prevent any sun light exposure and provide darkness for rooting zone. During the growing period, plants were watered daily and Osmocote granular and Miracle Growth were added to the soil mixture and cups inside the boxes as nutrient supplement monthly. After 5 months, based on the low space for massive roots, the cups were replaced with a 9L container. Intermingling of roots among adjacent saplings was prevented by weekly manual separation of new root growth.

Herbicide application. 44 cottonwood plants with sufficient root density were exposed to the four herbicides (three new: Dithiopyr, Triclopyr, and Penoxsulam and an existing commercially available product: Dichlobenil) with three different concentrations after seven months of plant growth. The concentrations tested for each herbicide as their active ingredients (a.i.) are summarized in Table 4.1. The active ingredients are based on the typical commercially available herbicide concentrations used for sewer root intrusion treatment (Raffensperger, 2012). Four plants were used for each herbicide and four additional plants served as a control. Each of the four plants was selected randomly using SAS Randomized Complete Block Design (RCBD) (Statistical Software, 2002). Figure 4.1 displays the selection pattern of the plants from the greenhouse.

Table 4.1. Treatments and Rates

Treatment #	Treatment	Rate (% Active Ingredients)	Rate (mg/500ml)
1	Dithiopyr	1	21.04
2	Dithiopyr	0.25	5.22
3	Dithiopyr	0.1	2.08
4	Triclopyr	1	8.38
5	Triclopyr	0.25	2.08
6	Triclopyr	0.1	0.83
7	Penoxsulam	0.5	11.57
8	Penoxsulam	0.15	3.46

Table 4.1. Continued

9	Penoxsulam	0.05	1.15
10	Dichlobenil	0.55	24
11	Control	-	-

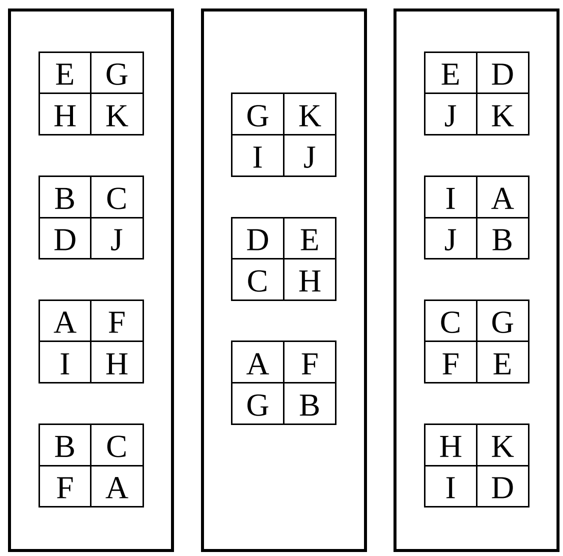


Figure 4.1. Selection Pattern of Trees for Herbicide Testing: A= Dithiopyr 1%, B= Dithiopyr 0.25%, C= Dithiopyr 0.1%, D=Triclopyr 1%, E= Triclopyr 0.25%, F= Triclopyr 0.1%, G= Penoxsulam 0.5%, H= Penoxsulam 0.15%, I= Penoxsulam 0.05%, J= Dichlobenil, K= Control

Each herbicide was prepared with 500 ml water in 18 oz. plastic cups that were placed at the bottom of each deepot. Each tree root was placed inside each cup to expose them to the herbicide solution for one hour.

The roots were washed with water and all roots growing outside of the deepots except the upper 1 in. were placed in cups filled with the herbicides. After one hour of exposure, the herbicide was discarded, the roots were washed with water from a hose, and the plants were placed back into the boxes (Figure 4.2). Daily watering was performed to maintain the same growing conditions prior to the herbicide exposure for a period of 3 weeks.

Figure 4.2. Picture of Roots a) Before Applying Herbicide, b) After Applying Herbicide

TTC test. After 3 weeks, the Triphenyl Tetrazolium Chloride (TTC) test was performed to assess the tissue viability of the roots (Ruf et al., 2003). TTC is colorless until the dehydrogenase enzymes in living cells reduce it to a bright red pigment called formazan (Briskin, 2000), which can be easily extracted and measured by spectrophotometer (Steponkus et al., 1967). The additional presence of live microorganisms on the surface of the plant roots was not considered since Steponkus et al. (1967) found that their reduction of TTC was less than 2% of the total reduction.

Roots were washed and six replicate samples were performed per plant. 0.4 grams (Steponkus et al., 1967) were collected for each sample and obtained from different parts of the roots (top, middle, and bottom portion) to perform the TTC test. In the TTC method, the roots were placed in 15ml test tubes and 3 ml of 0.6% TTC in 0.05 M phosphate buffer plus 0.05% wetting agent (Steponkus et al., 1967). All the samples were incubated for 18 hours at room temperature, rinsed with 10 ml distilled water, and extracted with 10 ml of 95% ethanol in a boiling water bath (Steponkus et al., 1967). The extracts were cooled (Figure 4.3) and the absorbance of the solution was recorded at 530 nm (Steponkus et al., 1967). The data was compared to the absorbance of untreated roots (control) and boiled roots (boiled roots are the dead tissue that are killed by the boiling process for 20 minutes).

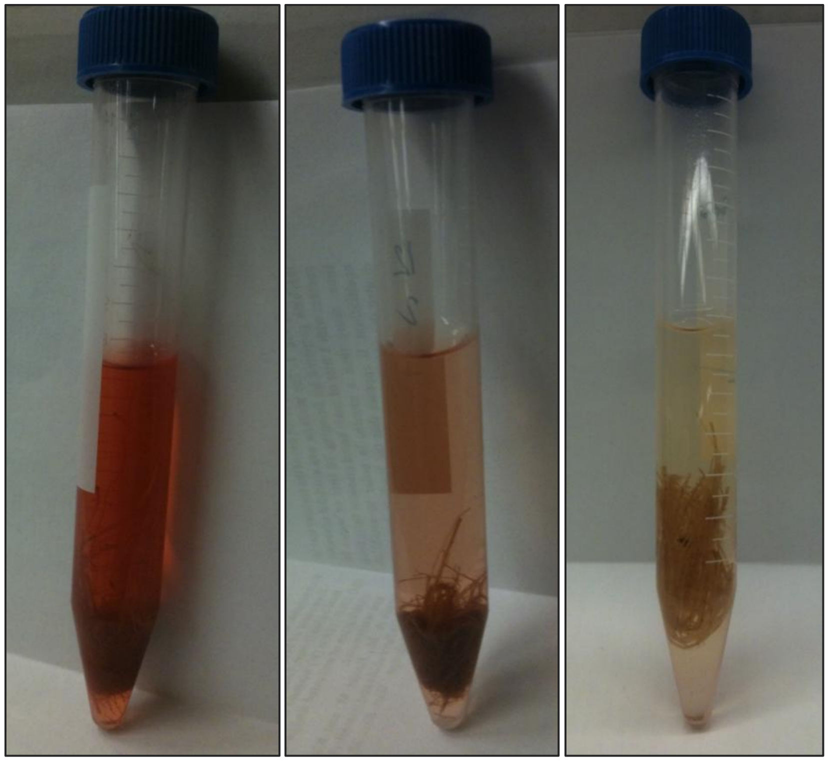


Figure 4.3. Pictures of TTC Results a) Control, b) Herbicide, c) Boiled

Experimental design and data analysis. Treatments were arranged in a completely randomized design. Each concentration of each treatment was performed on 4 trees with six replicates. Data were subjected to analysis of variance (ANOVA) using Proc GLM (Statistical Software, 2002) procedure and averages were separated using Fisher's protected LSD test at the 5% probability level (SAS institute, 1989).

Herbicide Testing on Nitrification Process

Two set of experiments were performed using two levels of herbicide concentrations to test the effect of herbicide on the nitrification process; 1) applied concentration injected at the point of root treatment line (1X dilution factor) and 2) potential diluted concentrations entering the activated sludge system (100X and 1000X dilution factor).

Reactors setup. Experimental Setup 1: Four batch reactors made of polyethylene were used for nitrification inhibition tests. Air was supplied at the bottom of the reactor using flexible spiral diffusers at a flow rate of 2.2 $Lmin^{-1}$ to maintain oxygen above 2 mg L^{-1} and to provide mixing for the system. Two ports for effluent and influent were designed at 2 and 4 liters of the reactors with the diameter of 3cm to remove the supernatant and feed the reactors, respectively. The reactors were operated at 25°C. The diagram of reactors, pumps, feed tank, and effluent tank is presented in Figure 4.4.

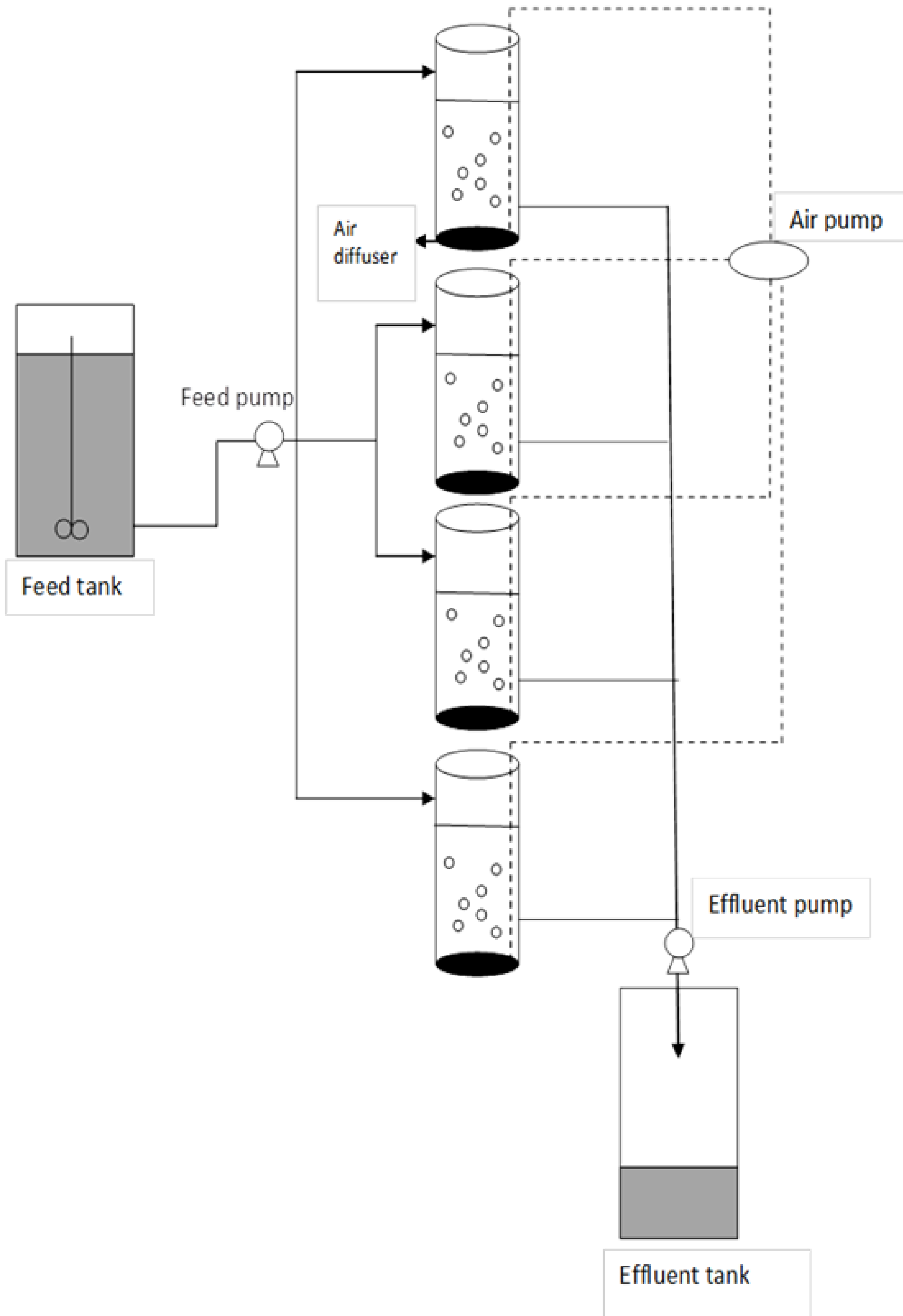


Figure 4.4. Reactor Set-Up for Exposure of Nitrification Process to Herbicide Concentration at Point of Application

Experimental Setup 2: Eight 1000 ml beakers were used as batch reactors for nitrification inhibition tests; three reactors for three herbicides (Dithiopyr, Triclopyr, and Penoxsulam) using 100X dilution factor, three reactors for three herbicides using 1000X dilution factor, one as a microbially active control, and one as a microbially inactive control. An air stone was placed at the bottom of each reactor to distribute the air and provide mixing for the system. Each air stone was connected to a manifold with eight outlets that incorporated separate valves to control the air flow rate at 2.2 $Lmin^{-1}$ to maintain oxygen level above 2 mg L^{-1} in the reactors. All reactors were operated at 25°C.

Activated sludge. Experimental Setup 1: Four batch reactors were inoculated with 4 L of activated sludge obtained from the North Cary wastewater treatment plant. The activated sludge was aerated for 1 hour followed by a 0.5 hr settling time. 2 L of supernatant was removed from each reactor and then fed with 2 L of synthetic wastewater. The composition of the synthetic wastewater is described in Table 4.2 (Rhee et al. (1997). Aeration was restarted to maintain an oxygen level above 2 mg/L for 4 hours followed by 0.5 hour settling time. After the second settling period, the supernatant removal and synthetic wastewater addition were repeated.

Experimental Setup 2: All reactors were inoculated with 1 L of activated sludge from North Cary wastewater treatment plant. The activated sludge in 7 reactors was aerated for 1 hour followed by a 0.5 hr settling period. The activated sludge for the microbial inactive control was autoclaved during the aeration period of other reactors. 500 ml of supernatant was removed from all reactors and then fed with 500 ml of synthetic wastewater described in

Table 4.2. Aeration was started to maintain the oxygen concentration above 2 mg/L for 4 hours followed by a 0.5 hour settling period. After the second settling period, the supernatant removal and synthetic wastewater addition were repeated.

Table 4.2. Synthetic Wastewater Composition

Chemical	mg/L	Chemical	mg/L
Glucose	280	$CaCl_2$.2H2O	3.75
Acetate	485	KH_2PO_4	44
$(NH_4)_2SO_4$	235.96	Na_2CO_3	66
$MgSO_4.7H_2O$	50	$NaHCO_3$	105
$FeCl_3$	0.25	$MnSO_4.H_2O$	5

Root control chemicals. Experimental Setup 1: Three proposed herbicides (Dithiopyr, Triclopyr, and Penoxsulam) with three concentrations were subjected to nitrification inhibition tests. The concentrations tested with the percentage of active ingredients for each herbicide are summarized in Table 4.3. The concentrations were based on the percent root kill results to achieve a minimum of 75% root mortality.

Table 4.3. Herbicides and Rates (Full Concentration Test)

Treatment #	**Treatment**	**Rate (% AI)**	**Rate (mg/500ml)**
1	Dithiopyr	1	21.04
2	Triclopyr	0.1	0.83
3	Penoxsulam	0.5	11.57
4	Control	**-**	**-**

Experimental Setup 2: Three herbicides (Dithiopyr, Triclopyr, and Penoxsulam) with concentrations of 100X and 1000X dilution factor were used for the second set of tests. The concentrations tested with the percentages of active ingredients for each herbicide are summarized in Table 4.4. The diluted concentrations of herbicides are used to simulate potential exposure levels of microorganisms after entering the activated sludge system in a wastewater treatment plant.

Table 4.4. Herbicides and Rates (Diluted Test)

Treatment #	**Treatment (D.F)**	**Rate (% AI)**	**Rate (mg/500 ml)**
1	Dithiopyr (100X)	0.0025	0.0522
2	Dithiopyr (1000X)	0.00025	0.00522
3	Triclopyr (100X)	0.001	0.0083
4	Triclopyr (1000X)	0.0001	0.00083
5	Penoxsulam (100X)	0.0005	0.0115
6	Penoxsulam (1000X)	0.00005	0.00115
7	Live Control	-	-
8	Dead Control	-	-

Analytical methods for nitrification inhibition. For the first set of testes after 15 minutes of aeration and for the second set of test immediately after starting the aeration, herbicides with the concentrations presented in Table 4.3 and 4.4 were added to each reactor. Immediately after the addition of herbicides, samples for analysis of COD, ammonia, nitrite, and nitrate concentrations were taken directly from reactors using a 10 ml glass pipette from the top of each reactor during aeration period. Sampling continued at a frequency of 1-1.5 hour for a period of 4 hours. COD, ammonia, nitrite, and nitrate concentration was measured using Standard Methods (APHA, 1995). All Samples were taken in triplicates.

4.3. RESULTS AND DISCUSSION

Herbicide Testing on Roots

The percent root kill was calculated using the following equations and the results are presented in Table 4.5. Six samples were used for each replicates reported in Table 4.5.

$$C_A = A - B$$
$$T_A = T - B \qquad (4.1)$$
$$\%Root\,kill = 1 - \left(\frac{T_A}{C_A}\right) \times 100 \qquad (4.2)$$

A=absorbance of untreated control
B=absorbance of boiled control
T=absorbance of treated control
C_A=control absorbance
T_A = treated absorbance

Table 4.5. Percent Root Kill

Treatment #	Treatment	Rep 1	Rep2	Rep 3	Rep 4
1	Dithiopyr-%1	76.6	89.3	52	69.3
2	Dithiopyr-%0.25	76.9	74.7	75.5	56.2
3	Dithiopyr-%0.1	67.2	60.5	54.5	51.57
4	Triclopyr-%1	94.3	85.5	79.6	86.6
5	Triclopyr-%0.25	60.5	81.6	81.9	78.4
6	Triclopyr-%0.1	97.8	94.7	94.5	95.7
7	Penoxsulam-%0.5	78.8	75.8	76.3	82.7
8	Penoxsulam-%0.15	81.4	74.3	78.6	75.6
9	Penoxsulam-%0.05	43.3	83.4	86.3	91.3
10	Dichlobenil-%0.55	97.2	97.9	94.9	96.7
11	Control	0	0	0	0
12	Boiled	100	100	100	100

As discussed earlier, SAS software (Statistical Software, 2002) was used to indicate the level of statistical significance between the different chemical treatments. The treatments were compared with each other and with the boiled samples and reported in Tables 4.6-4.8.

Table 4.6. ANOVA Table for Root Kill

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	14	31611	2258	51.85	<.0001
Error	33	1437	43.5	-	-
Corrected Total	47	33048.4	-	-	-

Table 4.7. The GLM Procedure Result for Root Kill

Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Dithiopyr 1%-0.25%	1	119.89	119.89	2.75	0.1065
Dithiopyr 1%-0.1%	1	871.11	871.11	20	<.0001
Dithiopyr 0.25%-0.1%	1	344.66	344.66	7.91	0.0082
Penoxsulam 0.5%-0.15%	1	339.53	339.53	7.8	0.8478
Penoxsulam 0.5%-0.05%	1	1564.77	1564.77	35.93	0.6186
Penoxsulam 0.15%-0.05%	1	446.52	446.52	10.25	0.7592
Triclopyr 1%-0.25%	1	2163.57	2163.57	49.68	0.0261
Triclopyr 1%-0.1%	1	1121.24	1121.24	25.75	0.0572
Triclopyr 0.25%-0.1%	1	343.84	343.84	7.9	0.0001
Dithiopyr-Penoxsulam	1	339.53	339.53	7.80	0.01
Dithiopyr-Triclopyr	1	1564.77	1564.77	35.93	<.0001
Penoxsulam-Triclopyr	1	446.52	446.52	10.25	0.003
Dithiopyr-Dichlobenil	1	2163.57	2163.57	49.68	<.0001
Penoxsulam-Dichlobenil	1	1121.24	1121.24	25.75	<.0001
Triclopyr-Dichlobenil	1	343.84	343.84	7.9	0.0083
Dithiopyr-Boiled	1	2737.93	2737.93	62.87	<.0001
Penoxsulam-Boiled	1	1544.17	1544.17	35.46	<.0001
Triclopyr-Boiled	1	593.12	593.12	13.62	0.0008
Dichlobenil-Boiled	1	22.51	22.51	0.52	0.4772

Table 4.8. t Tests (LSD) for Root Kill

t Grouping	**Mean**	**N**	**Herbicide**
A	100	12	Boiled
A	96.6	12	Dichlobenil
B	85.9	12	Triclopyr
C	77.3	12	Penoxsulam
D	69.8	12	Dithiopyr
E	0	12	Control

In Table 4.6, the overall F test is significant (F=51.85, p<0.001) at the 95% level for the variable "Treatment" indicating that the model as a whole accounts for a significant portion of variation in treatment compared to the control. As presented in Table 4.7, the Pr values for comparisons of Dithiopyr at the 1% and 0.25% active ingredient level, all three active ingredient levels of Penoxsulam, and Triclopyr 1% and 0.1% levels are higher than 0.05, indicating no difference in treatment performance among the levels tested . Dichlobenil was the best performing herbicide in killing the roots by displaying similar results as the boiled samples (The Pr value is 0.48, which is higher than 0.05). Also in the t test table (Table 4.8), averages with the same letter are not significantly different suggesting that the boiled samples and Dichlobenil has the same function.

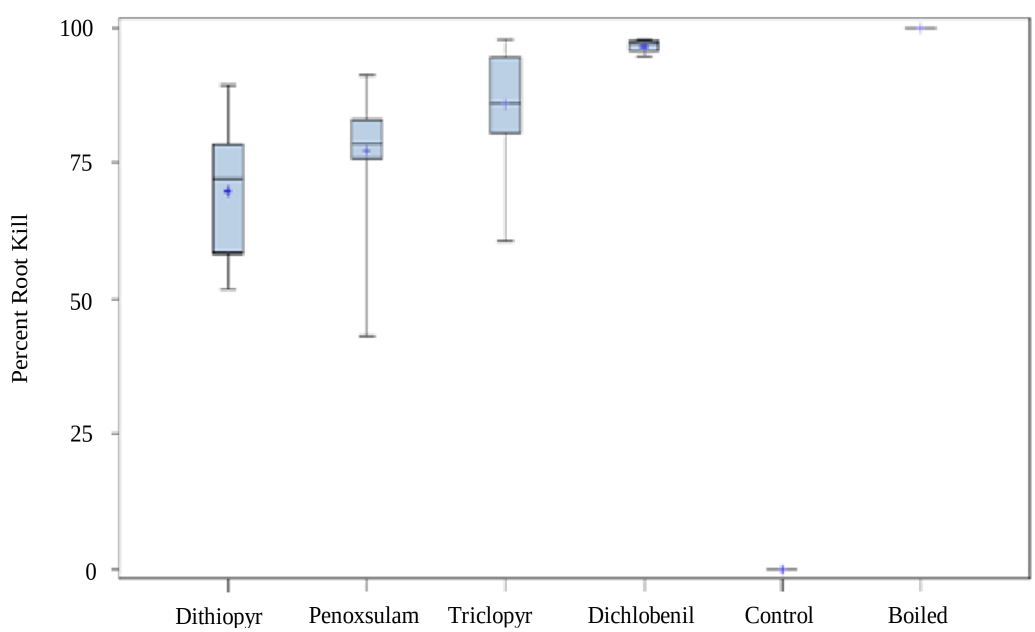


Figure 4.5. Overall Percent Root Kill- SAS Result

Figure 4.5 illustrates the overall percent root kill of each herbicide. Figure 4.6 summarizes the root kill efficiencies for the three herbicides tested as well as the commercial Dichlobenil product at the different active ingredient levels. In Figure 4.6, Dithiopyr with the highest active ingredient (1% a.i.) displayed only 76% root kill. As the Dithiopyr active ingredient decreases, the root kill efficiency decreased as low as 58% at the lowest tested level. Dithiopyr is considered a growth inhibitor and interferes with mitosis process and cell growth. Triclopyr displayed higher root kill efficiency than Dithiopyr with the same active ingredient level. The 0.1% a.i. Triclopyr displayed a very high percent root kill but did not follow the trend observed with Dithiopyr (i.e., a decreasing root kill percentage with decreasing herbicide active ingredient). A minimum root kill percent root kill was found at the intermediate active ingredient level. This Triclopyr root kill profile over the three tested ingredient levels is related to the herbicide's mechanism of action, which is categorized as a growth regulator. Growth regulators interfere with the plant cell's growth hormones balance and have been reported to present a higher activity at lower concentrations (Moore, 1998). Penoxsulam displayed little variation among the tested active ingredient levels and the percentage root kill remained relatively constant (about 73%). Penoxsulam is considered an amino acid synthesis inhibitor that blocks the protein production and increase the formation rate of toxic chemicals. The Penoxsulam results suggest that no additional inhibition of amino acid synthesis or accumulation of toxic chemicals was gained with increasing active ingredient over the range tested. Dichlobenil displayed the highest root control with 93% root kill at 0.55% a.i. Dichlobenil has the same mechanism of action as Dithiopyr, which interferes with the growth process of the cells. Dithiopyr inhibits the microtubule (component of the cytoskeleton for maintaining cell shape and helping in intracellular transport process) assembly and Dichlobenil inhibits the cell wall synthesis (Kramer et al., 2012).

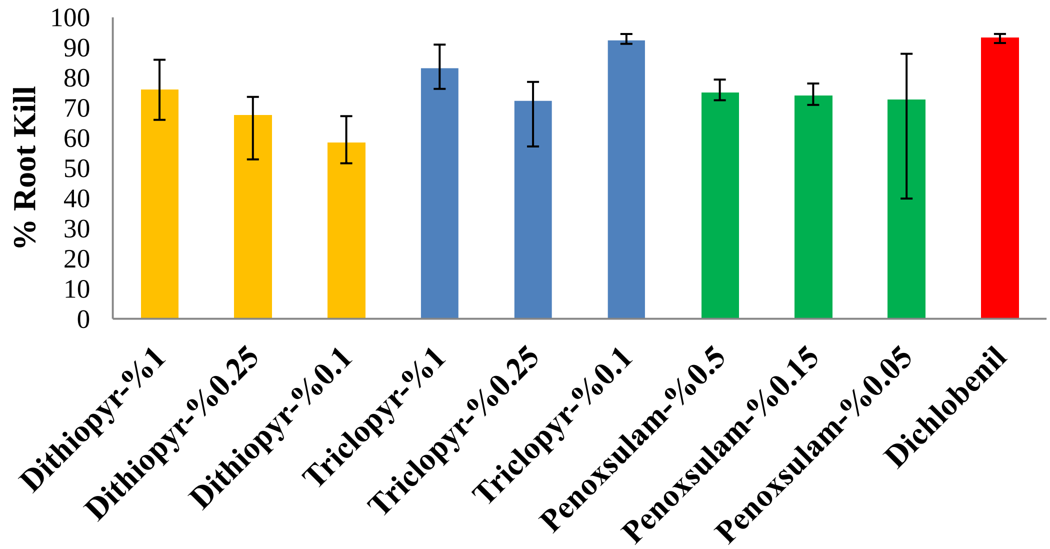


Figure 4.6. Average Root Kill Efficiency for Tested Herbicides

The impact of the herbicide products on root re-growth, shoot growth, leaf damage, and overall health was evaluated against untreated control by visual inspection. The overall health of the plants is illustrated in Figure 4.7. The upper portions of the plants displayed complete death after Triclopyr and Dichlobenil application. Re-growth was observed after three weeks of application on roots that were exposed by Dithiopyr. The upper portion of the plants treated with Dithiopyr also displayed some regions of death but based on root re-growth, shoot re-growth was also observed after three weeks (Figure 4.8). No root re-growth was observed on plants treated with Penoxsulam and the aboveground portion of those trees displayed low shoot damage after chemical exposure. The damage was higher than plants treated with Dithiopyr (based on insufficient live roots) but the trees remained viable and maintained their green leaves.

Figure 4.7. Overall Health of the Plants- a) Dithiopy, b) Penoxsulam, c) Triclopyr, d) Dichlobenil

Figure 4.8. Shoot and Root Re-growth- Plant Treated with Dithiopyr

Sample Size Analysis

JMP software (JMP, 1989-2007) was used to calculate the minimum sample size for the experiment to make sure the numbers of replicates were sufficient to provide significant difference between averages. For this experiment, 24 replicates were used for each treatment. Since this number is higher than the result of sample size calculation with JMP software, which is 14, the number of replicates used for this experiment was sufficient to provide significant difference between treatments.

Cost Estimation

Figure 4.9 illustrates the herbicide application rate and the cost associated with achieving a 75% root kill. Dithiopyr and Penoxsulam displayed 75% as the maximum root kill and Triclopyr displayed 75% as the minimum root kill using selected application rates of herbicides in the current study. Therefore, 75% was selected as an available point among the three herbicides for cost estimation. This level was also considered acceptable by root control product manufacturers for root kill in sewer lines (Raffensperger, 2011). Since the results of Dithiopyr 1% and 0.25% and all three Penoxsulam application rates were not significantly different (Table 4.7), Dithiopyr 0.25% and Penoxsulam 0.05% were used to calculate the usage cost. In addition, Triclopyr at the 0.1% application rate was selected for the cost estimation analysis since the lowest concentration of Triclopyr achieved 95% root kill. Dichlobenil data was estimated using results from Carringer's report (2003). As shown in Figure 4.9, Triclopyr represents the lowest rate and cost among the tested herbicides followed closely by Penoxsulam. Dichlobenil and Dithiopyr were a distant third and fourth, respectively. Although Triclopyr had the lowest cost for the rate applied, Penoxsulam may be a more appropriate alternative candidate for use as an alternative root control treatment since it had a lower adverse impact on the upper portion of the trees than Triclopyr. However, one question still remains about its impact on the wastewater treatment plant, which will be explored in the next section.

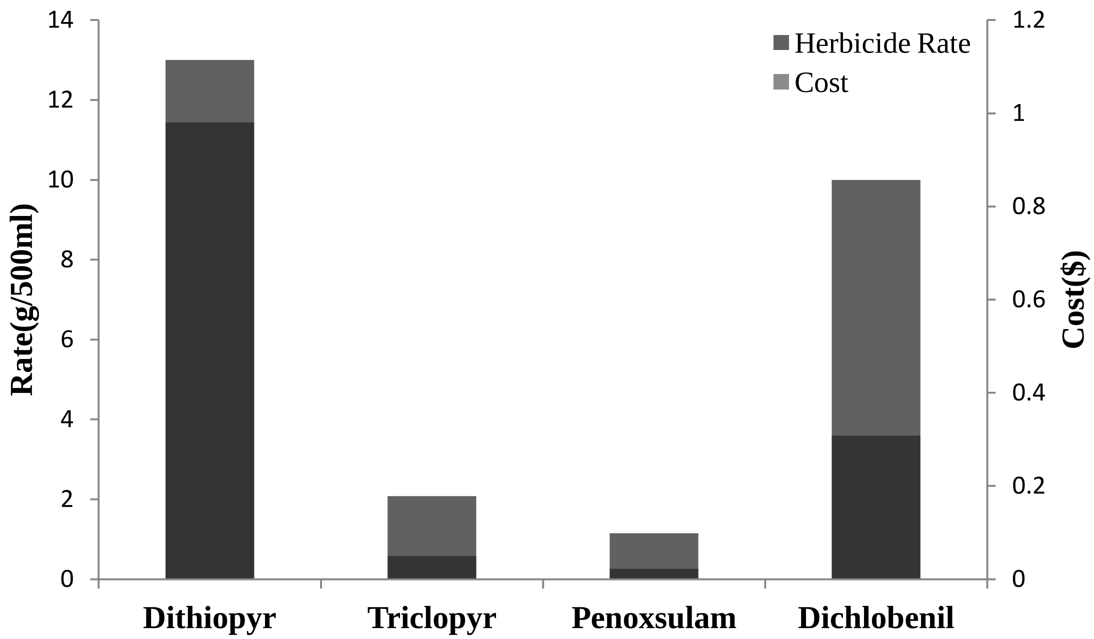


Figure 4.9. Rate and Cost Estimation for %75 Root Kill

Impact of Herbicide Chemical Addition on Nitrification Process

COD, ammonia, nitrite and nitrate concentrations were measured over a period of 4 hours to determine the probability of nitrification inhibition in all batch reactors. The results of reactors with herbicides were compared to the control reactors. A decrease in nitrite production or ammonia consumption indicates the inhibition of ammonia oxidation. The accumulation of nitrite and low nitrate concentration indicates the inhibition of nitrite oxidation.

Figure 4.10 illustrates the changes in ammonia concentration for the control reactor and the ammonia, nitrite, and nitrate concentration when the herbicides are applied over the 4 hours sampling period for Experimental Setup 1.

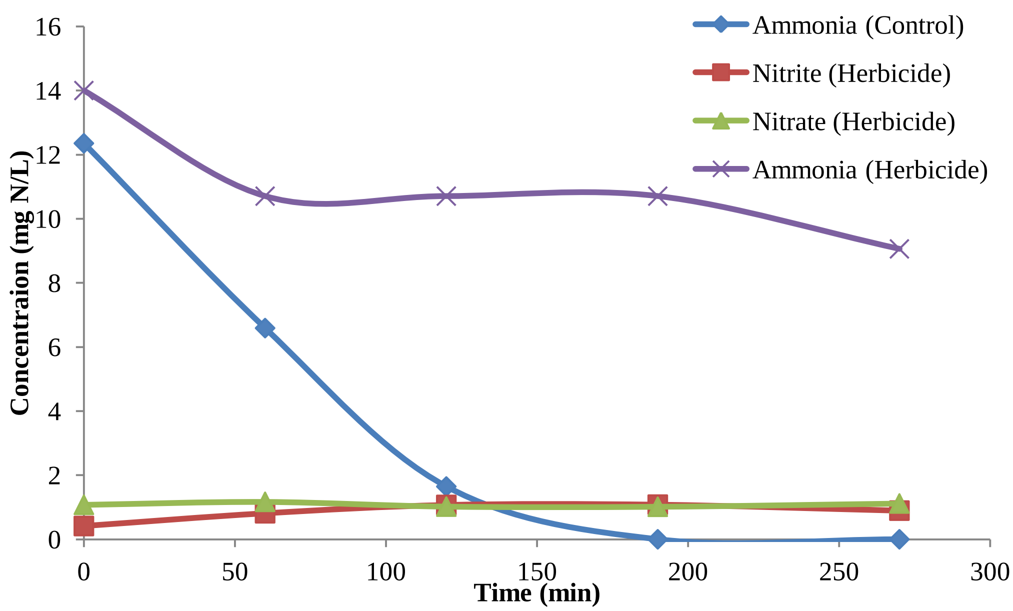


Figure 4.10. Ammonia, Nitrite and Nitrate Concentration- Control and Herbicide (Full Concentration Test)

In the control reactor, ammonia is completely oxidized to nitrite after 180 minutes. The addition of the three herbicides, however, inhibited ammonia oxidation. The chemical oxygen demand (COD) was also measured for each sample. The results for COD measurement are presented in Figure 4.11.

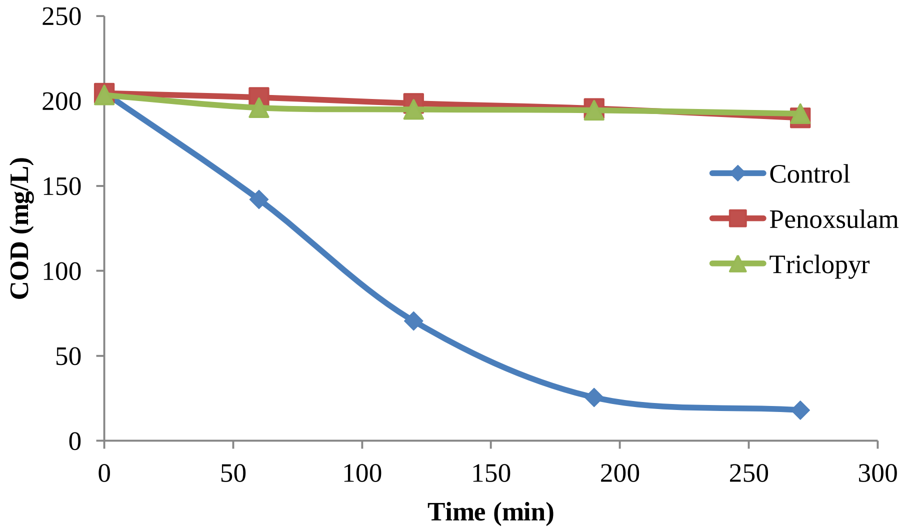
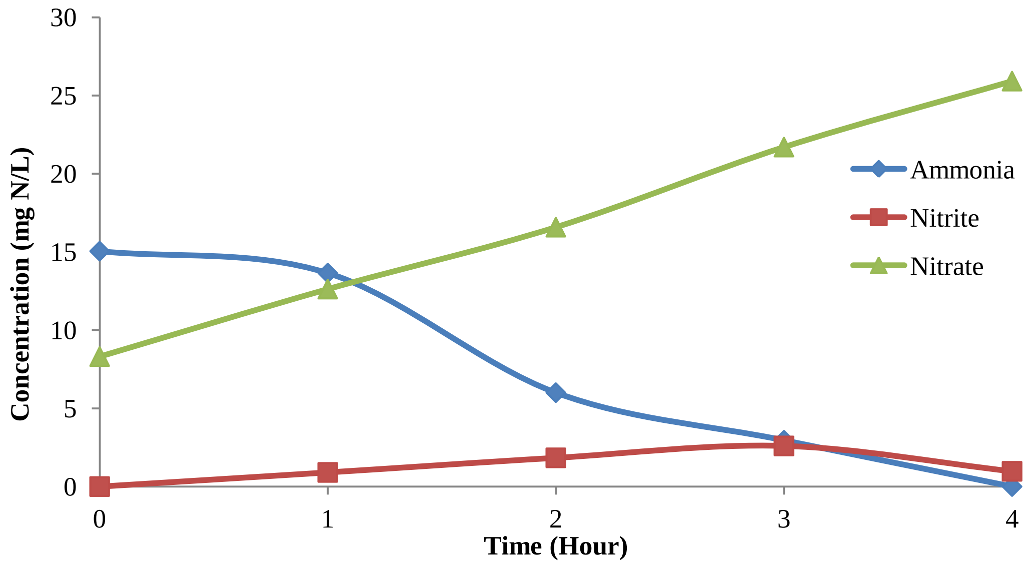


Figure 4.11. COD Measurements (Full Concentration Test)

As illustrated in Figure 4.11, the COD was consumed in the control reactor but remained constant in the reactors with herbicides, which indicated that the hetrotrophic bacteria were dead in the system. Unfortunately, Dithiopyr interferes with COD measurements and the results were unreadable.

Figure 4.12 illustrates the changes in ammonia, nitrite, and nitrate concentrations over the 4 hours sampling period for the control reactors (Live and dead) in Experimental Setup 2 with the diluted concentrations used to represent the likely level of herbicides at the inlet of the activated sludge system.

a)



b)

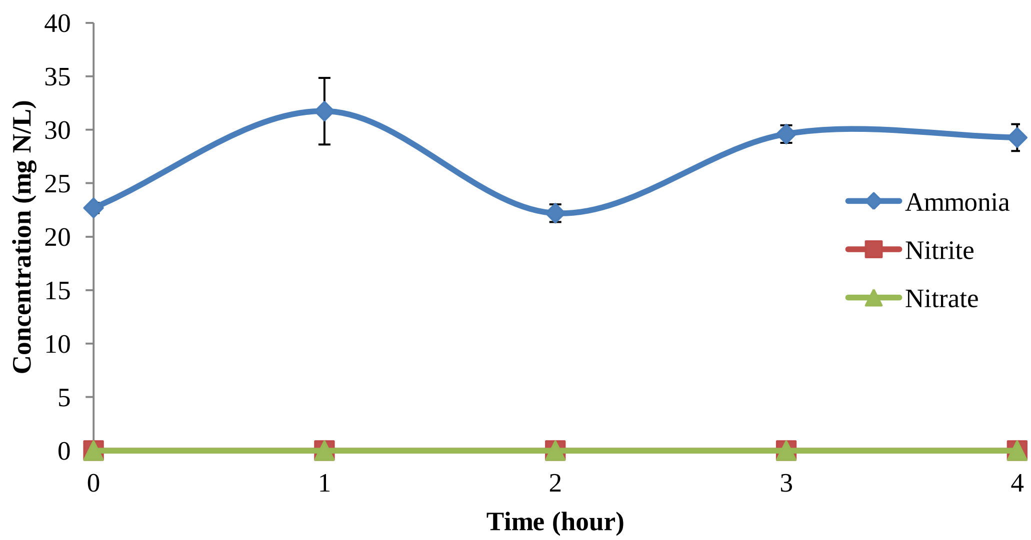
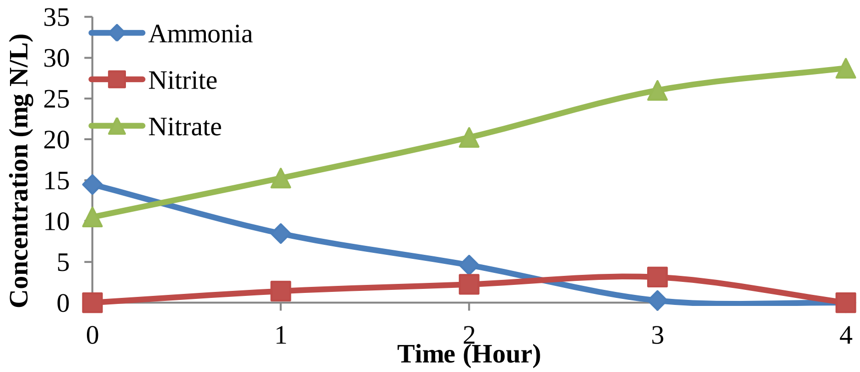


Figure 4.12. Ammonia, Nitrite and Nitrate Concentration- a) Live Control (Diluted Test), b) Dead Control (Diluted Test)

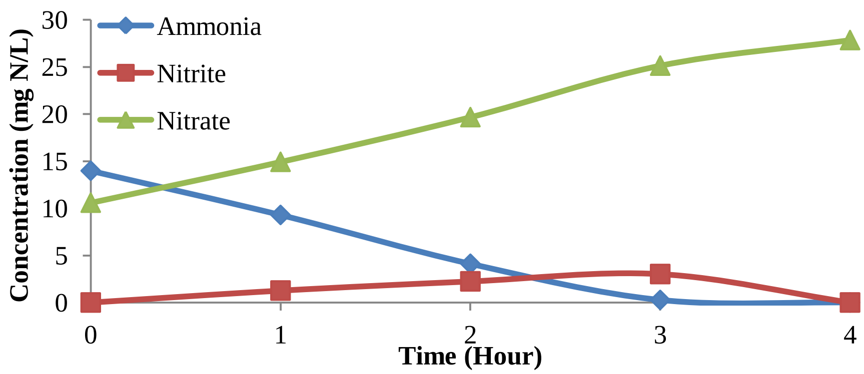
As illustrated in Figure 4.12.a, in the live control reactor ammonia oxidized completely to nitrite after 4 hours. Also nitrite accumulated to 2.5 mg NO_2/L in the first 3 hours but subsequently oxidized to nitrate. As shown in Figure 4.12.b, based on autoclave process and no activity of nitrifying bacteria, no conversion of ammonia to nitrite and nitrate had occurred. The ammonia concentration in the dead control reactor was higher than other reactors because only 500 ml of the supernatant was removed from the reactor after the first 4 hours of aeration, which resulted in some remaining ammonia in the system before the second feeding process. The results of ammonia, nitrite, and nitrate concentration for reactors with Dithiopyr, Penoxsulam, and Triclopyr are presented in Figure 4.13.

Figure 4.13. Ammonia, Nitrite, and Nitrate concentration. A) Triclopyr 1000X, B) Triclopyr 100X, C) Penoxsulam 1000X, D) Penoxsulam 100X, E) Dithiopyr 1000X, F)Dithiopyr 100X (Diluted Test)

A)


35
30
25
20
15
10
5
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
3
4
Time (Hour)

B)


30
25
20
15
10
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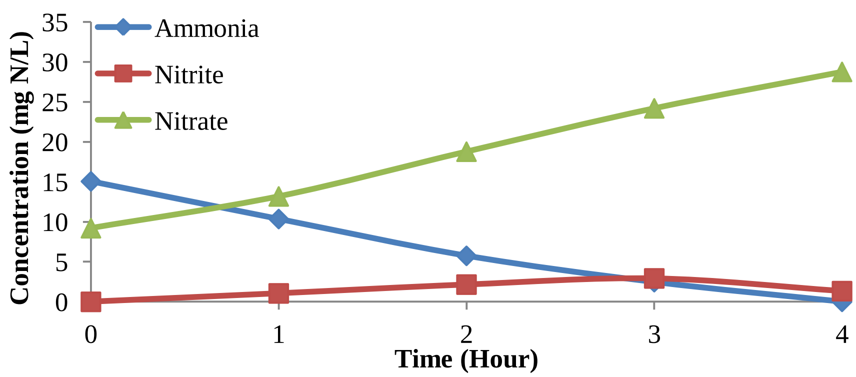
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
3
4
Time (Hour)

C)


35
30
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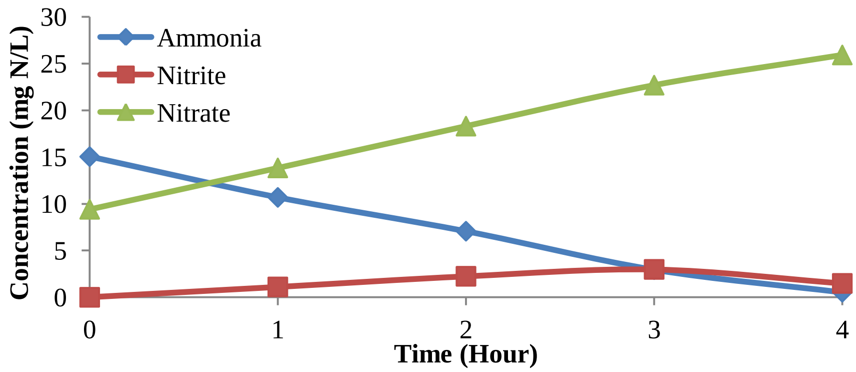
15
10
5
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
3
4
Time (Hour)

D)



30
25
20
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5
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
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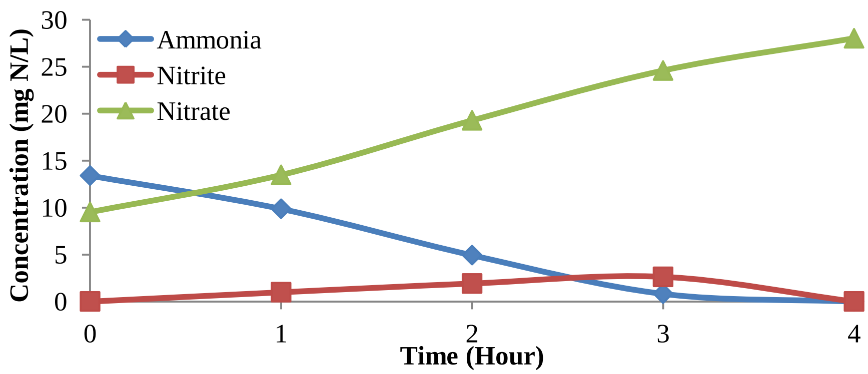
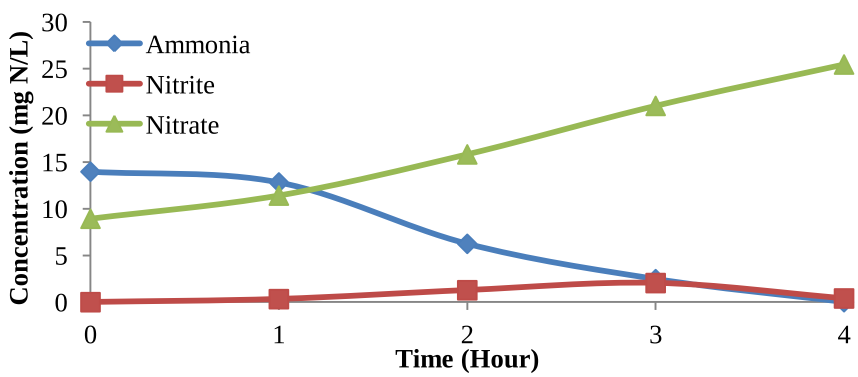
4
Time (Hour)

E)



30
25
20
15
10
5
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
3
4
Time (Hour)

F)



30
25
20
15
10
5
0
Concentration (mg N/L)
Ammonia
Nitrite
Nitrate
0
1
2
3
4
Time (Hour)

The addition of the three herbicides did not inhibit nitrification process and all reactors displayed the same results as live control reactor. Table 4.9 presents the ammonia consumption rates in all reactors.

Table 4.9. Ammonia Consumption Rate (Diluted Test)

Treatment	Ammonia Concentration (mg/L)		Ammonia Consumption Rate (mg/L.min)
	t=0 min	t=180 min	
Live Control	19	3	0.088
	18	4	0.077
	18	4	0.077
Triclopyr 1000X	17	0	0.094
	18	0	0.1
	18	1	0.094
Triclopyr 100X	17	0	0.094
	16	1	0.083
	18	0	0.1
Penoxsulam 1000X	19	3	0.088
	18	3	0.083
	18	3	0.083
Penoxsulam 100X	19	4	0.083
	19	5	0.077
	17	2	0.083
Dithipoyr 1000X	15	1	0.077
	17	1	0.088
	17	1	0.088
Dithiopyr 100X	16	3	0.072
	17	3	0.077
	18	3	0.083

A SAS code, presented in Appendix C-SAS program III, was used to indicate the level of statistical significance between the ammonia consumption rates of the reactors. The triplicate results of ammonia consumption rates were subjected to analysis of variance (ANOVA) using Proc GLM procedure and averages were separated using Fisher's protected LSD test at the 5% level of probability (Statistical Software, 2002). Each reactor containing herbicide was compared to the live control reactor and the results are presented in Tables 4.10 and 4.11.

Table 4.10. The Glm Procedure Result for Ammonia Consumption Rate (Diluted Test)

Contrast	DF	Contrast SS	Mean Square	F Value	Pr>F
Live-Dithiopyr 1000X	1	0.00001667	0.00001667	0.54	0.4762
Live-Dithiopyr 100X	1	0.00002017	0.00002017	0.65	0.4341
Live-Penoxsulam 1000X	1	0.00000017	0.00000017	0.01	0.9427
Live-Penoxsulam 100X	1	0.000024	0.000024	0.77	0.3945
Live-Triclopyr 1000X	1	0.000204	0.000204	6.57	0.0226
Live-Triclopyr 100X	1	0.000352	0.000352	11.34	0.0046

Table 4.11. t Tests (LSD) for Ammonia Consumption Rate (Diluted Test)

t Grouping	**Mean**	**N**	**Herbicide**
A	0.096	3	Triclopyr 1000X
A	0.0923	3	Triclopyr 100X
B,C	0.0846	3	Penoxsulam 1000X
B,C	0.0843	3	Dithiopyr 1000X
B,C	0.081	3	Penoxsulam 100X
C	0.0806	3	Live Control
C	0.077	3	Dithiopyr 100X

As presented in Table 4.10 the Pr values for comparisons of live reactor and Dithiopyr and Penoxsulam are higher than 0.05, indicating that the treatments are the same. Although both concentration levels of Triclopyr displayed a significant difference in comparison to the live control reactor, the ammonia consumption rates were higher and Triclopyr did not show an inhibition effect. Also in the t test table (Table 4.11), the averages with the same letter are not significantly different showing reactors with herbicides have the same or better nitrification performance as live control reactor and no significant inhibition was observed by the diluted root control chemicals entering activated sludge system.

The chemical oxygen demand (COD) was also measured for each sample. The results for COD measurement are presented in Figure 4.14.

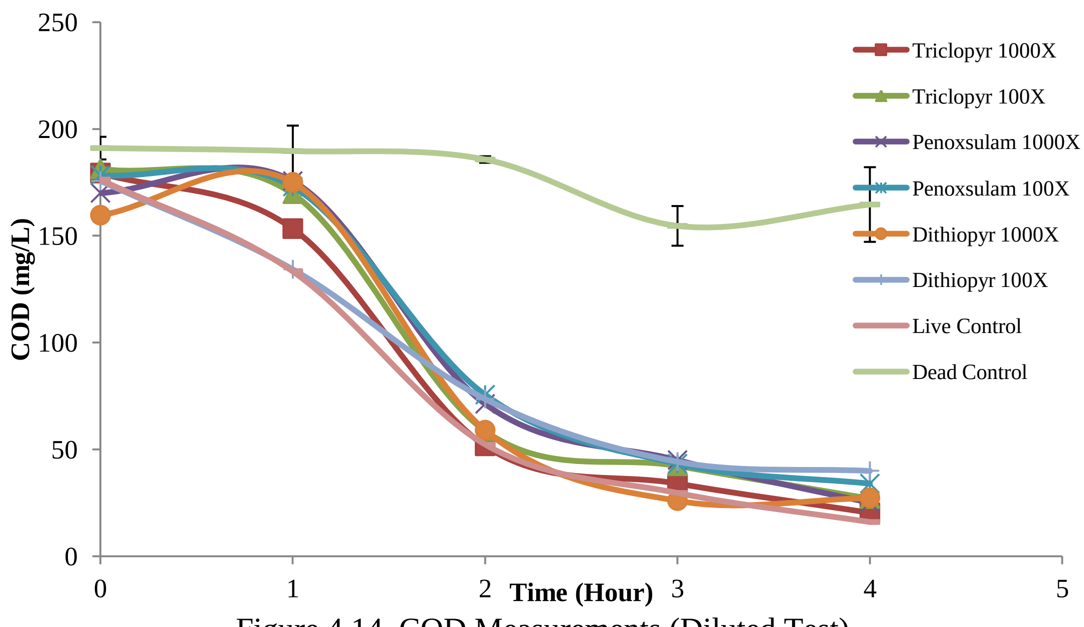


Figure 4.14. COD Measurements (Diluted Test)

As illustrated in Figure 4.14, the COD was consumed in all reactors with herbicide as the same pattern as live control reactor after 4 hours but remained constant in the dead control reactor.

Although the diluted herbicide tests used concentrations with 100X and 1000X dilution factor, it is anticipated that the actual herbicide concentration will be more diluted when they enter the wastewater treatment plant. An analysis was performed to determine the likely inlet concentration at a wastewater treatment plant and is shown in Table 4.12. In Table 4.12, the North Cary wastewater treatment plant, with a capacity of 12 MGD, was used as the test case. In addition, 50% of all sewer lines were assumed have root intrusion problems. We also assume 30% of each root intruded line needs treatment and the highest concentration of herbicide (Dithiopyr= 42.08 mg/L) was used for the treatment.

The results of this sample calculation in Table 4.12 suggest that the potential herbicide concentration entering the wastewater treatment plant will be 6 orders of magnitude lower than the diluted concentration tested in this study and constitute a conservative estimate on the impact of herbicides on nitrification processes. As such, the application of tested root control chemicals will not influence the wastewater treatment plant under real sewer conditions. Overall, the results of the percent root kill performance and the impact analysis on the wastewater treatment plant suggest that Penoxsulam should be selected as the best alternative option among the tested herbicides.

Table 4.12. Herbicide Dilution at Wastewater Treatment Plant

Assumption	50% of all sewer lines and 30% of each intruded line has root intrusion problem.
Capacity of North Cary WWTP	$12*10^6$ gal/day= $5*10^5$ gal/hr= 1900000 L/hr
Number of main sewer lines	10
Number of main sewer lines with root problem	5
Capacity of each line	$5*10^4$ gal/hr= 190000 L/hr
Distance between two manholes	400 feet= 121.92 m
Typical diameter of a sewer pipe	8 inch= 0.2032 m
Volume of sewer between two manholes	708 gal= 2.68 m^3=2680L
Volume of root intruded parts	57000 L
Volume of sewer without herbicide	133000 L
Herbicide dilution in each line	$3.16*10\text{-}^{4}$ mg/L
Herbicide dilution entering the WWTP per hour	$3.35*10^{-10}$ mg/L

4.4. CONCLUSION

This study compared the effectiveness of three herbicides (Triclopyr, Dithiopyr, and Penoxsulam) in controlling the root intrusion in sewer lines and compared the results with an existing commercial product based on Dichlobenil formula. Dichlobenil and Triclopyr displayed the highest root control compared to the other tested herbicides (about 93% and 78%) but the upper portions of the plants displayed complete death after applying these herbicides. Dithiopyr, with the highest herbicide level (1% a.i.) displayed only 76% root kill but significantly loss its effectiveness as concentration decreased (i.e.,the root kill efficiency decreased to 58%). The upper portion of the plants treated with Dithiopyr displayed some regions of death but based on root re-growth, shoot re-growth was also observed after three weeks. Penoxsulam displayed little variation among the tested concentrations and the percentage root kill remained above the industry acceptable limit (about 75%). The aboveground portion of the trees whose roots were treated with Penoxsulam showed little shoot damage after chemical exposure. The damage was higher than plants treated with Dithiopyr (based on insufficient live roots) but the trees remained viable and maintained their green leaves.

The second part of the study assessed the effect of herbicides on wastewater treatment plant nitrification process in activated sludge system with two herbicide concentration levels. All three alternative herbicides inhibit nitrification when the full treatment concentrations applied at sewer lines containing roots were used. When the herbicide concentrations were diluted by the factors of 100X and 1000X, no nitrification inhibition was observed with all three herbicides. It is anticipated that concentrations significantly lower than the diluted amount tested in this study will be observed at full scale treatment systems.

Penoxsulam represents a low cost that achieved acceptable root kill efficiency among the tested herbicides. Since Penoxsulam was the only herbicide that achieved the minimum requirements for potential commercial herbicide usage, had a low cost to achieve that requirement, and had the least impact on the above ground plant health, it was recommended as the best performing alternative herbicide. In places where the health of the above ground portion of trees is not an issue, based on higher percent root kill and lower cost, Trilcopyr could be an alternative option.

5. FUTURE WORK

Future work for this research should include measuring the required time for nitrite and nitrate recovery. In addition, lower concentrations of Triclopyr and Dichlobenil should be tested since lower concentrations of these herbicides may not kill the above portion of the plant yet provide sufficient root kill efficiency. Finally, tests with the addition of simultaneous herbicides that utilize different mechanisms of action on the treatment of intruded roots should be performed. For example a combination of 70% Penoxsulam and 30% Triclopyr could provide effective treatment to achieve a high percent root kill as well as low nitrification inhibitory effect in wastewater treatment plants. This decision is based on Triclopyr high efficiency in killing roots, low nitrification inhibition of Penoxsulam and low chemical cost for both of the herbicides.

6. REFERENCES

Ahrens, J., O. Leonard, and N. Townley (1970). Chemical control of tree roots in sewer lines. *Water Pollution Control Federation journal* 42 (9):1643-1655

Ake, T. (1995). The evaluation of Metam sodium and dichlobenil impacys on activated sludge nitrification". *Thesis for masters of science in environmental engineering, Virgina, Polythechnic and State University*.

APHA, 1995. Standard mehods. *American Health Association*, 19 th Edition, Washing, DC.

Arvin, E., S. Dyreborg, C. Menck, and J. Olsen (1994). A mini-nitrification test for toxicity screening, MINTOZ. Water Resources. 28(9):2029-2031.

Briskin, D. (1994). Membranes and transport systems in plants: An overview". *Weed Science*, 42:255-262.

Bromilow, R., K. Chamberlain and A. Evans (1990). Physicochemical aspects of phloem translocation of herbicides. *Weed Science* 38:305-314.

Carringer, R. (2003). Comparison of RootX and Dithiopyr for root control in simulared sewer system. *Study Report prepared for General Chemical Company*.

Colquhoun, J. (2001).How herbicides work: uptake, translocation, and mode of action. *Oregon State University. Extension Service*, 8785: 1-21.

Comas, L., D. Eissenstat, and A. Lakso (2000). Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytol*, 147:171-178.

Ducoste, J., J. Wood, T. Aziz, J. Groninger, L. Holt, and K. Keener (2008a). Rooting out SSOs. *Water Environement Federation Magazine*.

Ducoste, J., K. Keener, J. Groninger and L. Holt (2008b). Fats, roots, oils, and grease (FROG) in centralized and decentralized systems. *WERF Report 03-Cts-16T.*

Duke, K. and E. Jessen (2005). Sewer line chemical root control. *EPA Report*.

Raffensperger, P., 2011, Personal Communication.

Goodman, R. (1988). Herbicide selectivity. *Trends in Biotechnology*, 6(2).Grimes, H., R. Slay and Th. Hodgez (1988). Plant plasma membrane proteins. *Plant Physiol* 88, 444-449.

Groninger, J. and J. Bohanel (2000). Effects of Diquat applied to exposed roots of Black Willow. *Plant Growth Regul* 19:453-456.

Groninger, J., Sh. Zedaker, and J. Seiler (1997). Herbicides to control tree roots in sewer lines". *Journal of Agriculture* 23(5).

Hogan, M. (2001). Problems caused by roots in sewers. *Water World Continuing Education Report*.

JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007

Kantharaj. G. (2000). Plant cell biology. <http://plantcellbiology.masters.grkraj.org/>

Kramer, W., U. Schirmer, P. Jeschke, and M. Witschel (2012). Modern crop protection compounds. Vol. 1:443-446

Lindeburg, M. (1975). Chemical Engineering reference manual for the PE exam.

Malavenda, A., and K. Duke (1999).Method for controlling root growth using Diquat Bromide". *United States Patent*, Patent Number: 5919731.

McGraw-Hill (1999). <http://www.mhhe.com/biosci/esp/2001_gbio/folder_structure/ce/m3/s1/>

Mosier, D., D. Peterson, and D. Regehr (1990). Herbicide mode of action. *Kansas State University. Cooperative Extension Service*, 715:3-12.

Moore, G. (1998). Tree growth regulators: Issues of control, matters of management. *Arboriculture and Urban Forestry Online* 24(1).

Newton, B. and L. Townsend (2001). Sewer root control. *Kentucky Pesticide Applicator Training Program*.

Pike, D. and A. Hager. How herbicides work. *National Agricultural Pesticide Impact Assessment Program Report*.

Randall, C., E. Cokgor, Z. Kisoglu, W. Punrattanasin, U. Erdal, and T. Sriwiriyarat (2003). The effects of Diquat Divromide on biological wastewater treatment plants. Journal of Environmental Science and Health, A38(10):2453-2463.

Rhee, S., J. Lee, and S. Lee (1997). Nitrate accumulation in a sequencing batch reactor during the aerobic phase of biological nitrogen removal. *Biotechnology Letters*, 19(2):195-198.

Richardson, M. (1985). Nitrification inhibition in the treatment of Sewage. *The Royal Society of Chemistry*.

Rolf, K. and O. Stal (1996). Tree roots in sewer systems in MALMO, SWEDEDN. *Journal of Arboriculture* 20(6).

Rubin, A. (2004). Metam Sodium (Sodium N-Methyldithiocarbamate), Risk characterization document. *Medical Toxicology Branch, Department of Pesticide Regulatoin, California Environmental Protection Agency*,1-120.

Ruf, M. and I. Brunner (2003). "Vitality of tree fine roots: reevaluation of the tetrazolium test. *Tree Physiology* 23:257-263.

Scholar, F. and J. Wrobleski (2002). Controlling root intrusion. *REEVES Journal*.

Statistical software, Statistical Software (2002–2003), SAS software, Version 9.1, Statistical Software, Cary, NC.

Steponkus, P. and F. Lanphear (1967). Refinement of Triphenyl Tetrazolium Chloride method of determining cold injury. *Plant Physiol*. 42:1423-14267.

Webb, D. (2001). Vegetative Growth.
<http://www.biologie.unihamburg.de/bonline/library/webb/BOT201/Angiosperm/MagnoliophytaLab99/MagnoliosidaLab.htm>

Yeung, Ch. And C. Criddle (2007). Inhibition of Activated Sludge Nitrification by Root Control Chemicals: An Initial Evaluation of Dosage and Contact Time. *Department of Civil and Environmental Engineering, Stanford University*. 2-12.

APPENDIX

APPENDIX A – Herbicides characteristics

Table A.1. Penoxsulam Characteristics

Molecular formula	$C_{16}H_{14}F_5N_5O_5S$
Molecular weight	483.37 g/mole
Description	Light tan liquid
Density	1.61 g/ml (20 C)
Melting point	212 C
Boiling point	NA
Vapor pressure	9.55* 10-14 Pa (25 C)
Solubility in water	5.7 mg/L (pH 5, 19 C); 410 mg/L (pH 7, 19 C); 1460 (pH 9, 19 C)
Stability	Hydrolytically stable in pH 5 to 9 water; thermally stable at typical use temperatures
pKa	NA
Kow	Log Kow=-0.354

Table A.2. Dithiopyr Characteristics

Molecular formula	$C_{15}H_{16}F_5NO_2S_2$
Molecular weight	401.41 g/mole
Description	Crystalline colorless, faint odor
Density	1.42 g/ml (20 C)
Melting point	65 C
Boiling point	52 C ($1.01*10^{-5}$ Pa)
Vapor pressure	$5.33*10^{-4}$ Pa (25 C)
Solubility in water	1.38 mg/L (20 C)
Stability	>2 yr (>0 C)
pK_a	None (non-ionizable)
K_{ow}	56,250

Table A.3. Triclopyr Characteristics

Molecular formula	$C_{13}H_{16}Cl_3NO_3$
Molecular weight	356.63 g/mole
Description	Fluffy white solid
Density	1.85 g/ml
Melting point	148-150 C
Boiling point	Decomposes before boiling
Vapor pressure	1.6* 10^{-4} Pa (25 C)
Solubility in water	23 mg/L (25 C)
Stability	Decomposes by UV light; decomposes at 290 C
pK_a	2.68 (weak acid)
K_{ow}	2.64 (pH 5); 0.36 (pH7); 0.11 (pH 9)

Table A.4. Dichlobenil Characteristics

Molecular formula	$C_{17}H_3Cl_2N$
Molecular weight	172.01 g/mole
Description	White to slightly yellow crystals, aromatic odor
Density	0.6 g/ml (20 C)
Melting point	145-146 C (pure); 139-145 C (technical)
Boiling point	270 C (1.0 * 10^5 Pa)
Vapor pressure	7.33* 10^{-2} Pa (20 C); 1.33* 10^{-1} Pa (25 C); 2 Pa (50 C); 147 Pa (100 C)
Solubility in water	5.7 mg/L (pH 5, 19 C); 410 mg/L (pH 7, 19 C); 1460 (pH 9, 19 C)
Stability	Water (25 C), 20.5 mg/L (pH 5); 21.2 mg/L (pH 7); 21.9 mg/L (pH 9)
pK_a	NA (NON-IONIZABLE)
K_{ow}	500

APPENDIX B – Absorbance results

Table B.1. Absorbance results

Dithiopyr Rate	Absorbance at 530 nm						Average Abs	Treated Sample Abs	%root kill	Ave. %root kill
1%	0.25	0.157	0.377	0.36	0.287	0.581	0.3353	0.2493	76.64325	79.3286
	0.077	0.333	0.162	0.158	0.335	0.138	0.2005	0.1145	89.274	
	0.409	0.19	0.176	0.341	0.219	0.331	0.2777	0.1917	82.04528	
	0.626	0.497	0.333	0.573	0.238	0.212	0.4132	0.3272	69.35207	
0.25%	0.101	0.072	0.386	0.684	0.19	0.559	0.3320	0.2460	76.9555	70.8353
	0.585	0.118	0.108	0.385	0.301	0.639	0.3560	0.2700	74.70726	
	0.321	0.266	0.26	0.286	0.327	0.628	0.3480	0.2620	75.45667	
	0.338	0.363	0.777	0.222	0.493	1.127	0.5533	0.4673	56.2217	
0.01%	0.267	0.371	0.371	0.513	0.382	0.713	0.4362	0.3502	67.1975	58.4582
	0.237	0.484	0.631	0.534	0.545	0.613	0.5073	0.4213	60.53084	
	0.656	0.347	0.633	0.343	0.699	0.75	0.5713	0.4853	54.53552	
	0.392	0.286	0.248	0.168	1.127	1.397	0.6030	0.5170	51.56909	
Triclopyr Rate	Absorbance at 530 nm						Average Abs	Treated Sample Abs	%root kill	Ave. %root kill
1%	0.0287	0.048	0.265	0.17	0.141	0.229	0.1470	0.0610	94.2904	86.5866
	0.16	0.132	0.301	0.375	0.2	0.276	0.2367	0.1507	85.8829	
	0.109	0.29	0.384	0.408	0.278	0.353	0.2990	0.2130	79.6000	
	0.229	0.166	0.195	0.149	0.347	0.29	0.2293	0.1433	86.5730	
0.25%	0.237	0.484	0.631	0.534	0.545	0.613	0.5073	0.4213	60.5308	75.6284
	0.115	0.443	0.25	0.228	0.237	0.42	0.2822	0.1962	81.6237	
	0.159	0.259	0.14	0.696	0.183	0.237	0.2790	0.1930	81.9204	
	0.413	0.36	0.168	0.253	0.259	0.444	0.3162	0.2302	78.4387	
0.01%	0.035	0.072	0.063	0.113	0.224	0.15	0.1095	0.0235	97.7986	95.6987
	0.122	0.133	0.16	0.204	0.105	0.13	0.1423	0.0563	94.7229	
	0.144	0.071	0.105	0.118	0.255	0.173	0.1443	0.0583	94.5355	
	0.064	0.074	0.105	0.118	0.255	0.173	0.1315	0.0455	95.7377	
Penoxsulam Rate	Absorbance at 530 nm						Average Abs	Treated Sample Abs	%root kill	Ave. %root kill
0.50%	0.551	0.244	0.197	0.188	0.436	0.26	0.3127	0.2267	78.7666	78.3958
	0.277	0.181	0.212	0.461	0.452	0.48	0.3438	0.2578	75.8470	
	0.196	0.109	0.474	0.669	0.21	0.378	0.3393	0.2533	76.2685	
	0.184	0.115	0.491	0.331	0.191	0.312	0.2707	0.1847	82.7010	
0.15%	0.169	0.133	0.314	0.247	0.415	0.429	0.2845	0.1985	81.4052	77.4902
	0.133	0.233	0.304	0.612	0.488	0.392	0.3603	0.2743	74.3013	
	0.416	0.214	0.123	0.288	0.402	0.442	0.3142	0.2282	78.6261	
	0.227	0.388	0.258	0.246	0.527	0.431	0.3462	0.2602	75.6284	
0.05%	0.4	0.472	1.392	0.382	0.772	0.733	0.6918	0.6058	43.2475	76.0500
	0.18	0.342	0.166	0.41	0.324	0.157	0.2632	0.1772	83.4036	
	0.368	0.156	0.281	0.238	0.153	0.2	0.2327	0.1467	86.2607	
	0.167	0.211	0.173	0.239	0.191	0.093	0.1790	0.0930	91.2881	

Table B.1. Continued

Dichlobenil	Absorbance at 530 nm						Average Abs	Treated Sample Abs	%root kill	Ave. %root kill
	0.113	0.141	0.115	0.078	0.121	0.128	0.116	0.03	97.1897	96.6432
	0.052	0.062	0.147	0.141	0.106	0.147	0.1092	0.0232	97.8298	
	0.105	0.091	0.131	0.148	0.169	0.204	0.1413	0.0553	94.8165	
	0.081	0.105	0.145	0.106	0.145	0.143	0.1208	0.0348	96.7369	

	Absorbance at 530 nm						Average Abs
Control	0.725	1.299	1.42	1.281	0.422	1.258	1.0675
Boiled	0.064	0.092	0.116	0.098	0.076	0.073	0.0865

Table B.2. Average Absorbance at 530nm

Treatment #	Treatment	Rep 1	Rep2	Rep 3	Rep 4
1	Dithiopyr-%1	0.335	0.201	0.273	0.413
2	Dithiopyr-%0.25	0.332	0.356	0.348	0.553
3	Dithiopyr-%0.1	0.436	0.507	0.571	0.603
4	Triclopyr-%1	0.147	0.236	0.299	0.224
5	Triclopyr-%0.25	0.507	0.282	0.279	0.316
6	Triclopyr-%0.1	0.109	0.142	0.144	0.132
7	Penoxsulam-%0.5	0.313	0.344	0.339	0.271
8	Penoxsulam-%0.15	0.284	0.360	0.314	0.346
9	Penoxsulam-%0.05	0.692	0.264	0.233	0.179
10	Dichlobenil-%0.55	0.116	0.109	0.141	0.121
11	Control	1.299	1.42	1.281	1.258
12	Boiled	0.064	0.092	0.073	0.076

APPENDIX C – SAS Program

C1. SAS Program I

```
proc factex;
     factors block / nlev=4;
output out=blocks
           block nvals=(1 2 3 4);
  run;
     factors trt / nlev=11;
output out=rcbd
           designrep=blocks
randomize (101)
trt cvals=('A' 'B' 'C'
                   'D' 'E' 'F'
'G' 'H' 'I'
                    'J' 'K'   );
  run;
proc print data=rcbd;
run;
```

C2. SAS Program I- Result

Obs	block	trt
1	3	E
2	3	G
3	3	H
4	3	K
5	3	B
6	3	C
7	3	D
8	3	J
9	3	A
10	3	F
11	3	I
12	2	H
13	2	B
14	2	C
15	2	F
16	2	A
17	2	G
18	2	K
19	2	I
20	2	J
21	2	D
22	2	E
23	1	C
24	1	H
25	1	A
26	1	F
27	1	G
28	1	B
29	1	E
30	1	D
31	1	J
32	1	K
33	1	I
34	4	A
35	4	J
36	4	B
37	4	C
38	4	G
39	4	F
40	4	E
41	4	H
42	4	K

43	4	I
44	4	D

C3. SAS Program II

```
data Root;

input ph$ k no @;

do rep=1 to 4; input y @;

output; end;

cards;

Di 1 1 69.35 76.64 82.05 89.27

Di 0.25 2 56.22 74.71 75.46 79.95

Di 0.1 3 51.57 54.54 60.53 67.19

Pe 1 4 75.85 76.27 78.76 82.70

Pe 0.25 5 74.3 75.63 78.63 81.41

Pe 0.1 6 43.25 83.4 86.26 91.29

Tr 0.5 7 79.61 85.51 86.58 94.29

Tr 0.15 8 60.53 78.44 81.62 81.92

Tr 0.05 9  94.53 94.72 95.73 97.79

Dichlobenil 0 10 94.82 96.74 97.19 97.83

No 0 11 0 0 0 0

Boil 0 12 100 100 100 100

;

proc glm;

class no rep;
```

```
model y= no rep;

contrast 'D1-D2' no 1 -1 0 0 0 0 0 0 0 0 0 0;

contrast 'D1-D3' no 1 0 -1 0 0 0 0 0 0 0 0 0;

contrast 'D2-D3' no 0 1 -1  0 0 0 0 0 0 0 0 0;

contrast 'P1-P2' no 0 0 0  1 -1 0 0 0 0 0 0 0;

contrast 'P1-P3' no 0 0 0  1 0 -1 0 0 0 0 0 0;

contrast 'P2-P3' no 0 0 0  0 1 -1 0 0 0 0 0 0;

contrast 'T1-T2' no 0 0 0  0 0 0 1 -1 0 0 0 0;

contrast 'T1-T3' no 0 0 0  0 0 0 1 0 -1 0 0 0;

contrast 'T2-T3' no 0 0 0  0 0 0 0 1 -1 0 0 0;

 contrast'1-2' no 1 1 1 -1 -1 -1 0 0 0 0 0 0;

contrast'1-3' no 1 1 1 0 0 0 -1 -1 -1 0 0 0;

contrast'2-3' no 0 0 0 1 1 1 -1 -1 -1 0 0 0;

contrast'1-4' no 1 1 1 0 0 0 0 0 0 -3 0 0;

contrast'2-4' no 0 0 0 1 1 1 0 0 0 -3 0 0;

contrast'3-4' no 0 0 0 0 0 0 1 1 1 -3 0 0;

contrast'1-boiled' no 1 1 1 0 0 0 0 0 0 0 0 -3;

contrast'2-boiled' no 0 0 0 1 1 1 0 0 0 0 0 -3;

contrast'3-boiled' no 0 0 0 0 0 0 1 1 1  0 0 -3;

contrast'4-boiled' no 0 0 0 0 0 0 0 0 0 1 0 -1;
```

```
proc boxplot data=root;

plot y*ph;


data modified;

set Root;

if k=0 then delete;

proc glm data=modified;

class ph rep k;

model y=k|ph rep;

means ph/LSD;

run;
```

C4. SAS Program II- Result

The GLM Procedure

Class Level Information

Class	Levels	Values
no	12	1 2 3 4 5 6 7 8 9 10 11 12
rep	4	1 2 3 4

Number of Observations Read 48
Number of Observations Used 48
The SAS System

The GLM Procedure

Dependent Variable: RootKill

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	31611.35713	2257.95408	51.85	<.0001
Error	33	1437.07457	43.54771		
Corrected Total	47	33048.43170			

R-Square	Coeff Var	Root MSE	RootKill Mean
0.956516	8.840308	6.599069	74.64750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
no	11	30402.66255	2763.87841	63.47	<.0001
rep	3	1208.69458	402.89819	9.25	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
no	11	30402.66255	2763.87841	63.47	<.0001
rep	3	1208.69458	402.89819	9.25	0.0001

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
D1-D2	1	119.892613	119.892613	2.75	0.1065
D1-D3	1	871.113800	871.113800	20.00	<.0001
D2-D3	1	344.662512	344.662512	7.91	0.0082
P1-P2	1	1.629013	1.629013	0.04	0.8478
P1-P3	1	10.998050	10.998050	0.25	0.6186
P2-P3	1	4.161612	4.161612	0.10	0.7592
T1-T2	1	236.313800	236.313800	5.43	0.0261
T1-T3	1	169.096050	169.096050	3.88	0.0572
T2-T3	1	805.208450	805.208450	18.49	0.0001

1-2	1	339.528038	339.528038	7.80	0.0086
1-3	1	1564.773504	1564.773504	35.93	<.0001
2-3	1	446.516267	446.516267	10.25	0.0030
1-4	1	2163.573075	2163.573075	49.68	<.0001
2-4	1	1121.236669	1121.236669	25.75	<.0001
3-4	1	343.844602	343.844602	7.90	0.0083
1-boiled	1	2737.932300	2737.932300	62.87	<.0001
2-boiled	1	1544.167969	1544.167969	35.46	<.0001
3-boiled	1	593.121102	593.121102	13.62	0.0008
4-boiled	1	22.512050	22.512050	0.52	0.4772

The GLM Procedure

Class Level Information

Class	Levels	Values
Herbicide	3	Di Pe Tr
rep	4	1 2 3 4
k	6	0.05 0.1 0.15 0.25 0.5 1

Number of Observations Read	36
Number of Observations Used	36

The GLM Procedure

Dependent Variable: RootKill

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	4828.089328	438.917212	9.68	<.0001
Error	24	1088.568061	45.357003		
Corrected Total	35	5916.657389			

R-Square	Coeff Var	Root MSE	RootKill Mean
0.816016	8.669815	6.734761	77.68056

Source	DF	Type I SS	Mean Square	F Value	Pr > F
k	5	2585.400801	517.080160	11.40	<.0001
Herbicide	1	339.528037	339.528037	7.49	0.0115
Herbicide*k	2	351.000300	175.500150	3.87	0.0350
rep	3	1552.160189	517.386730	11.41	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
k	4	1357.716967	339.429242	7.48	0.0005
Herbicide	1	339.528037	339.528037	7.49	0.0115
Herbicide*k	2	351.000300	175.500150	3F.87	0.0350
rep	3	1552.160189	517.386730	11.41	<.0001

The GLM Procedure

t Tests (LSD) for RootKill

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	45.357
Critical Value of t	2.06390
Least Significant Difference	5.6746

Means with the same letter are not significantly different.

t Grouping	Mean	N	Herb
A	100.000	12	Boil
A			
A	96.645	12	Dichlobe
B	85.939	12	Tr
C	77.313	12	Pe
D	69.790	12	Di
E	0.000	12	Control

C5. SAS Program III

```
Data Ammonia;

input trt$ @;
do rep=1 to 3; input rate @;
output; end;
cards;
Di1000 0.077 0.088 0.088
Di100 0.072 0.077 0.083
Live 0.088 0.077 0.077
Pen1000 0.088 0.083 0.083
Pen100 0.083 0.077 0.083
Tri1000 0.094 0.1 0.094
Tri100 0.094 0.083 0.1
;

Proc glm;

class trt;

model rate=trt;

contrast 'Live-Di1000' trt 1 0 -1 0 0 0 0;

Contrast 'Live-Di100' trt 0 1 -1 0 0 0 0;

Contrast 'Live-Pen1000' trt 0 0 -1 1 0 0 0;

Contrast 'Live-Pen100' trt 0 0 -1 0 1 0 0;

Contrast 'Live-Tri1000' trt 0 0 -1 0 0 1 0;

Contrast 'Live-Tri100' trt 0 0 -1 0 0 0 1;


means trt/lsd;

run;
```

C6. SAS Program III- Result

```
                              The GLM Procedure

                           Class Level Information

            Class         Levels    Values

            trt                7    Di100 Di1000 Live Pen100 Pen1000 Tri100 Tri1000


                    Number of Observations Read          21
                    Number of Observations Used          21


                              The GLM Procedure

Dependent Variable: rate

                                        Sum of
        Source                      DF         Squares     Mean Square    F Value    Pr > F

        Model                        6      0.00080590      0.00013432       4.32    0.0113

        Error                       14      0.00043533      0.00003110

        Corrected Total             20      0.00124124


                      R-Square     Coeff Var      Root MSE     rate Mean

                      0.649275      6.545697      0.005576      0.085190


        Source                      DF       Type I SS     Mean Square    F Value    Pr > F

        trt                          6      0.00080590      0.00013432       4.32    0.0113


        Source                      DF     Type III SS     Mean Square    F Value    Pr > F

        trt                          6      0.00080590      0.00013432       4.32    0.0113


        Contrast                    DF     Contrast SS     Mean Square    F Value    Pr > F

        Live-Di1000                  1      0.00001667      0.00001667       0.54    0.4762
        Live-Di100                   1      0.00002017      0.00002017       0.65    0.4341
        Live-Pen1000                 1      0.00000017      0.00000017       0.01    0.9427
        Live-Pen100                  1      0.00002400      0.00002400       0.77    0.3945
        Live-Tri1000                 1      0.00020417      0.00020417       6.57    0.0226
        Live-Tri100                  1      0.00035267      0.00035267      11.34    0.0046
```

The GLM Procedure

t Tests (LSD) for rate

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	0.000031
Critical Value of t	2.14479
Least Significant Difference	0.0098

Means with the same letter are not significantly different.

t Grouping		Mean	N	trt
	A	0.096000	3	Tri1000
	A			
B	A	0.092333	3	Tri100
B				
B	C	0.084667	3	Pen1000
B	C			
B	C	0.084333	3	Di1000
	C			
	C	0.081000	3	Pen100
	C			
	C	0.080667	3	Live
	C			
	C	0.077333	3	Di100

APPENDIX D- Figures

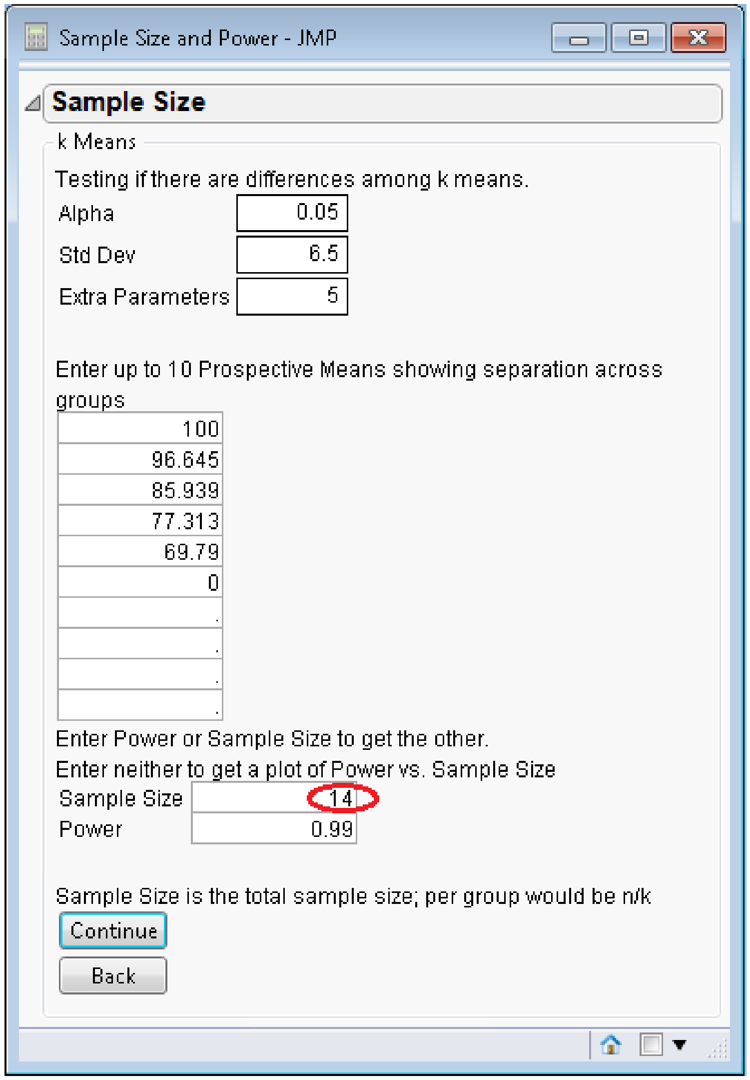


Figure D.1. Sample Size Result- JMP Software (JMP, 1989-2007)

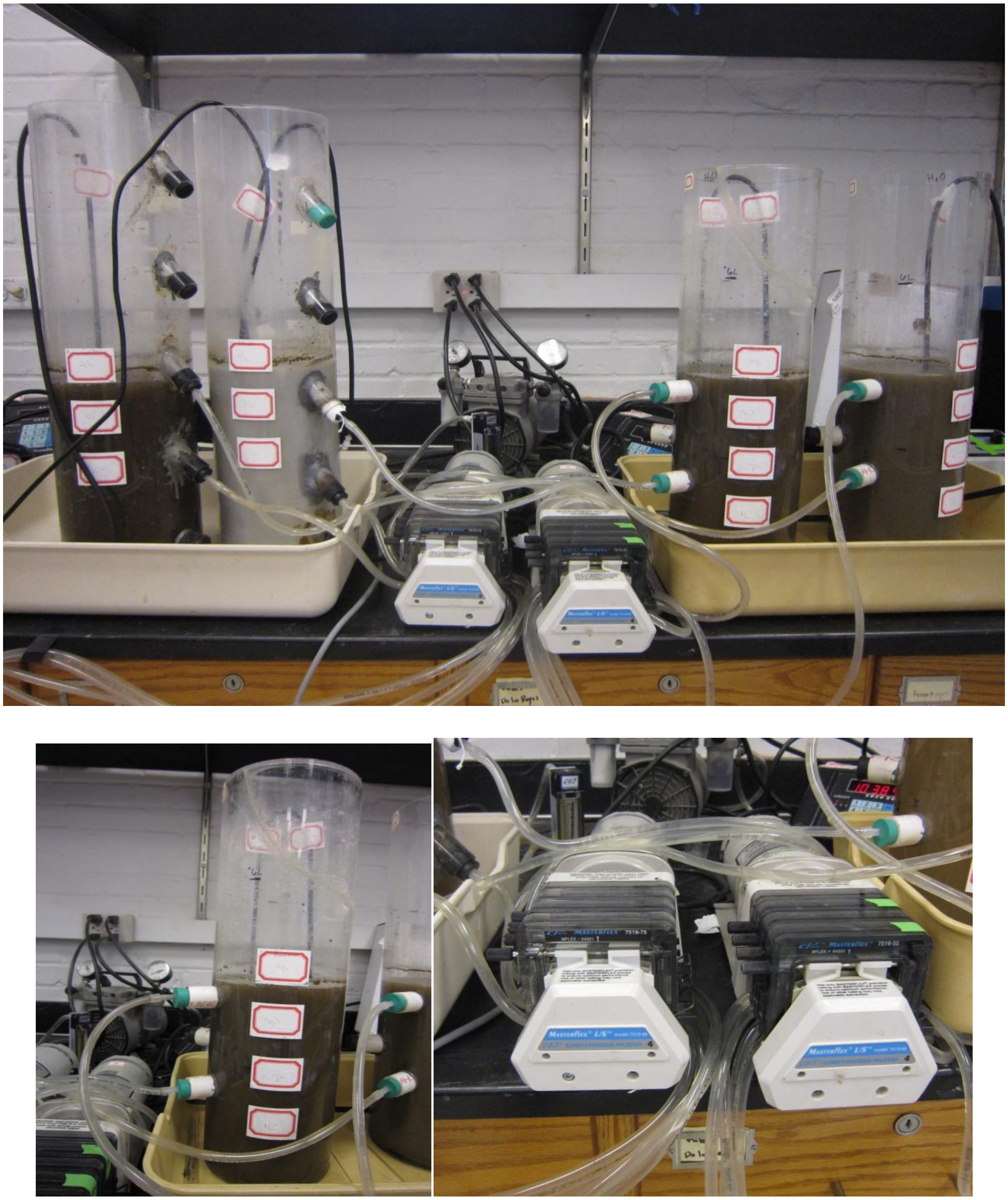


Figure D.2. Set-Up a) The Overall Set Up, b) Reactor, c) Pumps