THE CONTINUATION OF NATIVE NON-INVASIVE PLANT SPECIES RESEARCH OF ENGINEERED WETLANDS

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Abstract

Wetlands are vital ecosystems for many reasons and can provide strategies to be implemented in wastewater treatment. However, their loss around the world as a result of urbanization has caused larger bodies of water to become heavily polluted by excess nutrients such as phosphorus. Although there is extensive research on implementing constructed wetlands as a solution, there is a lack of information regarding the use of native non-invasive macrophytes. Thus, the first part of this study examined the use of select native non-invasive plant species in laboratory-based floating engineered wetland mesocosms to assess their potential for successful growth and use in nutrient removal strategies. The blue flag iris, cardinal flower and sneezeweed were chosen for the experiment as they developed best under the laboratory conditions. Additionally, while their productivity (biomass) increased with phosphorus additions, the phosphorus concentrations in the mesocosms remained high and were not reduced. The results are preliminary and further research is necessary regarding engineered wetland development and their role in nutrient sequestration.

In the second part of this thesis, the composition of native wetland species located in southern Ontario was evaluated. The Palgrave Forest and Wildlife Area was the chosen site.

Assessments included the identification and presence of species as well as the seasonality of the community. A total of 15 plant species were identified in this wetland area, and while five are considered to have serious invasive characteristics, their inclusion in a biodiverse, relatively unimpacted habitat appeared to keep these deleterious features in check. As expected, seasonality had an impact on wetland productivity, biomass and species presence.

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Table of Contents

List of Tables

List of Figures

List of Appendices

1.0 INTRODUCTION

1.1 Overview

Flowing or stagnant, fresh or salty, the area where land meets water is one of the most biodiverse and productive ecosystems in the world (Ramsar Convention on Wetlands, 2018). These habitats are known as wetlands, and aside from their aesthetically pleasing beauty, they have a long list of responsibilities that exceed those of terrestrial ecosystems. Whether it's habitat for wildlife, flood attenuation from snow melt and storms, mitigating the impacts of climate change through CO_2 sequestration or providing us with food and recreational opportunities, there is no doubt that wetlands present society and the environment with enormous value (USEPA, 1994; Capotorto, 2006; Ontario Ministry of Natural Resources and Forestry, 2017; Ramsar Convention on Wetlands, 2018). This thesis intends to focus on their ability to purify wastewater by phytoremediation. And to continue the work started by Tiley (2013) and Fernandes (2017).

Despite their evident value in treating wastewater, wetland habitats around the world are constantly being threatened by urban development and land use, which consequently threatens water quality and human health as pollutants and nutrients accumulate in aquatic environments (Bradford, 2016; Rai, 2018). The overabundance of phosphorus in lakes via urban runoff is of particular concern because it can have detrimental effects on the ecosystem by causing eutrophication and harmful algal blooms (Picard *et al*., 2005; Filippelli, 2016; Environment and Climate Change Canada and USEPA, 2017; Geng *et al*., 2017; Lake George Association, 2017; Cook *et al*., 2020).

Constructed wetlands are systems that are designed using vegetation, soil and associated microorganisms to utilize and mimic the processes that occur in natural wetlands. They can be implemented in the environment or used for lab-based experiments for various purposes but are typically used as a wastewater treatment strategy (Vymazal, 2007; Zhang *et al*., 2010; Pavlineri *et al*., 2017). Constructed wetlands come in many forms, designs, sizes and shapes and provide an aesthetically pleasing, cost-effective and sustainable alternative to conventional wastewater treatment strategies (Wallace and Knight, 2006; Kadlec and Wallace, 2009; Pavlineri *et al*., 2017; Lucke *et al*., 2019; Xiao *et al*., 2019). The issue with most constructed wetlands currently being implemented for wastewater treatment is their use of exotic and/or invasive plant species. Species such as *Typha* and *Phragmites* are commonly used in constructed wetlands because of their fast growth, tolerance to various stressors and proven ability to successfully remove pollutants from the environment. However, their aggressive and invasive characteristics can significantly reduce the diversity of ecosystems by displacing, dominating and outcompeting other vital native species (Brisson and Chazarenc, 2009; Zhang *et al*., 2010; Ijaz *et al*., 2016).

Few studies have focused on the use of native and non-invasive species in constructed wetlands. The small amount that has been conducted have demonstrated that these species may also be suitable for wastewater treatment (Kao *et al*., 2009; McAndrew *et al*., 2016; Fernandes, 2017). With that being said, there are still enormous gaps in the knowledge and literature with respect to whether or not native non-invasive species can effectively remove pollutants from the environment to the point where they can replace commonly used invasive species, such as *Typha*, in future constructed wetlands.

1.2 Objectives

The overall objective of this thesis is to continue on the existing work regarding native non-invasive wetland macrophytes in the laboratory and in field. This objective was accomplished by selecting, acquiring and growing various native non-invasive plant species and using them to develop an engineered wetland system where different concentrations of phosphorus were added and chemically and biologically analyzed. The second part of this study was accomplished by conducting field work at a local wetland site to determine the presence of native non-invasive species and observe seasonality (shifts in temperature, hydrology, growing season) of the community over time.

2.0 LITERATURE REVIEW

2.1 Wetlands

Wetlands are considered a transitional area between two biological communities, linking terrestrial and aquatic habitats, making them one of the most productive and diverse ecosystems in the world (Bardecki, 1989; USEPA, 1994; Fraser *et al.*, 2004; Mayer *et al.*, 2005; National Geographic Society, 2012; Rai, 2018). A wetland has the unique characteristic of being constantly or seasonally saturated with water less than 2 meters in depth, consisting of aquatic plants that have uniquely adapted to the wet conditions (USEPA, 1994; Vymazal, 2007; Ducks Unlimited Canada, 2015). In Canada, natural wetland ecosystems are widely distributed, covering 14% of the total land area (Environment and Climate Change Canada, 2016). The four major types of wetland plants include: emergent (e.g., *Typha* and *Iris versicolor*), submerged (e.g., *Ceratophyllym demersum*), floating-leaved (e.g., *Nuphar variegate*) and free-floating (e.g., *Lemna minor*) (Environment Canada, 1996; Brix, 2003; Vymazal, 2007). Emergent plants are rooted in the sediment while their photosynthetic and reproductive structures grow above the water surface. Floating-leaved species are also rooted in the sediment, but their leaves float on the surface of the water. Free-floating species are similar as they also float on the water surface, however, their roots are unattached and hang in the water column. Lastly, submerged macrophytes are rooted in the sediment but grow completely underwater (Capotorto, 2006; Vymazal, 2007).

2.1.1 Classification of Wetlands

These biologically diverse ecosystems are home to a unique mixture of plants and animals found in different types of climates around the world, except Antarctica, and can be categorized into swamps, marshes, bogs, fens and shallow water (USEPA, 1994; National Wetlands Working Group, 1997; National Geographic Society, 2012; Ducks Unlimited Canada, 2015). Wetlands can be further classified as palustrine (lack flowing water), marine (saltwater), estuarine (where a river meets the sea), riverine (adjacent to rivers) and lacustrine (open freshwater) depending on their water source, depth, location and microclimates (Cowardin *et al.*, 1979). Nutrient concentration can also be used to classify wetlands as oligotrophic (low nutrients), mesotrophic (moderate nutrients) and eutrophic (high nutrients) (Wetzel, 1983). Although all types of wetlands are ecologically significant, marshes are the most well-known as they are a transitional area containing emergent macrophytes that receive from precipitation, runoff, groundwater and stream inflow. These wetlands are commonly protected or restored for their services (Ducks Unlimited Canada, 2015).

2.1.2 Importance of Wetlands

The Ramsar Convention on Wetlands (2018) states that wetlands are one of the most productive and vital ecosystems on the planet for both organisms and human beings, providing countless ecological and economic services (USEPA, 1994; National Geographic Society, 2012; Ducks Unlimited Canada, 2015; Ontario Ministry of Natural Resources and Forestry, 2017; Ramsar Convention on Wetlands, 2018). Their functions and values can be divided into three major categories: hydrology, biogeochemistry and habitat. With regards to hydrology, wetlands can reduce the chance of floods, recharge groundwater and reduce soil erosion. The vegetation and soil in wetland ecosystems absorb and temporarily store water that can be slowly released or penetrated into the ground for aquifer recharge (Capotorto, 2006; Ducks Unlimited Canada, 2015; Ontario Ministry of Natural Resources and Forestry, 2017). Additionally, the roots of aquatic plants prevent erosion during storms by stabilizing the soil. Biogeochemistry relates to the ability of wetlands to enhance water quality through sediment deposition, absorption of nutrients and contaminants, microbial communities and release of oxygen (Capotorto, 2006). They also provide habitat for aquatic and terrestrial organisms, including threatened and endangered species. Many fish, reptile, amphibian and bird populations rely on wetland ecosystems for habitat, nutrients, breeding and/or energy restoration during migration (USEPA, 1994; Mayer *et al.*, 2005; Doran and Kahl, 2014; Bradford, 2016; Ontario Ministry of Natural Resources and Forestry, 2017). This creates an aesthetically pleasing area for recreational opportunities and economic value from natural products (USEPA, 1994; Ontario Ministry of Natural Resources and Forestry, 2017). The emergent and submerged macrophytes surrounding wetlands are also important because they prevent contaminants from being carried away to other areas by wind, rain and groundwater and provide substrate such as roots, stems and leaves that allow different microorganisms to grow and break down organic materials (Zhang *et al.*, 2010).

2.1.2.1 Palgrave Forest and Wildlife Area

Outdoor areas and parks are vital for city regions and developed areas as they contribute to healthy living, communities and ecosystems. The Palgrave Forest and Wildlife Area is located in Palgrave, a suburban community within the Town of Caledon in Peel Region. This area was chosen as the experimental site for this thesis. It is situated on the Oak Ridges Moraine and is property of the Toronto and Region Conservation Authority (TRCA) (TRCA, 2016b). The TRCA strives to protect and restore the integrity and health of the local natural environment and its associated services of various watershed communities in Southern Ontario (TRCA, 2016a).

The TRCA is the single largest landowner of Caledon as 55% of the population is located within its watershed jurisdictions (TRCA, 2016a).

The Palgrave Forest and Wildlife Area is part of the Humber River watershed, which is one of nine watersheds comprised in TRCA's jurisdiction (TRCA, 2016a; TRCA, 2020b). The Humber River watershed is one of the largest watersheds that drain the City of Toronto and neighbouring areas by discharging into Lake Ontario. Approximately 46% of the drainage area is agricultural production of livestock and cash crops in the Towns of Caledon and Vaughan, whereas 24% is residential, industrial or commercial land use (Struger and Fletcher, 2007). The Palgrave Forest and Wildlife Area is a 306-hectare greenspace that consists of forests, meadows, wetlands and ponds that accommodate a diverse community of organisms. In addition to providing various species, including species of concern with habitat, the Palgrave Forest and Wildlife Area is used for a wide range of recreational purposes such as hiking, mountain biking/cycling, horseback riding and cross-country skiing (TRCA, 2016b). This area was chosen for macrophyte acquisition because it is a conservation area that is considered representative of natural wetland ecosystems in Southern Ontario. This particular area can also be considered as a pristine wetland as it seems to be untouched by an abundant amount of anthropogenic sources such as road salts.

2.1.3 The Loss of Wetlands

Environmental scientists have acknowledged the significance of wetlands and their natural processes; however, they are sensitive ecosystems that are constantly being stressed by numerous factors such as climate change, pollution, nutrient loading and human development

(Haven *et al*., 1997; Capotorto, 2006; Zhang *et al*., 2010; Doran & Kahl, 2014; Geng *et al*., 2017). Despite their ecological and economic significance, 69-75% of the Earth's wetlands have been lost since 1900, and due to the rapid increase in human development and expansion, many wetlands are continuously being destroyed or degraded (Havens *et al*., 1997; Bradford, 2016; Kumar *et al*., 2017). Between 1990-2015 the average global rate of wetland loss was -0.78% of wetlands per year. These rates have significantly increased from -0.68 to -0.69% per year between 1970-1980 up to -0.85 to -1.60% per year since the year 2000 (Ramsar Convention on Wetlands, 2018). Various human activities impact the structure and function of natural wetlands by modifying natural disturbances or activating new ones (Bradford, 2016). Coastal wetlands along the Great Lakes have been altered or destroyed by human development resulting in a loss of hundreds of thousands of acres of wetlands that are critical for ecosystem services. In northwestern Ohio, Lake Erie originally comprised of 307,000 acres of wetlands. As of 2014, only 5% of those wetlands continued to exist (Doran & Kahl, 2014). Due to these major losses around the world, many wetland-dependent species are decreasing and are threatened with extinction (Ramsar Convention on Wetlands, 2018).

2.1.3.1 Wetland Loss in Ontario

Before European settlement, natural wetlands covered about 2,026,591 hectares of land in Ontario, but since 2002 that area has decreased to only 560,844 hectares (Ducks Unlimited Canada, 2010). Southern Ontario has suffered the most regarding wetland loss with about 85% converted for other uses such as agriculture, human development, mining, hydroelectric development and transportation, with greater loss of about 90% in southwestern Ontario (Bardecki, 1989; Bardecki, 1998; Zhang *et al.*, 2010; Bradford, 2016; Ontario Ministry of

Natural Resources and Forestry, 2017). Before 1982, wetland loss was mainly attributed to agriculture, however, recent studies suggest that urbanization is a significant factor responsible for wetland loss in Ontario. Many of the remaining natural wetlands in southern Ontario are small and are predominately swamps (Bradford, 2016).

Urbanization is an environmental stressor that interferes with the frequency, timing, pathway, quantity and quality of water entering a wetland (Bradford, 2016). As populations and urbanization increase, water systems are becoming more polluted from sewage, industrial and agricultural effluents that contain numerous contaminants including nitrogen and phosphorus (Rai, 2018). Other pressures such as climate change and invasive species are also an extremely significant cause for their degradation (Bardecki, 1998; Burkett & Kusler, 2000; Houlahan & Findlay, 2004). Changes in temperature, precipitation and atmospheric CO_2 linked to climate change can impact the overall structure and function of various wetland types around the world. Since they are a transitional zone, wetland flora and fauna species are highly vulnerable to changes in hydrology that are beyond their tolerance levels. The combination of increased global temperatures and inconsistent precipitation associated with climate change can alter the size of a wetland or completely dry it out; wetlands where the primary source of water comes from precipitation are especially vulnerable to this. Since plant species may respond differently to these impacts the plant community structure of a wetland can be altered as increased temperatures lengthen the growing season and higher atmospheric CO_2 levels boost plant growth (Burkett & Kusler, 2000). Overall, wetlands across Canada are being greatly influenced (Bardecki, 1998).

2.1.3.1.1 Is the Protection of the Great Lakes Helping Wetlands?

The Great Lakes are highly important ecologically, economically and socially as together they contain one fifth of the world’s fresh surface water which provides drinking water, to millions of people (Environment and Climate Change Canada (ECCC) and the United States Environmental Protection Agency (USEPA), 2017; The National Wildlife Federation, 2019b; USEPA, 2019). According to ECC and USEPA (State of the Great Lakes 2017), the water quality and ecosystem health of the Great Lakes are protected through various agreements and health indicators which can consequently protect the vital wetlands surrounding their coastlines that polish water and provide habitat for wildlife. The northern regions of the Great Lakes, specifically Lake Superior, have experienced significant progress of coastal wetland ecosystems such as restoration, increased animal diversity and decreased contaminants (Environment and Climate Change Canada and USEPA, 2017). Although Great Lake protection efforts have improved certain coastal wetlands, many continue to be destroyed by invasive plants, nutrient enrichment and sedimentation. Overall, the status of wetland habitats surrounding the Great Lakes vary significantly from good to poor depending on the location. Thus, it is unclear how well the protection of the lakes is supporting the health of wetlands (Mayer *et al.*, 2005; Environment and Climate Change Canada and USEPA, 2017).

2.2 Invasive Species

The term “native species” refers to species that are naturally located in a geographical ecoregion without any introduction via human activities (Cronk & Fennessy, 2001; The National Wildlife Federation, 2019a). On the other hand, invasive species are non-native species that are intentionally or accidentally introduced to an area that they predominate by spreading rapidly

(Cronk & Fennessy, 2001; Capotorto, 2006; Fernandes, 2017). However, some native species can possess invasive characteristics and become invasive when relocated to different sections of an area in which they reside (Tiley, 2013). This occurrence can be explained by multiple hypotheses that suggest: i) resources previously devoted towards defense are now devoted to reproduction; ii) an increased mutualistic relationship between plant and soil microbes; iii) increased vulnerability of native species (Capotorto, 2006; Fernandes, 2017).

Invasive species are a major ecological issue around the world (Houlahan & Findlay, 2004). In addition to animals, many plant species are considered invasive and can have negative effects on native plant species, ecosystem processes and community structure by becoming major competitors (Houlahan & Findlay, 2004; Trebitz & Taylor, 2007; Environment and Climate Change Canada, 2017). The different stages of invasion include transportation, introduction, establishment and spread (Azan *et al*., 2015). Of the plant species identified by The Nature Conservancy in 1998, 57% are estimated to be considered “possibly extinct”, “critically endangered” or “endangered” in part by invasive species competition (Houlahan & Findlay, 2004). Due to this, various organizations have been established to tackle invasive species including the World Conservation Union’s Global Invasive Species Program, U.S. National Invasive Species Council and the Ontario Phragmites Working Group (Houlahan & Findlay, 2004).

2.2.1 Invasive Plant Species in Wetlands

Aquatic and semi-aquatic plants that thrive in wet environments make up a significant amount of invasive species as they have a higher chance of becoming invasive than terrestrial plants. Floating aquatic plants usually spread across the surface of the water whereas the growth variability of submerged and emergent species allow them to occupy the entire water column and prevent the growth of other plants (Azan *et al*., 2015). Additionally, hydrological disturbances such as flood pulse, surface runoff and storm events can cause the accumulation of water, nutrients, sediment, heavy metals and contaminants in wetlands. These conditions can impact wetland features and make them more vulnerable to invasive plant species. As a result, invasive plants are more common in wetlands disturbed by eutrophication, sedimentation or hydraulic change than terrestrial ecosystems (Havens *et al*., 1999; Capotorto, 2006; Trebitz & Taylor, 2007; McAndrew *et al*., 2016). Their presence in wetlands can cause negative impacts such as decreased plant and animal richness and diversity, degraded habitat, sediment loss, altered nutrient cycling, modified food webs, decreased productivity and reduced societal value (Capotorto, 2006; Trebitz & Taylor, 2007). Invasive wetland plants are able to thrive mainly due to their ability to distribute seeds through flotation, tolerate extreme conditions, rapidly uptake nutrients and grow quickly (Capotorto, 2006).

2.2.2 Invasive Plant Species in Canada

Wetland invasion is the consequence of interspecific competition as invasive species are better at competing for resources and can endure environmental stress (Coleman *et al.*, 2001; Houlahan & Findlay, 2004; Environment and Climate Change Canada, 2017). Many wetland species in Ontario are considered invasive and are outlined in Table 1.

Table 1: Invasive Plant Species of Wetland Ecosystems in Ontario

Common Name	Scientific Name
• Black alder	• *Alnus gltinosa*
• Flowering rush	• *Butomus umbrellatus*
• Giant hogweed	• *Heracleum mantegazzianum*
• European frog's bit	• *Hydrocharis morsus-ranae*
• Himalayan balsam	• *Impatiens glandulifera*
• Yellow flag iris	• *Iris pseudacorus*
• Moneywort	• *Lysimachia nummularia*
• Purple loosestrife	• *Lythrum salicaria*
• European watermilfoil	• *Myriophyllum spicatum*
• Reed canary grass	• *Phalaris ardundinacea* subsp.
• Common reed	• *Phragmites australis*
• Curly-leaved pondweed	• *Potamogeton crispus*
• Narrowleaf cattail	• *Typha angustifolia*
• Broadleaf cattail	• *Typha latifolia*

(Taylor, 1992; Houlahan & Findlay, 2004; United States Department of Agriculture (USDA), 2006a; USDA, 2006c; Trebitz & Taylor, 2007; Tiley, 2013; City of Stratford, 2014; TRCA, 2020a).

For instance, the narrowleaf cattail (*Typha angustifolia*) is an invasive wetland species that is a strong competitor for light and can tolerate high salinity environments (Figure 1a). This species was either introduced to the U.S. by European settlement or has diffused from the Northeast coast of the U.S. and has since rapidly expanded its range across North America, including Ontario (Chow-Fraser, 2005; Capotorto, 2006; USDA, 2006c; Tiley, 2013). The invasive plant purple loosestrife (*Lythrum salicaria*) is also a highly competitive species that has eliminated and replaced many native species by invading fundamental wetlands throughout the temperate regions of North America (Figure 1b). Additionally, both *L. salicaria* and *Phalaris arundinacea* (reed canary grass) have successfully reduced plant diversity in many wetland environments (Houlahan & Findlay, 2004; Capotorto, 2006). *P. arundinacea* is a considerably aggressive invader that outcompetes native wetland plants when nutrient concentrations and sedimentation increase (Capotorto, 2006). A study on coastal wetlands along the Great Lakes basin found that emergent species *Typha*, *Phragmites australis* (common reed), *P. arundinacea* and *L. salicaria* had strong and widespread impacts on native wetland plants. These invasive species were more common near lakes surrounded by agriculture with less native wetland plants. In addition to agriculture, other environmental disturbances such as loss of land, population growth, human development and point source pollutants can cause invasive species to thrive over native species (Trebitz & Taylor, 2007). Overall, previous studies have demonstrated that the abundance and dominance of invasive species in wetlands was associated with low native species abundance, resulting in low overall biodiversity (Capotorto, 2006).

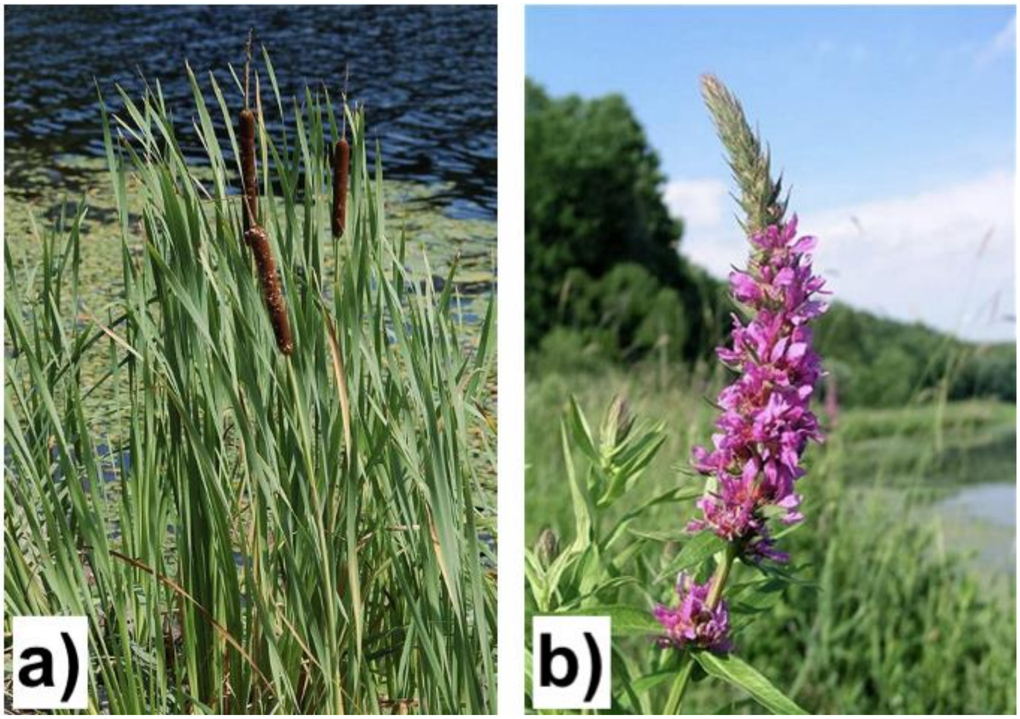


Figure 1: Invasive Plant Species of Wetland Ecosystems in Ontario

a) Cattail (*Typha*) (Boyt, 2009) b) Purple Loosestrife (*Lythrum salicaria*) (Wilson, 2013)

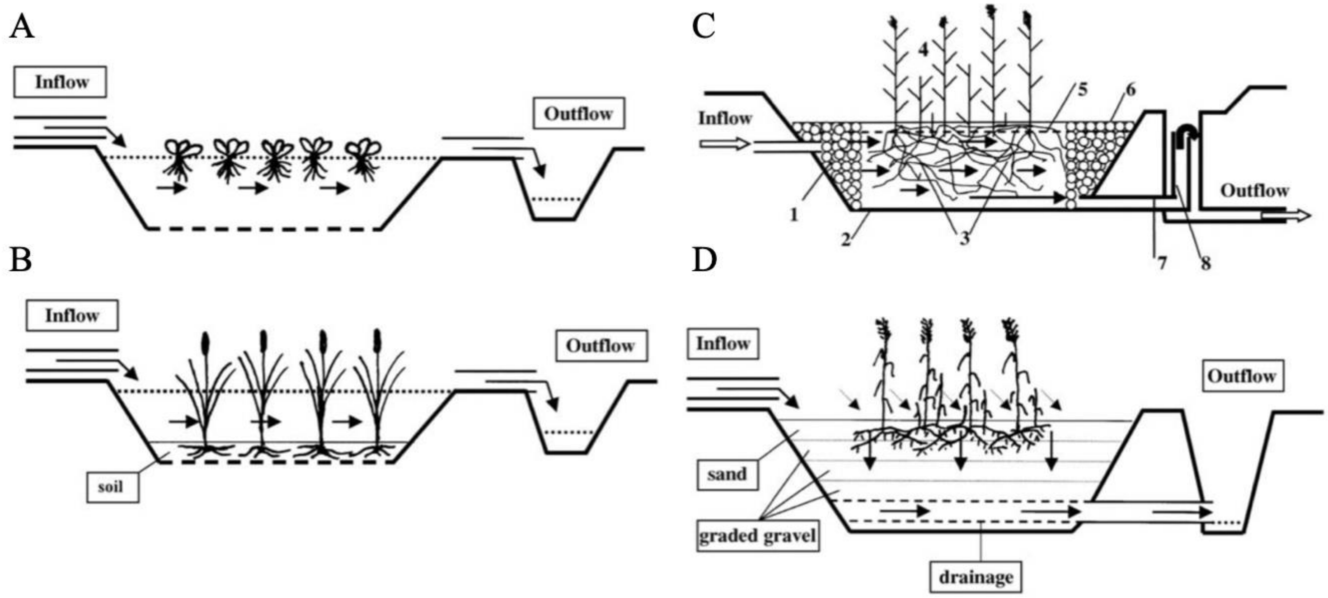
2.3 Constructed Wetlands for Wastewater Treatment

The presence of environmental contaminants from wastewater can have long-term impacts on ecosystems, organisms and humans (Hoang *et al*., 2013; Liu & Wong, 2013; Garcia-Rodriguez *et al*., 2014; Rai, 2018). Organisms that spend their entire life cycle in aquatic ecosystems are especially vulnerable as they are constantly exposed to these contaminants (Garcia-Rodriguez *et al*., 2014). Numerous technologies in wastewater treatment plants (WWTP) such as advanced oxidative processes, activated carbon adsorption, membrane filtration and membrane bioreactors can be used to effectively remove environmental contaminants including phosphorus, nitrogen, heavy metals, organic contaminants, pharmaceuticals, microplastics and personal care products (Hoang *et al*., 2013). For example, one study investigated the photo-oxidative degradation of aqueous polyvinyl alcohol polymer solutions (PVA), a common refractory pollutant, using a laboratory-scale UV/H_2O_2 photochemical reactor. Their results indicated that the structure of PVA was successfully altered and degraded by means of this method (Hamad *et al*., 2014). However, the high costs of such methods pose a great disadvantage (Hoang *et al*., 2013). Due to this, there is an increased interest in a cost-effective, alternative eco-technology treatment process such as constructed wetlands, which are becoming an increasingly popular approach for wastewater treatment since they do not cause the same negative environmental impacts that conventional methods do (Wallace & Knight, 2006; Kadlec & Wallace, 2009; Wang & Sample, 2014; Pavlineri *et al*., 2017; Johnson & Mehrvar, 2019; Lucke *et al*., 2019; Xiao *et al*., 2019).

A constructed wetland is an artificial wetland that has been designed and constructed to mimic the natural biological, physical and chemical processes using vegetation, soil and associated microbes to assist in wastewater treatment (Vymazal, 2007; Zhang *et al.*, 2010; Garcia-Rodriguez *et al.*, 2014; Johnson & Mehrvar, 2019). Although their main function is to improve water quality, *in situ* constructed wetlands can also be developed for restoration purposes to provide habitat for wildlife, protect littoral zones and create recreational and tourism opportunities (Havens *et al.*, 1999; Pavlineri *et al.*, 2017). Many constructed wetlands contain emergent vegetation and other characteristics that resemble natural marshes (USEPA, 1994; Zhang *et al.*, 2010; Zhang *et al.*, 2014a). The first documented constructed wetland was designed in 1901 to filter water through a vertical flow system with various layers of substrate (Wallace & Knight, 2006). However, macrophytes were not considered in wastewater treatment until 1953 when Dr. K*ä*the Seidel added *P. australis* to a hybrid vertical and horizontal flow system. His work proved that constructed wetlands were an effective method to remove pollutants (Kadlec & Wallace, 2009; Vymazal, 2011). Since then, constructed wetlands have been widely used for on-site wastewater treatment purposes. Many lab-based studies have been conducted which demonstrated total phosphorus removal averaging 48.75% in floating wetlands (Pavlineri *et al.*, 2017), 17.4-39.5% in floating wetlands and 74-81.1% in vertical subsurface flow wetlands (Lopardo *et al.*, 2019), and 28-58% in floating wetlands (Tanner & Headley, 2011). They can also be implemented at wineries for on-site treatment of winery wastewater where removal rates of chemical oxygen demand and total suspended solids are greater than 98% (Johnson & Mehrvar, 2019) or coupled with microbial fuel cells in WWTPs for successful nitrogen removal (Tao *et al.*, 2020).

2.3.1 Types of Constructed Wetlands

Since then, several designs of constructed wetlands including surface flow, subsurface flow, floating and hybrid systems (Figure 2) have been used for different purposes (USEPA, 1994; Vymazal, 2007; Zhang *et al.*, 2010).



<u>Figure 2: Various Designs of Surface and Subsurface Flow Constructed Wetlands</u>

A) Constructed wetland containing free-floating macrophytes, B) Surface flow constructed wetland containing emergent macrophytes, C) Horizontal subsurface flow constructed wetland containing emerged and submerged macrophytes, D) Floating constructed wetland with vertical subsurface flow (Vymazal, 2007).

Surface flow consists of influent entering wetlands from terrestrial runoff (Vymazal, 2007). Surface flow constructed wetlands are capable of decreasing velocity and increasing water retention time and contact between water, sediment and vegetation, which improves pollutant removal (Capotorto, 2006). Their design is most similar to natural wetland ecosystems where aerobic biodegradation and photodegradation are the main removal processes (Garcia-Rodriguez *et al*., 2014; Rozema *et al.*, 2016). Subsurface flow is considered the influent entering wetlands through the substratum (Capotorto, 2006; Vymazal, 2007). The movement of water in subsurface flow constructed wetlands takes place below ground across porous substrates such as rock, sand or gravel either vertically or horizontally (Capotorto, 2006; Johnson & Mehrvar, 2019). Vertical subsurface flow constructed wetlands mostly produce aerobic environments due to their greater oxygen transfer and provide nitrification. On the other hand, horizontal subsurface flow wetlands generally provide anaerobic environments that enhance denitrification processes (Garcia-Rodriguez *et al*., 2014; Zhang *et al*., 2014a). Both vertical and horizontal types of subsurface flow constructed wetlands are used to treat municipal sewage by removing organic compounds and suspended solids (Zhang *et al*., 2014a). These types of systems are better suited for colder climates, such as Canada and northern U.S., to insulate the microorganisms and prevent freezing effects (Picard *et al*., 2005; Zhang *et al*., 2010; Rozema *et al*., 2016; Johnson & Mehrvar, 2019). The Niagara region of Ontario currently uses this type of system to treat winery wastewater (Johnson & Mehrvar, 2019). Floating constructed wetlands are designed to mimic natural floating wetland islands that form when the vegetation combines with organic matter and sediment (Lucke *et al.,* 2019). They are typically developed for water quality improvement since the direct contact between the plant roots and polluted water enhances nutrient uptake (Zhang *et al*., 2014a). Lastly, hybrid systems combine various constructed wetland designs which can simultaneously provide aerobic and anaerobic conditions to ensure that wastewater from various sources is effectively treated. These types of systems are usually designed for municipal sewage treatment but have also been used to treat wastewater from lakes, hospitals, laboratories and wastewater treatment plants (Zhang *et al*., 2009; Zhang *et al*., 2014a; Rozema *et al*., 2016).

Different wetland macrophytes need different types of water flow in constructed wetland for optimal growth. Floating plants that grow at the surface of the water rely on surface flow systems so their roots can be continuously exposed to incoming nutrients. Submerged macrophytes on the other hand require subsurface water flow so their grounded roots can efficiently absorb nutrients. Emergent macrophytes can obtain nutrients in both surface and subsurface water flow wetland systems because part of the plant is exposed to the water column and grown above the water surface while their roots are grounded by sediment (Vymazal, 2007). They can also be grown hydroponically in floating constructed wetlands (Lucke *et al*., 2019).

2.3.2 Invasive Species in Constructed Wetlands

Although there is little research regarding native non-invasive plant species in constructed wetlands, the few studies available in the literature suggest that they can also be implemented in wetlands constructed for wastewater treatment and pollutant removal (White *et al*., 2000; Kao *et al.*, 2009; Zhang *et al.*, 2010; Fernandes, 2017). Kao *et al.* (2009) built a subsurface flow constructed wetland at a highway rest area for on-site wastewater treatment. They used 13 native non-invasive wetland species, several of which were either purchased/used in this experiment (Table 5) including sneezeweed, blue flag iris, prairie cordgrass, blue vervain; observed in-field (Table 18) blue vervain or found in Environment Canada's list of macrophytes suitable for engineered wetlands and/or wetland restoration (Table 2) such as sedges, blue flag iris, soft-stem bulrush and arrowhead (Environment Canada, 1996; Kao *et al*., 2009; Fernandes, 2017). In their research, the constructed wetlands decreased 5-day biological oxygen demand (BOD), total suspended solids (TSS) and nitrogen concentration by 65.8%, 78.9% and 42.1% respectively. Their findings were similar to previous studies (Kao *et al*., 2009). An additional study (McAndew *et al*., 2016) utilized five North American native non-invasive wetland species in a mixed constructed floating wetland system in a stormwater retention pond for nitrogen removal. The species chosen were water plantain, blue flag iris, tussock sedge, common rush and pickerelweed, all of which overlap with species used in this thesis or found in Environment Canada's list of macrophytes suitable for engineered wetlands and/or wetland restoration. The system as a whole removed a total of 65.8 g of nitrogen from the pond. It was found that the shoots of the sedges and rushes had the highest nutrient content, suggesting that these particular species absorb nitrogen more effectively, which corresponds with previous constructed wetland experiments (McAndrew *et al*., 2016). Overall, these studies demonstrate that native non-invasive species can be used for successful wastewater treatment.

Table 2: Common Marsh Plants for Wetland Restoration

Emergent	
Water Plantain	*Alisma plantago-aquatica*
Swamp Milkweed	*Asclepias incaranata*
Sedges	*Carex spp.*
Turtlehead	*Chelone glabra*
Spike Rushes	*Eleocharis spp.*
Water Horsetail	*Equisetum fluviatile*
Blue Flag Iris	*Iris versicolor*
Rushes	*Juncus spp.*
Pickerelweed	*Pontederia cordata*
Arrowhead	*Sagittaria latifolia*
Hard-Stemmed Bulrush	*Scirpus acutus*
Black Bulrush	*Scirpus atrovirens*
Soft-Stem Bulrush	*Scirpus validus*
Green Fruited Bur-Reed	*Sparganium chlorocaprum*
Giant Bur-Reed	*Sparganium eurycarpum*
Cattails	*Typha spp.*
American Brooklime	*Veronic americana*
Submergent	
Coontail	*Ceratophyllum demersum*
Waterweed	*Elodea canadensis*
Watermilfoil	*Myriophyllum exalbescens*
Sago Pondweed	*Potamogeton pectinatus*
Pondweed	*Potamogeton richardsonii*
Bladderworts	*Utricilaria vulgaris*
Tape Grass	*Vallisneria americana*
Floating-Leaved	
Yellow Water Lily	*Nuphar variegata*
White Water Lily	*Nymphaea odorata*
Water Smartweed	*Polygonum amphibium*
Variable-Leaved Pondweed	*Pontamogeton gramineus*
Floating Pondweed	*Pontamogeton natans*
Free-Floating	
Common Duckweed	*Lemna minor*
Star Duckweed	*Lemna trisulca*
Greater Duckweed	*Spirodela polyrhiza*

(Environment Canada, 1996; Fernandes, 2017).

However, constructed wetlands for wastewater treatment are still highly vulnerable to the impacts of invasive species because their efficiency is dependent on their natural processes and functions (Havens *et al*., 1999; Fraser *et al*., 2004; Capotorto, 2006; Geng *et al.*, 2017). Despite the fact that both exotic and native invasive plant species cause obvious harm to wetland ecosystems, known native invasive species such as bulrushes (*Scirpus*), cattails (*Typha)* and reeds (*Phragmites*) are commonly used in constructed wetlands across North America and Europe (Picard *et al.*, 2005; Capotoro, 2006; Vymazal, 2007; Kadlec & Wallace, 2009; Zhang *et al.*, 2014b; Johnson & Mehrvar, 2019). These species are considered most suitable for constructed wetlands and wastewater treatments due to their dominance, fast growth rate, easy establishment and tolerance to unfavourable conditions (Fraser *et al.*, 2004; Zhang *et al.*, 2010). Of these species, the most commonly used emergent macrophytes in North American constructed wetlands are *Typha.* (Houlahan & Findlay, 2004; Picard *et al.*, 2005; USDA, 2006a; USDA, 2006c; Vymazal, 2007; Kadlec & Wallace, 2009; Zhang *et al.*, 2010; Lucke *et al.*, 2019). This is because they are native to temperate regions in North America and have proven to successfully remove excess nutrients, specifically nitrogen and phosphorous, in surface flow and subsurface flow constructed wetlands (Brisson & Chazarenc, 2009; Tiley, 2013). However, *Typha* display invasive characteristics such as competitiveness and dominance in disturbed ecosystems across North America. Despite its invasive nature nevertheless, *Typha* species such as *Typha latifolia* continue to be used in constructed wetlands for wastewater treatment because they are tolerant to various stressors such as pollution, inconsistent water levels and soil salinity (Havens *et al*., 1999; Coleman *et al*., 2001; Capotorto, 2006; Ijaz *et al.*, 2016). *Typha* monoculture constructed wetlands can only be justified for closed systems that can not result in an outbreak that will impact the rest of the ecosystem (Fernandes, 2017). It is important to note that *T. latifolia* is not inherently invasive and is still considered an essential species, but evidence suggests that it has the ability to become invasive by aggressively displacing other native species and consequently, decreasing biodiversity (Tiley, 2013). *Phragmites* species also display similar invasive behaviour but are also continuously used in constructed wetlands in North America and Europe due to convenience and high pollutant removal (Havens *et al*., 1999; Vymazal, 2007; Manceau *et al*., 2008; Brisson & Chazarenc, 2009; Kadlec & Wallace, 2009; Zhang *et al.*, 2010).

In Tiley's (2013) research on the efficiency of *Typha latifolia* to remove pollutants in constructed wetlands conducted in our laboratory, it became evident that *T. latifolia* was a highly invasive species. Due to this, he concluded that macrophytes selected for constructed wetlands should be limited to native non-invasive species that are also capable of pollutant removal. Bringing invasive species into a laboratory-scale engineered wetland system is concerning due to their potential to spread rapidly. Native non-invasive species should be used as an alternative to current species such as *Typha* because it will reduce the negative ecological impacts of invasive species while also maintaining biological diversity. Thus, as outlined in the objectives of this study, only native non-invasive wetland species were used.

2.4 The Phosphorus Cycle

Phosphorus is an essential element required by all living organisms and is important for supporting healthy aquatic environments (Filippelli, 2016; Hartshorn *et al*., 2016). It is a key component of nucleic acids that form DNA and RNA, phospholipids that form cell membranes, ATP which is a significant energy carrier and bone material (Vymazal, 2007; Filippelli, 2016). Both phosphorus and nitrogen are crucial for normal functioning ecosystems; however, phosphorus is considered the limiting nutrient in freshwater ecosystems. Opposed to other biogeochemical cycles including water, carbon and nitrogen, the phosphorus cycle is a slow net transfer from land to water that does not include a gas phase in the atmosphere (Filippelli, 2016).

2.4.1 Geologic Process of Phosphorus

The ultimate and largest natural source of phosphorus in the environment is sedimentary rock and apatite minerals (Filippelli, 2016). Phosphorus in the form of phosphate (PO_4^{-3}) is released to soil and water from these rocks via weathering where it is stored or taken up by various organisms (Vymazal, 2007). In the soil, phosphorus is present in various forms that can be grouped into readily bioavailable and not readily bioavailable (Filippelli, 2016). Plants that obtain phosphorus from soils or water are consumed by herbivores and then carnivores through the food chain and return to the soil through animal waste or decomposition. Phosphorus can also enter aquatic ecosystems through runoff and transport by rivers where it is sedimented due to its low solubility. These phosphates can reenter the phosphorus cycle or become available to aquatic organisms if processes such as subduction and accretion occur in large scale natural wetland systems. Since phosphorus is critical for biological productivity, the concentration of phosphate in surface waters is very low as it is constantly being taken up by phytoplankton and its concentration increases with depth (Filippelli, 2016).

2.4.2 Anthropogenic Phosphorus

Over the last 150 years, human development has altered natural cycles by increasing the global flow of nutrients, specifically ntitrogen and phosphorus, in the environment (Picard *et al.*, 2005; Filippelli, 2016). During the 19th century, sedimentary rocks in Europe and the U.S. were mined, ground and applied to agricultural fields as a plant nutrient to increase crop yield. Although it was initially successful, crops began to decrease because the fertilizer contained heavy metals cadmium and uranium that are toxic to plants (Filippelli, 2016). This incident resulted in the collaboration of leaching techniques and inclusion of nitrogen and potassium in fertilizers during the “Green Revolution”. Although crop production increased, freshwater and coastal marine systems were impaired by eutrophication, hypoxia and fish mortality. Additionally, humans have altered the natural phosphorus cycle through wetland loss which reduces the amount of substrate for phosphorus to bind to, causing it to wash away (Filippelli, 2016).

2.4.3 Phosphorus in Aquatic Ecosystems such as Wetlands

Since coastal wetlands act as barriers along shores by reducing and preventing the amount of pollutants entering aquatic ecosystems via urban runoff, their loss around coastlines such as Lake Erie are a major cause of nutrient loading (Doran & Kahl, 2014). Due to various human activities such as agricultural practices, urbanization and industrialization, the input of nutrients such as nitrogen and phosphorus have rapidly increased in biogeochemical cycles, especially aquatic ecosystems (Geng *et al.,* 2017; Lake George Association, 2017). Granted that nitrogen inputs from non-point sources are also noteworthy, for the purpose of this thesis, phosphorus removal will be the main focus.

Dissolved, particulate, organic and inorganic forms of phosphorus can be categorized as total phosphorus (TP). Suspended solids (SS) ranging from 1 μm to > 100 μm are the main source of TP in both aquatic ecosystems and agricultural runoff (USEPA, 2000). In wetlands, phosphorus exists as phosphate in organic and inorganic compounds in two main forms: dissolved and particulate. Free orthophosphate (PO_4^{3-}) is the only readily bioavailable form of dissolved phosphorus (and also highly reactive) that can be directly assimilated by algae and aquatic plants (American Public Health Association, 1998; Reddy & D'Angelo, 1997; Vymazal, 2007). During its existence in wetland ecosystems, phosphorus can undergo multiple transformations (Reddy & D'Angelo, 1997).

Phosphorus is a common component of fertilizers, manure, and municipal and industrial organic wastes. Anthropogenic phosphorus can enter aquatic ecosystems through STP effluent, sewer overflows, atmospheric transportation and agricultural and stormwater runoff (Picard *et al.*, 2005; Tiley, 2013; Geng *et al.*, 2017; Lake George Association, 2017). Although phosphorus concentrations are lower during the growing season (spring/summer) due to plant assimilation, high inputs and overall concentrations of phosphorus in lakes and rivers is a worldwide water quality issue. In the United States alone, 61% of 2048 water bodies do not meet EPA standards for total N and P concentration (Picard *et al.*, 2005; Filippelli, 2016). Additionally, since phosphorus is a limiting nutrient in freshwaters, excess phosphorus inputs can have adverse effects and contribute to eutrophication (Schindler, 1974; Picard *et al.*, 2005; Hartshorn *et al*., 2016; Geng *et al.*, 2017). Eutrophic conditions encourage excessive algal growth that consequently leads to the loss of essential aquatic species and overall ecosystem function (Picard *et al*., 2005). Organisms that spend their entire life cycle in aquatic ecosystems are especially vulnerable as they are constantly exposed to these contaminants (Garcia-Rodriguez *et al*., 2014).

2.4.4 Eutrophication in Lakes

Algae are a critical component of aquatic ecosystems and food chains that require nutrients such as phosphorus for optimal growth. Despite this, excessive inputs of nutrients such as anthropogenic phosphorus have impacted the biotic and abiotic factors of many North American and European aquatic ecosystems, including Lake Erie, by triggering what is termed by the International Joint Commission as harmful algal blooms (HAB) (Ludsin *et al*., 2001; Bykova *et al*., 2006; International Joint Commission, 2014; Environment and Climate Change Canada and USEPA, 2017; Xiao *et al*., 2019).

Water quality issues in freshwater systems are correlated to anthropogenic activities. Many aquatic systems are considered eutrophic, or at risk of eutrophication, as a result of agricultural and urban development (Cook *et al*., 2020). Lake Erie, parts of Lake Ontario and other nearshore areas surrounded by agricultural lands are highly vulnerable to non-point source runoff. Agricultural runoff containing phosphorus can cause uncontrollable algal growth, making eutrophication a major concern (International Joint Commission, 2014; Hartshorn *et al*., 2016; Environment and Climate Change Canada and USEPA, 2017). Once these algal populations increase to the point where underside algae do not have access to sunlight for photosynthesis, they decay and create anoxic and hypoxic conditions at lower water levels (Environment and Climate Change Canada and USEPA, 2017). As a result of low oxygen levels, the richness of beneficial benthic macroinvertebrate species such as mayflies and fish communities have decreased (Ludsin *et al*., 2001; Bykova *et al*., 2006; Environment and Climate Change Canada and USEPA, 2017). Due to eutrophication, Lake Erie was considered to be the “Dead Sea of North America” during the 1960s and 1970s (Ludsin *et al*., 2001).

HABs are a growing global concern as they are detrimental to the environment, water quality and human health (Hartshorn *et al*., 2016; Meyer *et al*., 2017; Cook *et al.*, 2020). They can lead to the disruption and loss of habitats by affecting aquatic plants and organisms. Social and economic losses derive from the lack of tourism and recreational opportunities due to slime, unpleasant odors and invasive plants, while the decrease in fish populations impact commercial fishing industries. Cyanobacteria can also contaminate drinking water by impacting its odour and taste (Bykova *et al*., 2006; International Joint Commission, 2014; Environment and Climate Change Canada and USEPA, 2017). Cyanobacterial species including *Mycrocystis aeruginosa, Planktothrix, Anabaena, Cladophora,* and *Lyngbya* have been the most common species reported in Lake Erie throughout the last 50 years (Mayer *et al*., 2011; Meyer *et al*., 2017). Certain types of freshwater cyanobacteria can produce toxins known as microcystins, such as *Microcystis*. *Microcystis* are especially harmful as they can produce fatal neurotoxins and hepatotoxins that attack neurons and liver cells; exposure to water contaminated by microcystins can result in acute neurotoxicity, skin irritation and in serious cases liver cancer (Bykova *et al*., 2006; Mayer *et al*., 2011; Hartshorn *et al*., 2016; Environment and Climate Change Canada and USEPA, 2017; Cook *et al*., 2020). Additionally, *Microcystis* can decrease oxygen concentrations, reduce light availability, and alter CO_2 and pH levels (Cook *et al*., 2020). Concentrations of nutrients, such as phosphorus, from runoff or nutrient loading is an important factor that promotes the excessive growth of HABs. It was found that high phosphorus concentrations supported the growth of toxic *Microcystis* strains, while nontoxic strains were more common at lower concentrations (Hartshorn *et al*., 2016).

2.5 Phytoremediation

Wetland macrophytes can play a major role in purifying wastewater as they uptake and degrade organic and inorganic compounds (Bardecki, 1984; Rai, 2018; USEPA, 2018). The concept of using various plant species to control and remove environmental pollutants such as metals, pesticides and nutrients from soil, water and the atmosphere is known as phytoremediation (Salt *et al*., 1998; Pilon-Smits, 2005; Zhang *et al*., 2010). Resilient emergent plants such as bulrushes (*Scirpus),* spikerush (*Efeocharis),* sedges (*Cyperus*), rushes (*Juncus),* common reed (*Phragrnites)* and cattails (*Typha)* are commonly used for phytoremediation in constructed wetlands, however, their invasive qualities pose a threat (Brix, 2003; Brisson & Chazarenc, 2009; Zhang *et al*., 2010; Hoang *et al*., 2013;). Macrophytes in constructed wetlands must be able to tolerate continuous saturation and exposure to wastewater that contains high concentrations of contaminants (USEPA, 1994; Xiao *et al*., 2019). Factors such as solubility, polarity, lipophilicity, hydrophobicity, ionization state and partitioning coefficients can determine the fate of the contaminant and whether it can be taken up by a plant and transferred through the root membrane (Garcia-Rodriguez *et al*., 2014). Pollutants can be removed simultaneously by several processes shown in Figure 3 known as: phytoextraction, the use of plants to obtain pollutants from soil and water; phytodegradation, where plants and their associated microbes breakdown pollutants; phytostabilization, the use of different plants to prevent the mobility and bioavailability of pollutants; phytovolatilization, where substances are broken down into volatile components and released into the atmosphere; and rhizofiltration, when plant roots absorb and adsorb contaminants (Salt *et al*., 1998; Hoang *et al.*, 2013; Garcia-Rodriguez *et al*., 2014; Hartshorn *et al*., 2016).

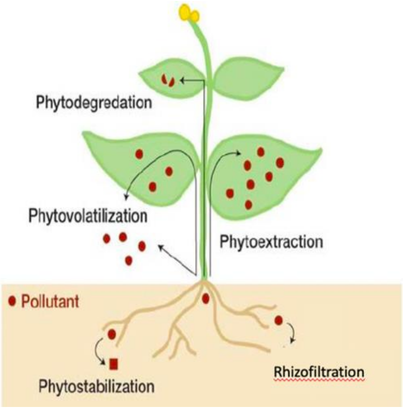


Figure 3: The Various Phytoremediation Processes (Pilon-Smits, 2005).

Macrophytes also enhance the removal of environmental contaminants by providing aerobic paths into sediment, increasing the microbial population and releasing plant exudates (Garcia-Rodriguez *et al*., 2014; Zhang *et al*., 2014b). The release of plant exudates from their roots increases the efficiency of microbial bioremediation in the rhizosphere, which plays an important role in phytoremediation processes because of their high metabolic activity (Salt *et al*., 1998; Hoang *et al*., 2013). The ability of constructed wetlands to improve water quality is highly dependent on the microbial communities present as they facilitate different biological processes such as ammonia oxidation, denitrification and nitrogen fixation (Fernandes *et al*., 2015). Microbe-assisted phytoremediation, where microbes along the rhizosphere of macrophytes assist in organic contaminant degradation, is an effective approach used on the removal of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Hoang *et al*., 2013).

Overall, phytoremediation through constructed and engineered wetlands provides an attractive, cost-effective and environmentally sustainable alternative to traditional wastewater treatment plants (Wallace & Knight, 2006; Kadlec & Wallace, 2009; Pavlineri *et al*., 2017; Lucke *et al*., 2019; Xiao *et al*., 2019). The natural processes of phytoremediation often take place on site, thus lowering costs and exposure of contaminants to humans, organisms and the surrounding environment (Pilon-Smits, 2005; Lucke *et al*., 2019). Although phytoremediation seems to be a promising biotechnology, it does have its limitations. Some plant species may be sensitive to environmental contaminants, thus reducing their growth and overall biomass for phytoremediation purposes. Another issue is that contaminated soils have a reduced number of microorganisms, hindering plant growth and the degradation of pollutants (Hoang *et al*., 2013; Xiao *et al*., 2019). Furthermore, a combination of biologically based wastewater treatment systems can effectively remove various pollutants from the environment. Previous research has shown that combined systems had an overall increased elimination rate. Additionally, constructed wetland systems can be incorporated into conventional methods such as activated sludge and advanced oxidation to increase removal (Garcia-Rodriguez *et al*., 2014).

2.5.1 How Wetlands Sequester Phosphorus

All forms of phosphorus can be converted or removed in natural and constructed wetland environments through peat/soil accretion, adsorption/desorption, precipitation/dissolution, plant/microbial uptake, fragmentation, leaching, mineralization, sedimentation or burial (Reddy & D'Angelo, 1997; Vymazal, 2007). The extent in which wetland macrophytes can store contaminants depends on macrophyte species, decomposition rates, leaching and translocation of phosphorus in biomass (Vymazal, 2007). The short-term or long-term uptake of phosphorus by wetland plants can reduce the effects of eutrophication downstream (Bardecki, 1984). Due to the high productivity of wetland macrophytes, a reasonable amount of nutrients can be found in their biomass (Vymazal, 2007; Brix 2003; Xiao *et al.*, 2019). Although a significant amount of the phosphorus present in wetlands is removed by plant uptake during the growing season when their biomass is highest, these rates are often lower compared to that of sedimentation, precipitation and burial during the rest of the year (Brix, 2003; Vymazal, 2007; Zhang *et al*., 2009). In order to increase removal rates and ensure that any nutrients incorporated by the plants are not released back into the environment from decaying litter during senescence in temperate regions, wetland macrophyte aboveground biomass must be harvested in the fall (Zhang *et al*., 2010; McAndrew *et al*., 2016; Geng *et al.*, 2107; Pavlineri *et al.*, 2017). Harvesting these plants can increase overall removal, especially for systems that are lightly loaded (Vymazal 2007). Lower removal rates via plant uptake may also be underestimated due to the fact that most harvesting methods only include above-water plant tissues and not below-water biomass. This does not accurately represent macrophyte removal potential in constructed wetlands since nutrients, especially phosphorus, are usually stored in the roots. Sequestered nutrients are remobilized and distributed to various parts of a plant as they go through the different growth phases in their lifecycle. Nutrient concentrations are highest in upper biomass during the summertime and are typically transferred to the roots in September. Thus, phosphorus removal efficiency can significantly increase if upper biomass is harvested in September. Due to this, whole plant harvesting should be considered as a constructed wetland management tool to increase plant uptake capacity (McAndrew *et al*., 2016; Pavlineri *et al.*, 2017). Floating constructed wetlands are especially advantageous because the floating plants, as well as the biomass suspended in the water column, can be harvested more easily compared to macrophytes rooted in the sediment (Geng *et al*., 2017). The harvested biomass can then be used as a food source, for livestock or humans, or used to produce bioenergy (Pavlineri *et al*., 2017). However, if macrophytes are not harvested prior to senescence, most of the nutrients that were assimilated during the growing season will be gradually released back into the environment by leaching and mineralization as only a small portion of these nutrients remain as long-term storage in the rhizome (Brix, 2003; Zhang *et al.*, 2009; Geng *et al.*, 2017; Pavlineri *et al.*, 2017). Phosphorus uptake by microbial populations is fast because microorganisms multiply quickly; however, their low biomass and high turnover rates reduce their long-term storage capacity (Vymazal, 2007). The reduction and oxidation (redox) potentials in natural and constructed wetland sediment determine the overall phosphorus retention. In natural wetlands, peat biomass typically contains less than 0.1% of phosphorus, whereas constructed wetlands contain higher amounts due to increased phosphorus loading rates (Nichols, 1983; Vymazal, 2007). Due to the production of organic acids via anaerobic bacteria reducing the decomposition rate of organic matter in the anoxic layers of constructed wetland sediment, peat accretion is the main long-term sequestration of organically-bound phosphorus (Nichols, 1983; Reddy & D'Angelo, 1997; Vymazal, 2007).

Currently, wastewater containing phosphorus is typically treated using traditional methods such as wastewater treatment plants (WWTP). Unfortunately, WWTPs are both energy and cost-intensive, thus constructed wetlands are an alternative method being considered as a possible solution to issues surrounding eutrophication and contaminants (Picard *et al*., 2005; Geng *et al*., 2017). Various studies have conducted research regarding the efficiency of wetlands to remove total nitrogen and phosphorus, however the results are inconsistent and have ranged from 3-98% and 31-99% respectively, with an average removal of 50% (Picard *et al*., 2005). Although some studies found that wetlands could not effectively remove phosphorus, several studies have concluded that constructed wetlands can efficiently remove phosphorus with removal ranging from 41-60% in mature constructed wetlands and over 90% in newly constructed wetlands (Vymazal, 2007). Another study on the Orlando Easterly Wetlands (OEW) found that the 1190 ha natural wetlands had an average TP removal rate of 67.71% over 17 years (Slayton, 2009). Thus, natural and constructed wetlands can be situated near WWTPs or along the coast of the Great Lakes to remove various contaminants from WWTP tertiary effluent or land runoff via pretreatment and/or polishing before entering other water systems (Toet *et al*., 2005; Zhang *et al*., 2010; Zhang *et al*., 2014a; Xiao *et al*., 2019).

2.6 Constructed Versus Engineered Wetlands

In the literature, the term "constructed wetland" is often used interchangeably with the term "engineered wetland" because they essentially both mean "man-made" (Vymazal, 2007). As previously mentioned, constructed wetlands are artificial wetlands that has been designed and constructed to mimic natural wetland ecosystems (Vymazal, 2007; Zhang *et al*., 2010; Garcia-Rodriguez *et al*., 2014). In temperate regions, constructed wetlands are constantly exposed to seasonal variability since they are typically developed in the natural environment for nutrient removal or restoration purposes. Although constructed wetlands have successfully improved water quality, these environmental changes may reduce their effectiveness, making them less consistent than conventional water treatment systems (Picard *et al*., 2005; Zhang *et al*., 2010). Overall, "constructed wetland" is used as an umbrella term.

On the other hand, engineered wetlands are considered to be advanced, semi-passive constructed wetland systems where conditions such as temperature, light, water level etc. are constantly monitored, manipulated and controlled to optimize contaminant removal through physical, chemical and biological processes (Zhang *et al*., 2010). These types of systems are usually laboratory-based or found in WWTPs. Like constructed wetlands, they have mainly been used to reduce flooding, reduce volume runoff and remove pollutants present in stormwater runoff, domestic wastewater and agricultural wastewater (Zhang *et al*., 2010; Geng *et al*., 2017). According to Zhang *et al*. (2010), "all engineered wetlands are constructed wetlands, but not all constructed wetlands are engineered wetlands".

Since the wetland systems in this study will be laboratory-based with controlled environmental conditions, they will be defined as engineered wetlands.

2.7 Building on Previous Research in the McCarthy Lab

Previous research conducted in our laboratory by Tiley (2013) tested the response of *Typha latifolia* to high nutrient rates in constructed wetlands. In his research, it was concluded that nutrient inputs increase the invasiveness of *Typha*, and thus only non-invasive species should be implemented in constructed wetlands.

Additional research carried out by Fernandes (2017) provided criteria for engineered wetland macrophyte selection, detailed protocols for germinating aquatic macrophytes under controlled laboratory conditions as well as floating and stationary constructed wetland model designs.

2.7.1 Contributing to Scientific Data Banks

iNaturalist is a social networking app that is a joint operation between the California Academy of Sciences and National Geographic Society that allows biologists and citizen scientists to record and share their observations with fellow scientists around the world (iNaturalist, 2020). It is considered a species identification and/or occurrence recording system that in turn provides open data on when and where species occur to scientists, resource managers, conservation agencies and the general public. All recorded observations are shared with scientific data banks such as the Global Biodiversity Information Facility (iNaturalist, 2020).

2.7.2 Gaps in the Knowledge

It is clear that strategies are needed to combat natural wetland loss and prevent nutrients from non-point sources from entering lakes. Although constructed wetlands are not a necessarily new technology, there are still many gaps in the knowledge. This research was conducted in order to add a piece of the puzzle to research on constructed wetlands that can be implemented in the environment, particularly along the edges of Lake Erie. Since only a few selected species (such as *Typha*) are continuously used in constructed wetlands, there is little information in the literature with regards to the use of native non-invasive plant species in these systems. Thus, it is important to study the potential of native non-invasive species for nutrient removal as environmentally sustainable alternatives that will not negatively impact the environment if they are implemented along coastlines.

2.8 Objectives

Similar to research on natural wetlands, research on constructed and/or engineered wetlands has mainly focused on the use of a few selected species for wastewater treatment. However, these certain species may cause environmental damage as a result of invasive characteristics.

Extensive literary review suggests that to date, there are limited laboratory-based engineered wetland studies using native non-invasive species. Therefore, the main objective of this thesis is to develop a laboratory-based engineered wetland system with native non-invasive species and study species composition in natural environments by building on the existing knowledge and research carried out by Mark Tiley and Francesca Fernandes, previous master's students in our laboratory. This will be achieved in two parts of sub-objectives:

Part 1:

1. Selecting and growing native non-invasive wetland macrophytes to create a laboratory-scaled engineered wetland system in KHN 302 (Ryerson lab).
2. Examining the effects that phosphorus has on the overall plant community and whether the selected plants are able to remove phosphorus from the aquatic environment.

Part 2:

3. Examining a natural wetland in Southern Ontario to study the presence of native species through identification.
4. Observe seasonal variation (temperature, hydrology, growing season) and senescence cycle of wetland community as it shifted from summer to fall.

3.0 MATERIALS AND METHODS

The materials and methods section has been divided into 2 parts:

Part 1: In-lab engineered wetland analyses

Part 2: *In situ* wetland analyses

3.1 PART 1: IN-LAB ENGINEERED WETLANDS

3.1.1 Macrophyte Acquisition:

Various wetland macrophytes were collected in September 2018 along the banks of wetlands located in the Palgrave Forest and Wildlife Area in Caledon, Ontario in order to populate the in-lab wetlands and generally get familiar with wetland macrophytes (Figure 4). Other members of the research team including Dr. Lynda McCarthy, Shenley Alkins and Rachel McNamee were asked to collect additional wetland plants which were obtained from Kitchener, Ontario; Callander, Ontario and High Park, Toronto, Ontario. It is important to note that this part of the procedure was performed prior to native non-invasive plant species becoming the focus of this thesis.

During the macrophyte acquisition, shovels were used to manually remove plants by digging around their root system and extracting them from the ground. Once removed, plants were placed into buckets filled with a garbage bag. A quarter of the bucket was filled with wetland water from the site to ensure the plant remained hydrated during transfer to Ryerson University. Some of the plants collected in field were identified as Canadian goldenrod (*Solidago canadensis)*, purple loosestrife (*Lythrum salicaria*) and cattail (*Typha*) (Figure 4).

Figure 4: Collecting Plants from a Natural Wetland in Palgrave Forest and Wildlife Area

a) extraction of wetland plants in Caledon, Ontario (Photo by Shenley Alkins)

b) collected plants transferred to buckets containing water from site (Photo by Joanna Tucci)

In addition to the plants collected in field, numerous organizations were contacted for the potential purchase of wetland macrophytes between September and November 2018.

Ontario universities and colleges with water research centres that were contacted, and the respective representative spoken to include the following:

- The University of Waterloo's Wetland Institute (Mary Anne Hardy)
- University of Toronto's Institute for Water Innovation (Elodie Passeport & Jennifer Drake)
- Queen's University (Shelley Arnott)
- Sir Sanford Fleming's Center for Advancement of Water and Wastewater Technologies

Ontario organizations that were contacted included the following:

- Ducks Unlimited Canada

- Canadian Wildlife Federation

Local Ontario plant nurseries that were contacted included the following:

- Royal Botanical Gardens
- Native Plant Nursery (Leah Wannamaker & Kristen Vincent)
- Verbinnen's Nursery
- Grow Wild Native Plant Nursery
- Glen Echo Nurseries

Several different macrophyte species were purchased from Glen Echo Nurseries during November 2018. Glen Echo is a family owned and operated garden centre and landscape business established in 1961 located on 35 acres of land in Caledon, Ontario where plants are grown on site (Glen Echo Nurseries, 2017). The macrophyte species included: bowles golden sedge (*Carex elata 'Aurea')*, black gamecock Louisiana iris (*Iris)*, corkscrew rush (*Juncus effuses 'Spiralis'*), forget-me-nots (*Myosotis*) and zebra rush (*Scirpus zebrinus*). These species were chosen as they were the only wetland and/or terrestrial plants available that can tolerate moist/wet environments. All plants were identified by their tag as well as information from literature. Plants were then transferred to Ryerson University and placed in buckets with water.

3.1.2 Development of In-Lab Engineered Wetlands:

Once wetland plants were obtained, an in-lab engineered wetland system was developed by building upon past research and methodologies conducted by Tiley (2013) and Fernandes (2017).

3.1.2.1 Stationary Engineered Wetland System:

The following materials (Table 3) and steps were used to develop an in-lab stationary engineered wetland system.

Table 3: Materials to Construct a Stationary Engineered Wetland System

• Adhesive	• Polyvinyl chlorine (PVC) tubes
• Buckets	• Water
• Light bank	• Wetland macrophytes
• Peat mix	• 0.5 mm polyester mesh

All plants that were collected in field as well as the larger plants purchased from Glen Echo either remained within garbage bags in buckets or were transplanted into polyvinyl chloride (PVC) tubes (acquired from Tiley and Fernandes experiments) to create a stationary constructed wetland (Figure 5). Buckets were tested for leaks by filling 25% of the bucket volume with water over a 24-hour period. 0.5 mm spacing polyester mesh sheets were attached to the bottom of 3 L open ended PVC tubes using adhesive to decrease substrate loss while still allowing root elongation. The macrophytes were then transplanted into the PVC tubes which were filled with a 1:1 mixture of peat and soil. 3 PVC tubes were arranged in each bucket which was filled to the brim with water. Buckets were then placed under light banks and maintained by adding water regularly (Fernandes, 2017).

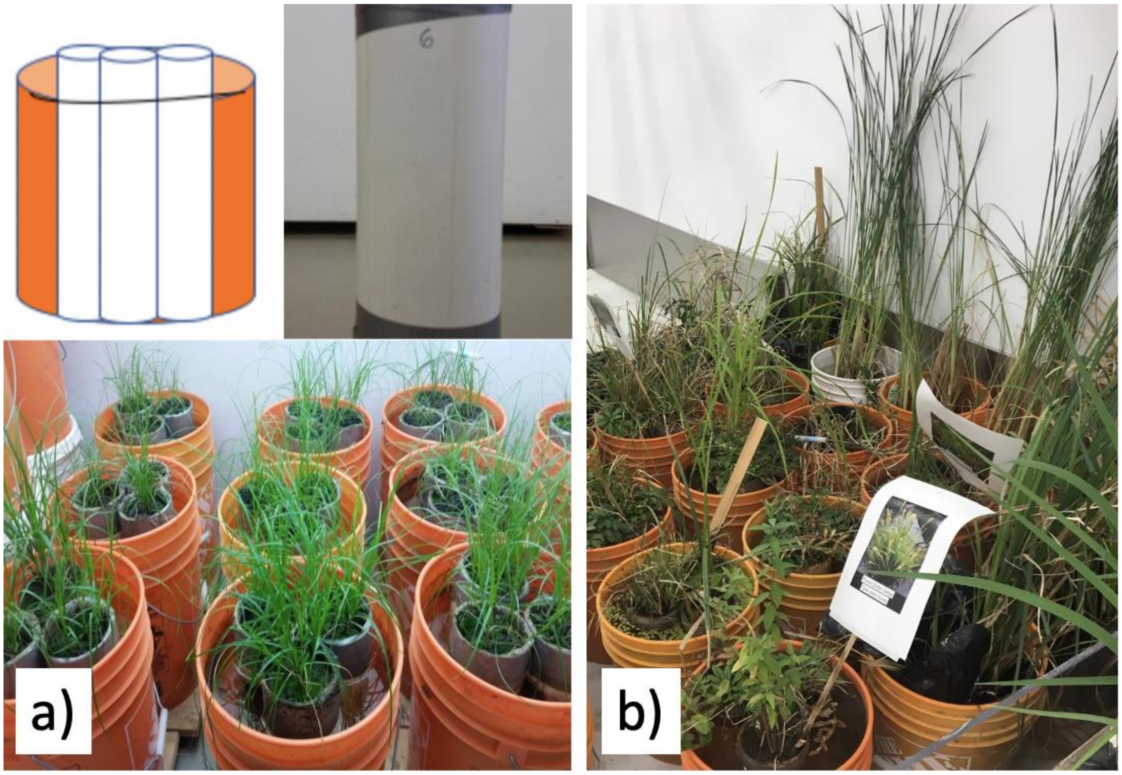


Figure 5: Experimental Setup of Stationary Engineered Wetland System

a) PVC tubes and stationary wetland setup (Fernandes, 2017) b) Stationary wetland setup from this thesis (Photo by Joanna Tucci)

3.1.2.2 Floating Engineered Wetland System:

The following materials (Table 4) and steps were used to develop an in-lab floating engineered wetland system modified from Fernandes (2017).

Table 4: Materials to Construct a Floating Engineered Wetland System

• Adhesive (Tuck Tape)	• Potting mix
• Ethylene propylene diene monomer (EPDM) pond liner	• Trough
• Foam pontoons	• Water
• Light bank	• Wetland macrophytes
• Peat mix	• Zip ties (24 in)
• Plastic planter (4.5 in x 4/5 in)	• 2 mm spacing mesh

3.1.2.2.1 Trough Setup

Troughs were lined with pond liner and tested for leaks by filling 25% of its volume with water and leaving it for 24 hours. If troughs were deemed leak proof, 0.1 m of 1:1 peat and soil mixture was added and 70% of its volume was filled with water.

3.1.2.2.2 Development of "Floating Mat"

Floating foam pontoons were cut into 10 cm fragments (Figure 6a). The 2 mm spacing plastic mesh was cut into pieces large enough to cover the bottom of each planter and were attached using Tuck Tape adhesive to decrease substrate loss while still allowing root elongation (Figure 6b&c). Four pontoon fragments were placed on a 70 cm zip tie which was then secured around each individual planter (Figure 6d). Each planter was half filled with peat mix before the macrophyte was added and their weight was evenly distributed to ensure stable buoyancy.

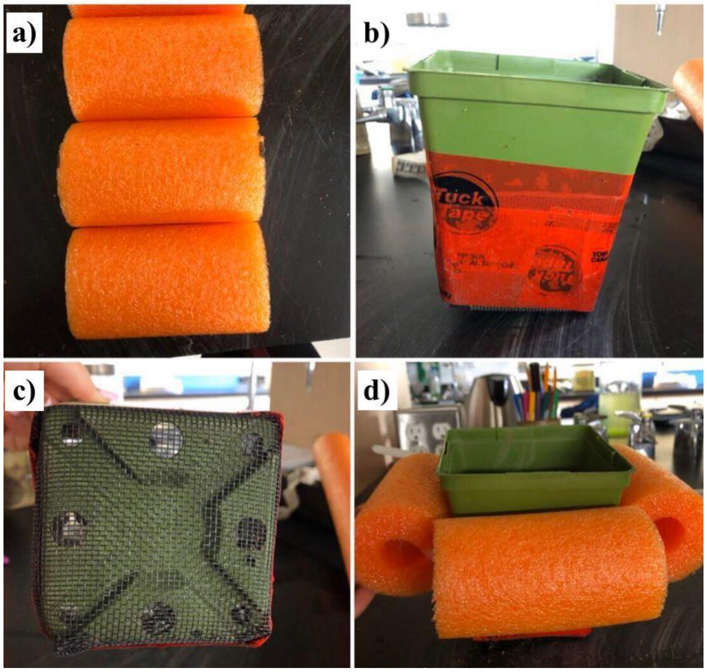


Figure 6: Step-by-Step Development of Buoyant Structure

(Photos by Joanna Tucci)

3.1.2.2.3 Floating Wetland Assembly

Once planters were stable, 3 were placed into each trough which was then filled with water up to the brim. Troughs were placed under light banks and in front of a rotatable table fan, both timed at 16h on, 8h off. Water levels were maintained by adding distilled water regularly. (Figure 7) (Fernandes, 2017).

Figure 7: Experimental Setup of Floating Engineered Wetland System (Giant Ironweed (*Veronia gigantean*) and Blue Flag Iris (*Veronia gigantean*))

(Photo by Joanna Tucci)

3.1.3 Reconnections:

Although the development of engineered wetlands was successful, the objectives of this thesis were adjusted to focus on native and non-invasive wetland plant species only, opposed to general wetland macrophytes. Thus, all existing macrophytes needed to be replaced.

3.1.3.1 Species Selection & Acquisition:

In order to select plant species, the following criteria modified from Wang and Sample (2014) was used:

1. Species must be native and non-invasive.
2. Species must be perennial.
3. Species must be emergent.
4. Species must be able to thrive in hydroponic conditions.

After reconnecting with certain organizations again during spring 2019, Native Plant Nurseries was the first to respond and quickly provided a detailed list of available native wetland macrophytes (pers. comm. Leah Wannamaker and Kristen Vincent). While I received other responses, my great interest was in indigenous native species, therefore Native Plant Nurseries was used for all plant purchases moving forward. Using the criteria above and additional research, the following species outlined in Table 5 were purchased from Native Plant Nurseries on various occasions during fall 2019. By the time the experiment started, the plant inventory consisted of 3 *Asclepias incarnata*, 9 *Helenium autumnale*, 9 *Iris versicolor*, 6 *Lobelia cardinalis*, 3 *Spartina pectinate* and 6 *Veronia gigantean*. *Chelone glabra*, *Thalictrum* *pubescens*, *Verbena hastata* did not survive. After purchase, all plants were grown in either a laboratory-scaled stationary of floating engineered wetland system until the experiment began.

Table 5: List of Species Purchased from Native Plant Nurseries

Common Name	Scientific Name
• Swamp Milkweed	• *Asclepias incarnata*
• Turtlehead	• *Chelone glabra*
• Sneezeweed	• *Helenium autumnale*
• Blue Flag Iris	• *Iris versicolor*
• Cardinal Flower	• *Lobelia cardinalis*
• Prairie Cordgrass	• *Spartina pectinata*
• Tall Meadow Rue	• *Thalictrum pubescens*
• Blue Vervain	• *Verbena hastata*
• Giant Ironweed	• *Veronia gigantean*

A few of the species purchased for the lab-based engineered wetlands overlapped with Environment Canada’s list of macrophytes suitable for engineered wetlands and/or wetland restoration in Table 2. The overlapping species included: swamp milkweed, turtlehead and blue flag iris (Environment Canada, 1996).

3.1.4 Experimental Design:

The overall design of this experiment was dependent on which species successfully grew under the laboratory conditions and the amount of biomass acquired by the end of January 2020. Out of all of the species outlined in Table 5, *H. autumnale, I. versicolor* and *L. cardinalis* thrived the most under the laboratory conditions and were considered for the experiment. For each species, the 3 plants that were in the best health and condition were used in the experiment. The design consisted of 2 types of mesocosms: 3 polycultures (multiple species) and 3 controls (no species). Each type of mesocosm experienced a different phosphorus treatment: 0 μg PO_4-P/L, 100 μg PO_4-P/L or 1000 μg PO_4-P/L as outlined in Figure 8. The order of the troughs was determined by random draw. Papers labelled with the different treatments were folded in half, placed into a glass jar and shaken for randomization. The order in which the papers were chosen was the order of the troughs lined up from left to right. Troughs were labelled accordingly.

1. Mixed Plants 1000 μg PO_4-P/L	2. No Plants 0 μg PO_4-P/L	3. No Plants 100 μg PO_4-P/L	4. Mixed Plants 0 μg PO_4-P/L	5. Mixed Plants 100 μg PO_4-P/L	6. No Plants 1000 μg PO_4-P/L

Figure 8: Experimental Design of Floating Engineered Wetlands

3 troughs will be considered the control group and will not contain any wetland plants. 3 other troughs will be considered polycultures and will contain multiple wetland plant species. 3 different phosphorus treatments will be added to each type of mesocosm.
(Photo by Joanna Tucci)

3.1.5 Assessing Phytoremediation of Phosphorus in Floating Engineered Wetlands:

In order to determine the removal of phosphorus from the system, a colorimetric analysis was performed.

3.1.5.1 Protocol for Cleaning Glassware and Other Items:

To eliminate contamination, all glassware and other objects were thoroughly cleansed prior to being used in experiments. The following materials (Table 6) were used to clean experimental equipment following a procedure from Fernandes (2017) (based on Environment Canada (1990) and modified by Puddephatt (2013)).

The following materials (Table 6) and steps were used for the cleaning protocol.

Table 6: Materials Required for Cleaning Protocol

• Dechlorinated municipal drinking water (DMDW)	• Hydrochloric Acid (HCl) 10% (v/v)
• Deionized water	• Plastic container
• Gloves	• Non-phosphate detergent (Extran)

In a large container, dechlorinated municipal drinking water and a non-phosphate detergent (Extran, in powder form) were mixed to a concentration of 2% w/v. Prior to use, all equipment was submerged in the soapy solution and soaked for a minimum of 30 minutes. Subsequently, the equipment was thoroughly finger scrubbed and rinsed with additional water. All items were then rinsed with hydrochloric acid (HCl) 10% v/v thrice to remove any remaining particulates, metals and/or bases. Finally, all items were rinsed with deionized water three times and left in an inverted position to air dry.

3.1.5.2 Nutrient Addition and Collection of Water Samples:

The following materials (Table 7) and steps were used for phosphorus addition and sample collection.

Table 7: Materials Required for Collecting Water Samples

• Dropper	• Marker
• Prepared 1M HCl	• 50 mL centrifuge tubes

Prior to the nutrient addition, initial water samples were collected in 50 ml centrifuge tubes from each trough and labelled with the trough number and date of extraction. Once labelled, 1 drop of 1M HCl was added to each collected sample and stored in the fridge. The preparation for the 1M HCl can be found in Appendix I.

After all initial water samples were collected and stored, the following amounts of phosphorus solution were added to each trough (Table 8). Calculations are outlined in Appendix II.

Table 8: Corresponding Nutrient Addition

Trough #	Treatment (μg PO_4-P/L)	Amount Added (mL)
1	1000	914
2	0	0
3	100	83.5
4	0	0
5	100	91.4
6	1000	914

After nutrient addition, water samples were collected in 50 ml centrifuge tubes from each trough and labelled with the trough number and date of extraction every other day over 14 days. Once labelled, 1 drop of 1M HCl was added to each collected sample and stored in the fridge until analyzed.

3.1.5.3 Ascorbic Acid Method for Chemical Analysis

A colorimetric determination of dissolved orthophosphate was also conducted using the Ascorbic Acid Method. Reactive phosphorus does not need to undergo preliminary hydrolysis or oxidative digestion in order to react to a colorimetric test such as the ascorbic acid method (USEPA, 1983; APHA, 1998; Fernandes, 2017).

3.1.5.3.1 Preparing Reagents

The materials and methods used to prepare stocks and the mixed reagent for the colorimetric phosphorus analysis can be found in Appendix III. Once all water samples were collected and ready to be analyzed, a mixed reagent was prepared. The following steps and materials (Table 9) were used to develop the mixed reagent for the ascorbic acid method.

Table 9: Materials for Preparing the Mixed Reagent

• Ammonium molybdate reagent	• Sulfuric acid reagent
• Antimonyl potassium tartate reagent	• 10 mL pipettes
• Ascorbic acid reagent	• 25 mL pipettes
• Pipette filler	• 120 mL beaker
• Stirring rod	

All individual reagents were combined to create 100 mL of mixed reagent. After all reagents reached room temperature, the mixed reagent was prepared by pipetting 50 mL of reagent 1, 5 mL of reagent 2, 15 mL of reagent 3 and 30 mL of reagent 4 into a beaker >100 mL in that order. The solution was mixed after the addition of each reagent. The mixed reagent was stable for 4 hours (APHA, 1998; Fernandes, 2017). When combined with reactive phosphorus in an acid medium, the ammonium molybdate and potassium antimonyl tartate form a heteropoly acid known as phosphomolybdic acid. When phosphomolybdic acid reacts with ascorbic acid, it gets reduced and produces an intense molybdenum blue colour (USEPA, 1983; APHA, 1998; Fernandes, 2017).

3.1.5.3.2 *Filtering Water Samples*

The following steps and materials (Table 10) were used to filter all water samples.

Table 10: Materials Required for Filtering Water Samples

• Marker	• Test tubes
• Syringe	• 0.22 μm pore filter

Prior to chemical analysis, all water samples were filtered in order from extraction date. A syringe was filled with the first sample and a 0.22 μm pore filter was placed in the filter holder and attached to the syringe. 5 mL of the water sample was then filtered into a test tube and labelled accordingly. This was repeated for all 42 water samples. Once all test tubes contained 5 mL of filtered sample water, 0.8 mL of mixed reagent was added to each test tube and mixed by inverting the tube several times. In addition, a blank solution was created by pipetting 0.8 mL of mixed reagent to a test tube containing 5 mL of distilled water.

3.1.5.3.3 Calibration Curve

A standard phosphorus solution was made for the calibration curve by diluting 100 mL of stock phosphorus solution to 1000 mL with distilled water. 1.0 mL = 0.01 mg P. The following concentrations in Table 11 were prepared. 0.8 mL of mixed reagent was added to each test tube.

Table 11: Calibration Curve Standard Dilutions

Test tube	Concentration of P (ppm) mg/L	Volume (ml) of stock per 50 ml of solution
1	0	0.00
2	0.20	1.00
3	0.60	3.00
4	1.00	5.00
5	1.40	7.00
6	1.80	9.00

3.1.5.3.4 Measuring Absorbance

Between 10-30 minutes after the mixed reagent was added to all test tubes containing water samples, the absorbance of each sample was measured using Agilent Cary 60 UV/Vis spectrophotometer. The spectrometer was turned on and allowed 10 minutes to warm up. While the spectrometer was warming up, the associated computer program was set up by opening "simple reads", clicking "set up" and setting the wavelength to 880 nm. A cuvette was rinsed with water, wiped with a Kimwipe, filled with the blank solution and placed in the sample compartment with the clear sides facing the path of light. Once everything was in place, the "zero" button on the computer was clicked and the program provided an absorbance. The blank was removed, and the cuvette was rinsed and wiped with a Kimwipe. This procedure was repeated for all 42 samples as well as the standard solutions for the calibration curve. The stronger the intensity, the higher the phosphorus concentration in the water sample.

3.1.6 Plant Measurements Upon Phosphorus Addition:

Overall plant biomass was also analyzed by measuring plant height with a measuring tape to the nearest mm at the beginning and end of the experiment to determine any affects phosphorus had on macrophyte growth (Fernandes, 2017). Pictures of each mesocosm were also taken at the same angle regularly in order to see changes in biomass (flowers, leaves, etc.).

3.2 Necessary Modifications to Methods

In the midst of the laboratory experiments, a virus known as COVID-19 was declared as a global pandemic, which had resulted in restrictions, quarantines and lockdowns across many countries in order to reduce its spread. On March 13th, Ryerson University officially cancelled on-campus classes and soon after, announced that building and laboratory access was authorized for "essential needs" only. Due to these unforeseen circumstances, further in-lab experimentation could not continue (which would've resulted in 4 months of data generation); thus, the *in situ* wetland field work experiments were incorporated.

3.3 PART 2: *IN SITU* WETLAND

Returning to Palgrave Forest and Wildlife Area for field work was always intended, even if for future research conducted by myself or succeeding students. The circumstances prompted me to go back into the environment and observe the presence of species at a biodiverse *in situ* wetland in an effort to decide what native non-invasive plant species could be used in future lab-based experiments. Thus, during summer and fall of 2020, the exact wetland in Palgrave Forest and Wildlife Area where macrophytes were obtained in September 2018 was revisited in order to conduct seasonal *in situ* plant identification. The seasonality of a wetland can be described as changes in temperatures and hydrology which influence the growing season (spring to summer) for plants (Picard *et al*., 2005; Zhang *et al*., 2010; Rozema *et al*., 2016)

3.3.1 Transect Sampling

This was not meant to be a rigorous ecological assessment. Instead, it was a plant identification evaluation and seasonal observation of the senescence cycle within a biodiverse wetland to determine the presence of native non-invasive species and what plants could potentially be used to further develop engineered wetlands.

The following steps and materials (Table 12) were used to conduct transect sampling.

Table 12: Materials for Transect Sampling

• Paper & pen	• Rope (10 m)
• Phone camera	• 1 m^2 quadrat (PVC pipe & string)

In order to ensure that the wetland habitat was well represented, different portions of the site were randomly targeted for plant inventory sampling (USEPA, 2002). A mixture of quadrat and systematic transect methods were used. The quadrat method is often used to count the number of individuals in a fixed area to determine species richness and abundance, as well as the composition of a plant community (Khan *et al*., 2013).

3.3.1.1 Summer (August 5th, 2020)

During the first field trip, an estimated transect line was established within 1-2 m of the wetland shoreline. Using a rope, every 10 m along the estimated transect a 1 m^2 quadrat was placed at the specific point of interest in a zone extending towards the water (USEPA, 2002; Toet *et al*., 2005; Khan *et al*., 2013; Bano *et al*., 2018; Janousek & Folger, 2017). Detailed notes and photos of each species within the quadrat were taken as additional reference for identification and seasonal comparison. This method was repeated for a total of 9 quadrats around the wetland area as shown in Figure 9. Since the 9 quadrat areas around the wetland were indicated during the first field trip, the physical 1 m^2 PVC pipe and string quadrat was no longer needed for the remaining trips.

3.3.1.2 Early Autumn (October 6th, 2020)

The estimated transect line that was established on August 5th was followed. Every 10 m, detailed photos and notes of each species were taken in an estimated 1 m^2 zone extending towards the water. This was repeated for a total of 9 quadrats around the wetland.

3.3.1.3 Late Autumn (November 4th, 2020)

The estimated transect line that was established on August 5th was followed. Every 10 m, detailed photos and notes of each species were taken in an estimated 1 m^2 zone extending towards the water. This was repeated for a total of 9 quadrats around the wetland.

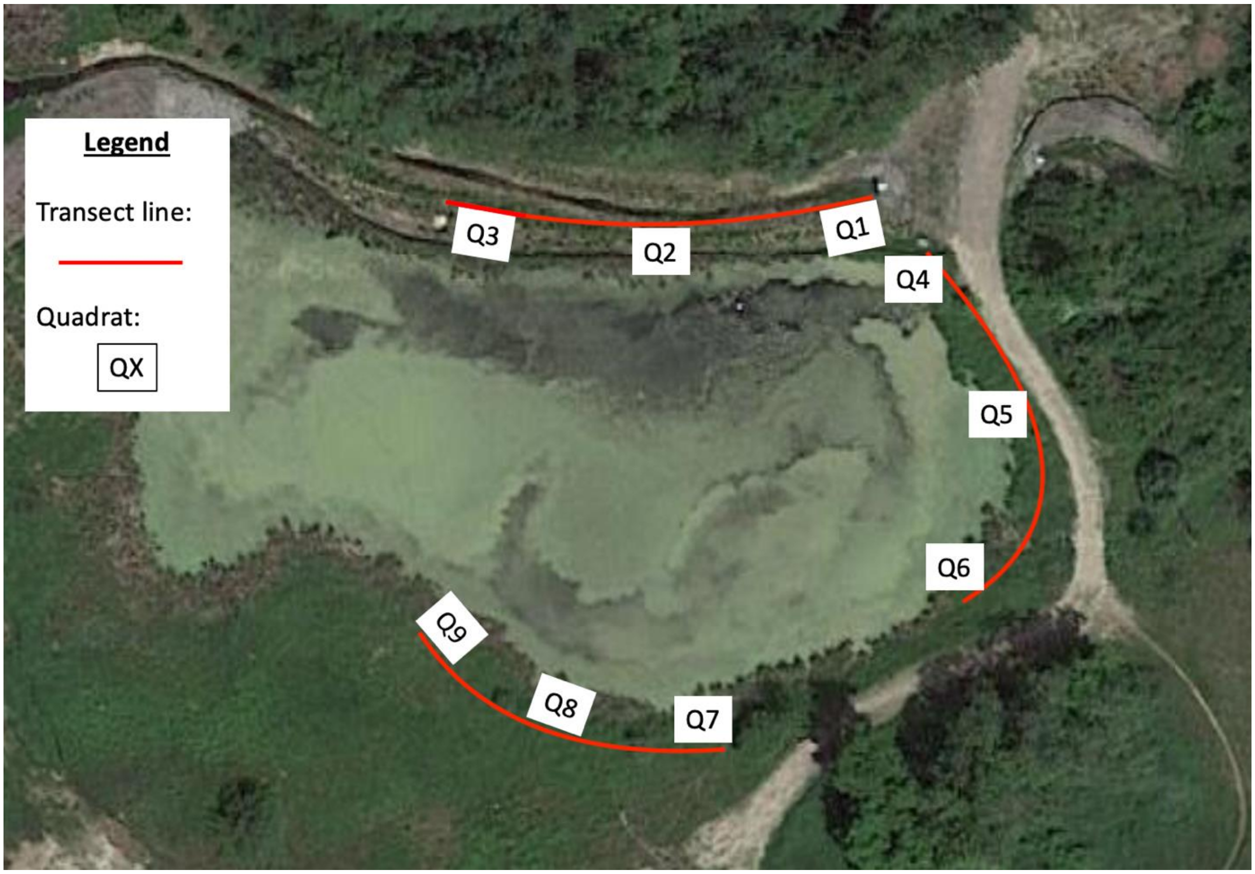


Figure 9: Map of Palgrave Forest and Wildlife Area

Satellite image of the research area, the locations of estimated transect lines and quadrats for seasonal plant identification (Google Maps, n.d.).

3.3.2 Plant Identification

As previously mentioned, visual observation, detailed notes and photo recording of numerous plant species were taken during the transect sampling. The pictures of each species were carefully analyzed and various field guides and identification apps such as the Field Guide to Common Wetland Plants of West Virginia, Native Plant Trust's "Go Botany", Ontario Wildflowers and iNaturalist were used to identify the species present at Palgrave Forest and Wildlife Area.

In order to use iNaturalist, its mobile application was downloaded, and an account was made. The observe icon was used to import pictures of a single species from camera roll that were taken during the various field trips. Once imported, the “What did you see” tab was used to either view species suggestions or look up species by name for identification. After a species was identified, the date and location of the observation were included, and the result was shared within the app itself as well as scientific databases. The results obtained from iNaturalist were used in collaboration with the data provided in other online wetland macrophyte field guides. All species were mainly identified visually by examining and comparing leaf composition and arrangement, flowers and fruits.

4.0 RESULTS AND DISCUSSION

The following results were obtained by performing the previously mentioned methodologies. The Results and Discussion section has been divided into two parts based on the objectives of this thesis which was to expand on the past research achieved in our lab with regards to the presence and use of native non-invasive plant species in constructed or engineered wetlands:

Part 1: In-lab engineered wetlands that examined i) engineered wetland development ii) phytoremediation of phosphorus, iii) plant biomass

Part 2: *In situ* wetlands that examined i) plant identification, ii) seasonal analysis

4.1 PART 1: IN-LAB ENGINEERED WETLANDS

4.1.1 Development of In-Lab Engineered Wetlands

Between the two different wetland setups, the floating engineered wetland systems were selected for the experiments over the stationary engineered wetland systems.

4.1.1.1 Floating Engineered Wetlands

The work initiated by Tiley (2013) and Fernandes (2017) provided the foundations for the further development of "floating engineered wetlands" in the current study. Section 3.0 (Materials and Methods) detailed the development of the engineered wetland system and its successful implementation in the laboratory can be reported here.

Natural floating wetlands, also known as floating islands or mats, are found around the world and are composed of an abundance of floating organic matter, sediment and wetland macrophytes that maintain buoyancy from oxygen and other gases from the plant roots and organic matter decomposition (Wang & Sample, 2014; Pavlineri *et al.*, 2017; Turner *et al.,* 2017; Lucke *et al.,* 2019). Within the limitations of the lab, we tried to emulate these natural systems. Additionally, floating engineered wetland designs are a relatively new phytoremediation approach with a wide range of applicability (Lopardo *et al.*, 2019) that the current study attempted to provide more information to. Over the years, artificial floating wetlands have been tested at the field scale in stormwater and retention ponds to recover habitat for aquatic waterfowl, protect littoral zones, decrease eutrophication and improve the quality of airport runway, sewage, agriculture and mine tailing runoff by mimicking the treatment processes that occur in natural floating wetlands (Pavlineri *et al.,* 2017; Lucke *et al.,* 2019). Floating engineered wetland systems incorporate emergent aquatic or terrestrial macrophytes grown hydroponically in a buoyant structure on the water surface, opposed to being rooted in the sediment (Tanner & Headley, 2011; Zhang *et al.*, 2014a; Pavlineri *et al.*, 2017; Lopardo *et al.*, 2019; Lucke *et al.,* 2019). As tested in the current study, artificial floating wetland systems are kept afloat by buoyant materials such as wood, plastic, inorganic matting, foam and/or fiberglass and contain a growth media to enhance biomass development (Tanner & Headley, 2011; Lucke *et al.*, 2019). This allows the upper parts of the plant to grow above the water, whereas a complex system of roots, rhizomes and biofilm are suspended in the water column beneath the floating mat (Zhang *et al.*, 2014a; Pavlineri *et al.*, 2017). Since the plants are not rooted in the sediment, they can only obtain nutrients directly from the floating mat or water column, thus, the increased direct contact between the roots and polluted water enhances the uptake of nutrients (Wang & Sample, 2014; Zhang *et al.,* 2014a; Hartshorn *et al.*, 2016; McAndrew *et al.*, 2016; Pavlineri *et al.*, 2017; Lopardo *et al.*, 2019; Lucke *et al.,* 2019). Additionally, the suspended root system supplies an extensive biologically active surface area for biofilm growth, where microorganisms sequester suspended particles, transform contaminants, filter water and uptake nutrients. For this type of wetland system, the combination of an extensive root system and microbial biofilm constitute the main removal pathway for nutrients (Tanner & Headley, 2011; Zhang *et al*., 2014a; Hartshorn *et al*., 2016; Lopardo *et al*., 2019; Lucke *et al.*, 2019). Unlike conventional wetland systems, the emergent plants in floating wetlands are not impacted by shallow water depths or total submergence as their buoyancy allows them to rise and drop with fluctuations in water level. Thus, if developed *in situ*, they will not be impacted by changes in water supply altered by extreme weather events (Erwin, 2009; Tanner & Headley, 2011; Zhang *et al*., 2014a; McAndrew *et al*., 2016; Pavlineri *et al.*, 2017; Lucke *et al.*, 2019). The efficiency of floating wetlands to uptake nutrients depends on plant species, biomass, root structure and nutrient storage capacity and structure (McAndrew *et al*., 2016; Pavlineri *et al*., 2017).

Due to these advantages, the floating engineered wetland setup was used for the experiments to enhance nutrient uptake.

4.1.1.2 Macrophyte Acquisition

Unfortunately, all organizations except for Glen Echo Nurseries were unable to help during October/November 2018 as the winter season was approaching and all aquatic macrophytes were either sold out or going into senescence. This is because wetland plants are capable of responding to environmental changes such as seasons. Before fall senescence, nutrients and important ions are translocated from the plant shoots to their roots and rhizomes where they are stored until early spring when they are used for growth (Vymazal, 2007). Since abiotic factors such as solar radiation and temperature influence wetland activity, many natural and engineered wetlands have displayed seasonal nutrient removal with the highest being during the growing season (June-October) and lowest in winter months (November-March) (Picard *et al.,* 2005).

4.1.1.3 Macrophyte Growth

As previously mentioned, Native Plant Nurseries was used for all plant purchases moving forward as the focus of this study shifted towards native non-invasive species.

The swamp milkweed (*A. incarnata*), turtlehead (*C. glarbra*), tall meadow rue (*T. pubescens*) and blue vervain (*V. hastata*) did not manage well under the laboratory conditions as they did not survive after being transferred to the lab. Prairie cordgrass (*S. pectinate*) did not decrease in biomass, but it also did not display any increases in production from when it was purchased in summer 2019 to when the experiment began March of 2020. Various factors could have impeded the growth of these species, the major one being that they were not able to adjust to the controlled laboratory conditions (temperature, light exposure, water levels etc.) or that these conditions were not optimal for their growth. Another reason could be the lack of strong water circulation within the floating and stationary wetland mesocosms, resulting in extreme anoxic conditions in the water column. Although all of these species are known to occupy moist to wet environments, the constant flooding or exposure to water could have been too much to tolerate.

On the other hand, the giant ironweed (*V. gigantean*) grew exceptionally well in the engineered wetland setup and had essentially doubled in height in only two short months to the point where it exceeded the height of the overhanging light banks. However, in November it was noticed that all three giant ironweed plants had been infected with powdery mildew (Figure 10).

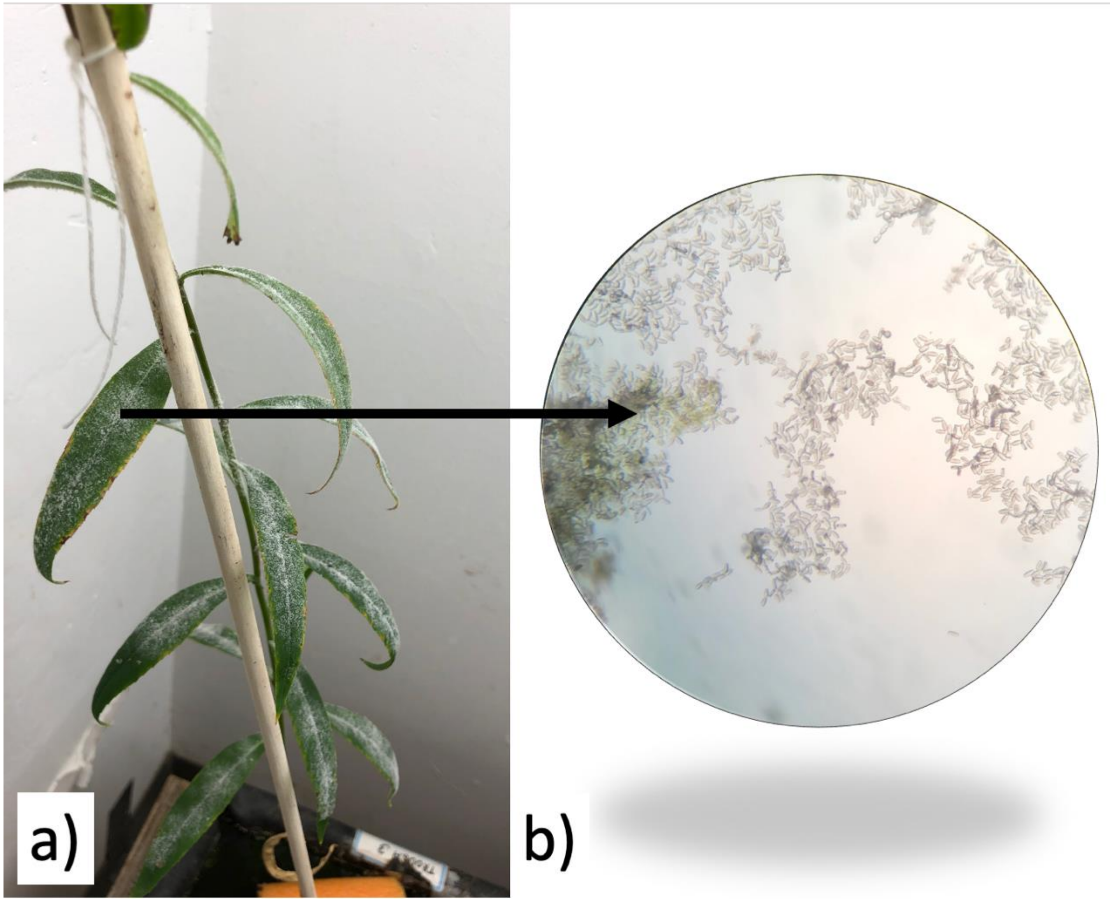


Figure 10: Giant Ironweed Infected with Powdery Mildew

a) Powdery mildew on the leaves of the giant ironweed (*V. gigantean*) in the engineered wetlands b) fungal sample observed under a light microscope (Photos by Joanna Tucci)

Powdery mildew is a widespread fungal disease that infects many plants. The disease is caused by various species of fungi in the order Erysiphales, which are specialized phytopathogens that alter the shape and function of host cells. The fungi thrive on dry surfaces in humid environments with temperatures between 15-28 °C, but do not grow well on wet surfaces such as wet foliage. Symptoms of powdery mildew include the growth of white hairy mycelium on the surface of young leaves which can result in curling and/or leaf fall of infected leaves, buds, flowers and other young tissues (Hückelhoven & Panstruga, 2011; Bolda & Koike, 2013;

Liyanage *et al*., 2017). Due to this infection, the giant ironweed macrophytes had deteriorated and could not be used for the experiment.

Out of the species purchased from Native Plant Nurseries listed in Table 5, only the sneezeweed (*H. autumnale*), blue flag iris (*I. versicolor*) and cardinal flower (*L. cardinalis*) developed well under the laboratory conditions and were used for the experiment (Figure 11). Sneezeweed is a plant native to North America (including Ontario) that is naturally occurring along rivers, wet meadows and wetlands (Muma, 2021c). Blue flag iris is a perennial macrophyte native to North America (including Ontario). It has adapted to tolerate constant inundation since it grows in wet soils found along shorelines, marshes, swamps, wet meadows and forested wetlands from Newfoundland to Manitoba, south to Minnesota and Virginia. It can grow 0.1 to 0.8 m high with 1 cm wide leaves and thick rhizomes (Harris *et al.*, 1997; USDA, 2002a; McAndrew *et al*., 2016). Lastly, the cardinal flower is a herbaceous perennial also native to North America (including Ontario) that was also used for the experiments. It can be found along stream banks and damp meadows, growing 60-120 cm tall with flowers 2.5-4 cm. It is relatively easy to grow and propagate and was used for various medicinal purposes by the Iroquois (Harris *et al*., 1997; USDA, 2003).

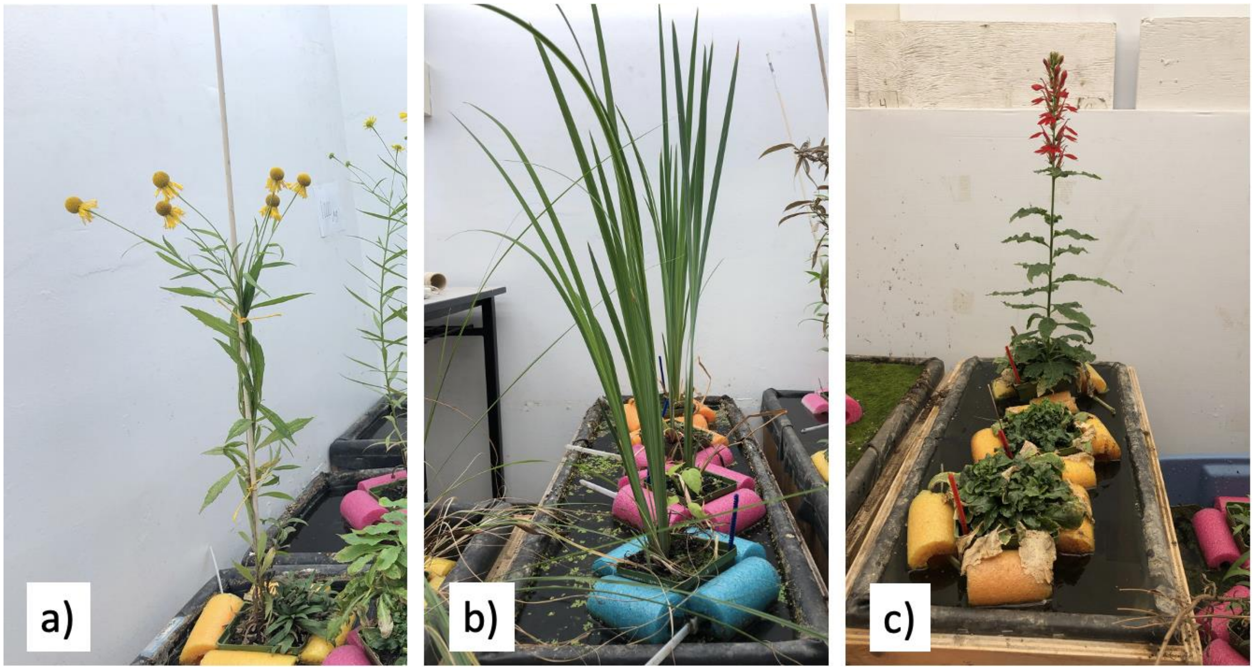


Figure 11: Macrophytes Chosen for Lab Experiments

a) Sneezeweed (*Helenium autumnale*) b) Blue Flag Iris (*Iris Versicolor*) c) Cardinal Flower (*Lobelium cardinalis*) (Photos by Joanna Tucci)

4.1.2 Plant Measurements Upon Phosphorus Addition

Another objective from the original experimentation was to examine whether phosphorus had any overall impacts on the plant community. The above-ground biomass of each macrophyte within the engineered wetland was used to observe any growth or decay. In order to determine potential changes in biomass among the different species and treatment concentrations (0, 100 and 1000 μg/L), the height of each macrophyte was measured and any changes in overall biomass, such as flowers and leaves, were observed at the beginning and end of the experiment.

Table 13 displays the overall height of the 3 species in each mixed culture prior to the beginning of the experiment.

Table 13: Initial Macrophyte Height on February 28, 2020

P Treatment	Blue Flag Iris (*I. versicolor*)	Cardinal Flower (*L. cardinalis*)	Sneezeweed (*H. autumnale*)
0 μg/L (Trough 4)	83.5 cm	17 cm	61 cm
100 μg/L (Trough 5)	71 cm	56.5 cm	65 cm
1000 μg/L (Trough 1)	86 cm	29 cm	81 cm

Table 14 displays the overall height of the 3 species in each mixed culture at the end of the experiment.

Table 14: Final Macrophyte Height on March 14, 2020

P Treatment	Blue Flag Iris (*I. versicolor*)	Cardinal Flower (*L. cardinalis*)	Sneezeweed (*H. autumnale*)	Average Growth + Standard Deviation
0 μg/L (Trough 4)	83.5 cm	27 cm	62.5 cm	**3.83 ± 5.39 cm**
100 μg/L (Trough 5)	71 cm	57 cm	65 cm	**0.16 ± 0.28 cm**
1000 μg/L (Trough 1)	86 cm	44 cm	83 cm	**5.66 ± 8.14 cm**
Average Growth + Standard Deviation	**0 cm**	**8.5 ± 7.36 cm**	**1.17 ± 1.04 cm**	

Despite the unusually high phosphorus levels in the troughs, the plants survived well and even increased their growth and biomass. The growth performance differed between species. Both *L. cardinalis* and *H. autumnale* produced extensive aerial tissues, roots and flowers and accumulated more aboveground biomass than *I. versicolor.* As seen in Table 14, *I. versicolor* did not experience any changes in plant height between February 28 and March 14, 2020. *L. cardinalis* grew by 10 cm in trough 4, 0.5 cm in trough 5 and 15 cm in trough 1. *H. autumnale* grew by 1.5 cm in trough 4, 0 cm in trough 5 and 2 cm in trough 1. Average plant height for *L. cardinalis* and *H. autumnale* were 8.5 cm and 1.17 cm respectively, whereas that for *I. versicolor* was 0 cm. Overall, the *L. cardinalis* species experienced the most growth over the 14-day experiment compared to the other 2 species.

The average plant growth for all 3 species under different P treatments of 0 μg/L, 100 μg/L and 1000 μg/L were 3.83 cm, 0.16 cm and 5.66 cm respectively. This indicates that the species grown in the trough with the highest P addition grew the most. The addition of P did not have any negative growth impacts on the macrophytes.

In addition to overall plant height, all 3 of the *H. autumnale* plants displayed an increase in biomass through number of flowers. The *H. autumnale* in troughs 1, 4 and 5 only had 2, 6 and 1 blooming flower respectively at the beginning of the experiment (March 2), but had a total of 10, 6 and 9 flowers respectively by the end of the experiment (March 14). Changes in overall aboveground biomass are displayed in Figures 12 and 13.

Figure 12: Overall Biomass of Mixed Cultures on March 2nd

a) Trough 1 (1000 µg/L) b) Trough 4 (0 µg/L) c) Trough 5 (100 µg/L) (Photos by Joanna Tucci)

Figure 13: Overall Biomass of Mixed Cultures on March 14th

a) Trough 1 (1000 µg/L) b) Trough 4 (0 µg/L) c) Trough 5 (100 µg/L) (Photos by Joanna Tucci)

Increases in plant height and overall biomass were expected as phosphorus is an essential nutrient required for plants to grow (Filippelli, 2016). The accumulation of elements such as nitrogen and phosphorus is an important factor in plant production and growth. However, nitrogen and phosphorus are usually co-limiting in most ecosystems, thus the growth and reproduction of plants is limited by the availability of these nutrients (Leng, 1999; Elser *et al.*, 2007; Chrysargyris *et al.*, 2016; Filippelli, 2016; Zeshan *et al.*, 2016). Numerous studies have shown that increased phosphorus concentrations in both aquatic and terrestrial habitats have positive impacts on plant growth and increased production (Elser *et al.*, 2007; Chrysargyris *et al.*, 2016).

4.1.2.1 Recommendations for Further Study

As previously mentioned, many of the species brought into the lab for engineered wetland development did not survive or have high productivity rates. As a possible consequence of many factors, future recommendations include increasing water circulation by the addition of submersible pumps to prevent anoxic conditions. The wetland design should also be evolved to provide macrophytes with "wet feet" while preventing constant flooding of the roots/planter. With regards to the stationary constructed wetlands, the PVC tubes can be placed at least 5 cm away from the bottom of the bucket on a growing tray, while the pontoons of the floating wetlands should be improved to further elevate the planter out of the water opposed to being submerged. Another suggestion would be to look over the species as often as possible to ensure their aboveground biomass is dry to inhibit fungal diseases such as powdery mildew. Lastly, it is recommended that future studies continue to elaborate on protocols for growing native non-invasive species under laboratory conditions.

4.1.3 Phytoremediation of Phosphorus

Tiley (2013) conducted a rigorous study regarding phosphorus sequestration, however, he performed these experiments on the invasive *Typha*. In this current study, one of the objectives from the initial experimentation was to measure phosphorus removal by native non-invasive macrophytes in engineered wetlands. However, this was not the major goal and only preliminary observations were made.

Table 15 and Figure 14 outline the resulting absorbance readings from the phosphorus standard curve. The standard curve was intentionally designed to cover a wide range of concentrations, including high concentrations of phosphorus in hopes to exceed the concentrations within the engineered wetlands so that the concentrations of unknowns could fall within the standard curve.

Table 15: Standard Curve Absorbance Readings Using UV/Vis Spectrophotometer

Test Tube	Concentration of P (ppm)	Absorbance
1	0.0	0.0000
2	0.2	0.0580
3	0.6	0.0807
4	1.0	0.1073
5	1.4	0.1357
6	1.8	0.1287

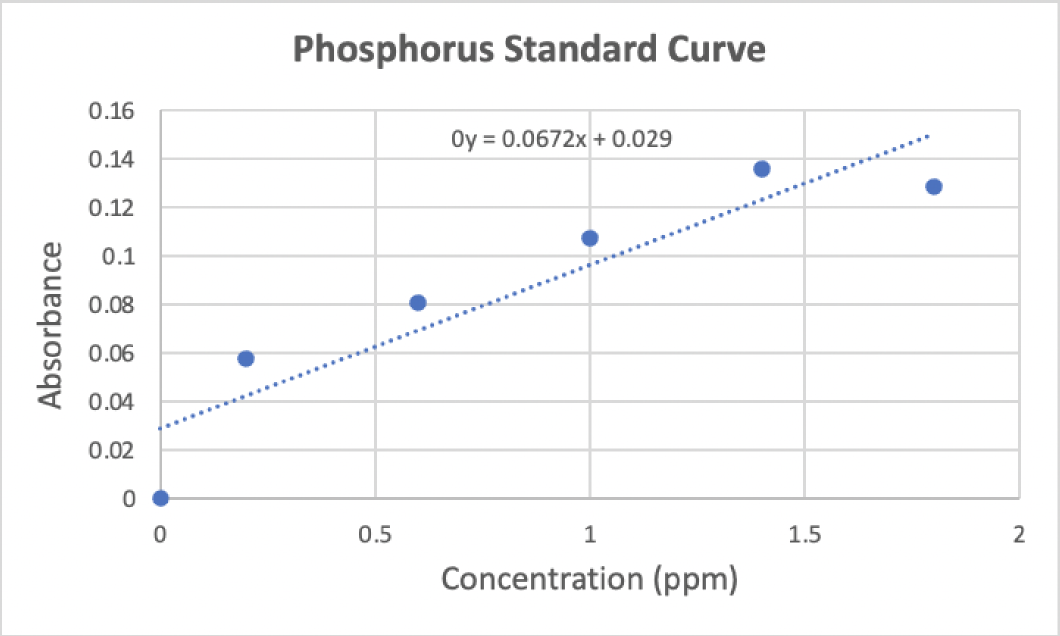


Figure 14: Phosphorus Standard Curve Using UV/Vis Spectrophotometer

Three treatments of 0, 100 and 1000 μg P/L were selected based on TP concentrations in natural wetlands in southern Ontario, with the 0 (no P added) treatment being used as a reference. Based on the literature, the treatments of 100 and 1000 μg P/L are fairly high compared to what is commonly found in natural systems but were selected as TP concentrations in non-point source agricultural runoff can exceed 300 μg P/L, and concentrations above 1000 μg P/L have been detected in parts of Canada. Thus, these values can mimic hypereutrophic waters (Yates & Prasher, 2009; Environment and Climate Change Canada, 2015).

Table 16 outlines the resulting absorbance readings from the water samples collected over 14 days from the mixed cultures (troughs 1,4 and 5) that experienced different phosphorus treatments. The corresponding graphs can be found in Appendix IV.

Table 16: Mixed Culture Absorbance Readings

Sample Day	Absorbance		
	$0\ \mu g$ PO_4-P/L (Trough 4)	$100\ \mu g$ PO_4-P/L (Trough 5)	$1000\ \mu g$ PO_4-P /L (Trough 1)
1 (Mar 2, 2020)	2.1040	2.3148	0.4370
2 (Mar 4, 2020)	1.8321	2.2114	0.9082
3 (Mar 6, 2020)	2.0036	2.4002	0.8568
4 (Mar 8, 2020)	2.1217	2.6910	0.8488
5 (Mar 10, 2020)	1.9330	2.3188	0.8575
6 (Mar 12, 2020)	1.9463	2.3535	0.8425
7 (Mar 14, 2020)	1.9785	2.3158	0.8001

Table 17 outlines the resulting absorbance readings from the water samples collected over 14 days from the controls (troughs 2,3 and 6) that experienced different phosphorus treatments. The corresponding graphs can be found in Appendix IV.

Table 17: Control Group (No Plants) Absorbance Readings

Sample Day	Absorbance		
	$0\ \mu g$ PO_4-P /L (Trough 2)	$100\ \mu g$ PO_4-P /L (Trough 3)	$1000\ \mu g$ PO_4-P /L (Trough 6)
1 (Mar 2, 2020)	0.0479	2.4387	0.0308
2 (Mar 4, 2020)	0.0544	2.2666	0.6035
3 (Mar 6, 2020)	0.0510	2.3489	0.5270
4 (Mar 8, 2020)	0.0460	2.7713	0.4842
5 (Mar 10, 2020)	0.0365	2.3503	0.4110
6 (Mar 12, 2020)	0.0362	2.2905	0.4215
7 (Mar 14, 2020)	0.043	2.4268	0.4136

This analysis was a first attempt pilot study to examine the ability of native non-invasive wetland macrophytes to remove TP from the environment and was not the major goal of this thesis. Constant and unusually high values of phosphorus were observed in all of the troughs over the two-week period of the experiment. As previously mentioned, the global pandemic known as COVID-19 had resulted in the shutdown of Ryerson labs. Due to this, the continuation of the experiments, which would have included standard procedures for diluting both the samples and the system as well as an extended period of time to determine if the wetland biomass would in fact decrease the phosphorus concentration over time, could not be completed. As a result of the unforeseen circumstances, the focus of this thesis shifted to the use of native non-invasive species for the extensive development of sustainable engineered wetlands in a laboratory setting and plant diversity and identification in natural wetland ecosystems.

The high absorbance readings represented in Tables 16 and 17 are correlated with constant, high values of phosphorus in the system. The highest absorbance value within the standard curve is 0.12, which corresponds to a concentration of 1.8 ppm. Based off of the results, the absorbance readings for most of the water samples are above the maximum limit of the standard curve (highest being 2.7713), so it can be assumed that the water sample concentrations are well above 5 ppm. These results are unexpected because the amount of phosphorus added (0.1 and 1.0 ppm) is very little compared to the final outcome. Absorbance range is typically within 0.1 and 1.0, and values above this are considered too high and concentrated. With an absorbance reading of 2.0, also interpreted as 1%T, indicates that 99% of the light is being blocked or absorbed by the sample and so on (Vernier, 2020).

4.1.3.1 Phosphorus Concentrations in Canada

The trends in phosphorus in water bodies across Canada varies widely as some areas are naturally low in phosphorus, while others are naturally high in phosphorus. According to the Environment and Climate Change Canada, TP concentrations between 2004 and 2006 ranged between <0.5 and 1880 μg/L, with an average of 14 μg/L (2015). The TP concentrations in streams within southern Ontario are usually low (<15 μg/L), but vary depending on geology, climate, land use and riparian zone coverage of the system (O’Brien *et al*., 2013). So, although the values observed from the troughs are extremely high compared to most natural systems, concentrations above 1 ppm (1000 μg/L) are found, which is why such concentrations were chosen for the standard curve.

4.1.3.2 Phosphorus Concentrations in Southern Ontario

In addition to the status of phosphorus across Canada, various studies have been conducted in southern Ontario specifically. Rutledge and Chow-Fraser conducted a study on the variation of phosphorus concentrations found in the Nottawasaga River Watershed, located in south central Ontario. They found that TP levels ranged from 6.1 to 44.2 μg/L and that the tributaries such as Innisfil Creek and Mark Creek had high concentrations of 40.4 and 41.0 μg/L respectively. The high levels were correlated to these specific areas being dominated by agricultural land use. One of the study areas, Willow Creek, flows through a portion of wetlands (Minesing Wetlands) prior to releasing into the Nottawasaga River. Despite the fact that wetlands are known to filter wastewaters, the TP concentrations at Willow Creek ranged from 12.1 to 180.8 μg/L, which was 5-30 times higher than the values obtained from the rest of the study sites. These findings suggest that since the wetlands were surrounded by agricultural areas, the constant nutrient loading led to the accumulation of phosphorus in the wetland sediment and under anoxic conditions the phosphorus was released back into the water column via internal loading (Rutledge & Chow-Fraser, 2019). A study on TP concentrations in small wetland-influenced streams in the Muskoka District of Ontario concluded that TP concentration averaged at 11.6 μg/L along the stream channel (O’Brien *et al*., 2013). Another study investigated the phosphorus dynamics and hydrology of the Hidden Valley wetland located in Kitchener Ontario over a span of 12 months. The base flow concentrations ranged from 0.00-650 μg/L of phosphorus, with an average of 30 μg/L when there were no storm events. However, the phosphorus concentrations reached a value of 2520 μg/L during storm event (Gehrels & Mulamoottil, 1990).

4.1.3.3 Phosphorus Concentrations in Constructed Wetlands

In addition to natural systems, phosphorus concentrations in full-scale and lab-scale constructed wetlands have also been documented. Of the multiple full-scale surface flow and subsurface flow constructed wetlands studied year-round in northeastern North America, the average influent and effluent TP concentrations were 39 mg/L and 13 mg/L respectively. This study also found that the wetlands performed well and were suitable for year-round wastewater treatment, even with the seasonal fluctuations (Rozema *et al*., 2016). Xiao and colleagues also studied nutrient removal efficiencies in wetland plants at decreased temperatures, using a lab-scale engineered wetland. During this experiment, the average TP concentration in the wetland influent was 14.05 mg/L and the effluent was much lower, which demonstrated that the plants in the engineered wetland successfully removed phosphorus from the system under low temperature conditions (Xiao *et al*., 2019).

4.1.3.4 Internal Nutrient Loading

The extremely high values obtained during this thesis may be a result of various factors. The substrate that was used in all of the troughs could have been nutrient rich, resulting in internal loadings of phosphorus. Although it is known that nutrients in the water can be removed via plant uptake, the sedimentation of nutrients is also a significant component of phosphorus removal in wetlands (Brix, 2003; Mayer *et al*., 2005; Vymazal, 2007; Zhang *et al.*, 2009). But the composition of the sediment and overall conditions of the aquatic system determine whether the sediment is a phosphorus sink or source (Mayer *et al.,* 2005). The sediment can act as a major source of phosphorus under certain conditions by releasing it back into the water column through diffusion, resuspension and/or bioturbation. This is known as internal loading, which occurs when physical, chemical and biological processes mobilize nutrients such as phosphorus from the sediment to the overlying water (Mayer *et al*., 2005; Orihel *et al.*, 2017). An experiment conducted at Cootes Paradise Nature Sanctuary, a coastal wetland located in southern Ontario that has experienced serious degradation due to nutrient loading, found that reflux from sediment was the major cause for ~57% of phosphorus loadings (Mayer *et al*., 2005). The removal of phosphorus via plant uptake can also decrease over time in wetlands that have phosphorus loading (Rozema *et al*., 2016).

Internal loading processes vary among different aquatic ecosystems but can be insignificant or in some cases, add more nutrients to the water than external phosphorus inputs. Although the release of phosphorus from sediments is a general process that happens in natural environments, most of the reported studies on internal loading are laboratory experiments that were performed under controlled conditions (Mayer *et al.*, 2005; Orihel *et al*., 2017). Therefore, internal phosphorus loading could have occurred in the engineered wetlands used for this thesis, which would explain why the amount of phosphorus added to the system was much smaller than the values obtained from the water samples throughout the experiment. This could be due to the fact that the wetland systems did not have strong enough water circulation from the table fan, creating anoxic conditions for phosphorus to be released from the sediment (Rutledge & Chow-Fraser, 2019).

4.1.3.5 Recommendations for Further Study

The high values can also be a result of the vegetation not reducing the phosphorus levels in the water, however there was not enough time allocated to observe the full potential of the macrophytes to successfully remove phosphates via phytoremediation. The model of the engineered wetland could have also impacted the results. In using rectangular troughs, more surface area of water was exposed to overhanging light banks, increasing the evaporation rates of the system. In a previous study performed in our lab these circumstances increased the concentration of nutrients in the troughs in one day, skewing the results (Fernandes, 2017). Although all water samples were stored under the appropriate conditions, another error may have been the fact that they were all analyzed on the same day at the end of the experiment. A future recommendation would be to analyze the samples the day of extraction or soon after. Another recommendation would be the inclusion of a water circulation system, such as submersible pumps, to encourage water flow and prevent anoxic conditions to improve nutrient removal rates. Lastly, it was clear that the two-week experimental period was too short to be able to reduce the very high values that were obtained. Thus, more time is required, and future studies should be prolonged.

4.2 PART 2: *IN SITU* WETLAND

It is important to reiterate that this component of the study was developed to be an overview and not an extensive ecological study.

4.2.1 Plant Identification

All of the plant species that were identified in the 1 m^2 quadrat regions, shoreline of wetland, or in the overall experimental site are listed in Table 18.

Table 18: List of Identified Plant Species from Palgrave Forest and Wildlife Area

Common Name	Scientific Name
• Common Milkweed	• *Asclepias syriaca*
• Aster ^	• *Aster*
• Canada Thistle	• *Cirsium arvense*
• Spotted Joe-Pye Weed	• *Eutrochium maculatum*
• Common Duckweed*^	• *Lemna*
• Cardinal Flower*	• *Lobelia cardinalis*
• White Water Lily*	• *Nymphaea alba*
• Reed Canary Grass	• *Phalaris arundinacea*
• Interior Sandbar Willow*	• *Salix interior*
• Canadian Goldenrod	• *Solidago canadensis*
• White Meadowsweet	• *Spiraea alba*
• Red Clover	• *Trifolium pratense*
• Cattail*^	• *Typha*
• Blue Vervain	• *Verbena hastata*
• Tufted Vetch	• *Vicia cracca*

(Faulkner & Byers, 2019; Muma, 2021a; Native Plant Trust, 2021a).

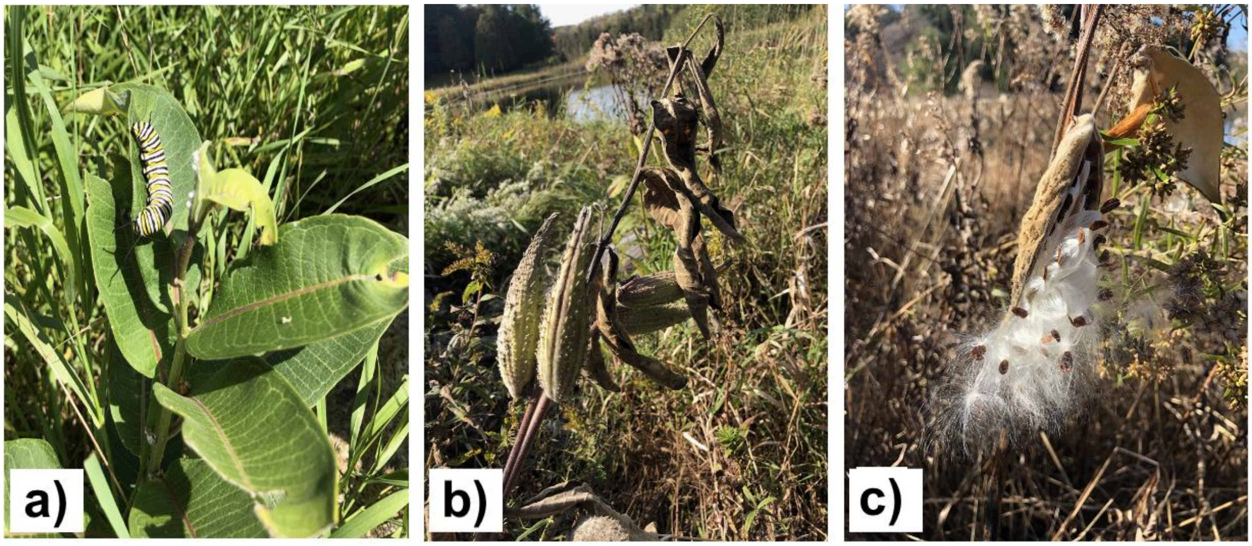
Plants indicated with a * were identified outside of the 1 m^2 quadrat zone.
Plants indicated with a "^" could not be identified to the species level.

The experimental wetland site at Palgrave Forest and Wildlife Area was highly diverse. Of the 15 identified species, 10 are native to Ontario. In addition to the species outlined in Table 18, many plants were not identified because they either were not in close proximity to the transect line, not within the quadrat areas, were rarely observed, or just unidentifiable. A few of the identified species found around the wetland overlap with Environment Canada's list of macrophytes suitable for engineered wetlands and/or wetland restoration in Table 2. The overlapping species included: common milkweed (*A. syriaca*), common duckweed (*Lemna sp.*), white water lily (*N. alba*) and cattail (*Typha*) (Environment Canada, 1996).

It is important to note that there was still high diversity, even in the presence of non-native and/or invasive plant species. This was surprising considering that *Typha*, *C. arvense (*Canada thistle), *P. arundinacea* (reed canary grass), *T. pratense* (red clover) and *V. cracca* (tufted vetch) are all either exotic, display invasive characteristics or both. Although all of these species have the potential to outcompete other species, they are all valuable to the overall community and contribute to the biodiverse population of the wetland as long as their populations are kept in check. *Typha* species are aggressive invaders of wetlands as they can rapidly grow and reproduce, forming dense monotypic populations. Under certain conditions, these traits cause *Typha* to outcompete other native plant species, reduce diversity and have overall negative impacts on the ecosystem (Haven *et al*., 1999; Coleman *et al.*, 2001; USDA, 2006a; USDA, 2006c; Trebitz & Taylor, 2007; Tiley, 2013; Ijaz *et al*., 2016). Despite this, the cattails did not appear to be overly dominating as the biodiversity of the wetland was maintained. This could be due to the fact that *Typha* are important indicators of ecosystem health since they only display invasiveness in anthropogenic, degraded or disturbed habitats. Their inadequate dominance may indicate that the Palgrave Forest and Wildlife Area is a fairly healthy ecosystem (USDA, 2006a; USDA, 2006c, Tiley, 2013). Although it is not clear whether the species observed at Palgrave Forest and Wildlife Area was *T. latifolia* or *T. angustifolia*, it is important to record as both species have the ability to become invasive. Canada thistle is considered one of the most invasive plants in the world and has become a serious invader of Canada, including Ontario. Despite its name, it is actually indigenous to Eurasia, where even there it is classified as one of the most detrimental agricultural weeds. It is harmful in places like Ontario because it outcompetes native plant species and degrades the quality of rangeland which results in crop-yield loss of canola, barley and wheat. Thistle can grow in open, disturbed environments including fields, meadows and roadsides (Ang *et al*., 1995; Tiley, 2010; Guggisberg, 2012; Nature Conservancy Canada, 2020). The tufted vetch is another invasive plant species that has infested North America but is native to parts of Europe and Asia. Throughout most of Canada, including Ontario, concerns about the negative impacts tufted vetch has on the environment have increased since it has been classified as exotic and invasive, as well as a weed by Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) (Seefeldt *et al*., 2007; Gibson *et al.*, 2019). It can be found in low soil fertility and disturbance areas such as roadsides, meadows and arable fields (Nordstrom, 1980; Seefeldt *et al.*, 2007). A study conducted at Boyd Conservation Area in Ontario concluded that tufted vetch was among the four most dominant plant species across various sample sites (Nordstrom, 1980).

The last invasive species that was observed throughout Palgrave Forest and Wildlife Area is reed canary grass. Both native (*Phalaris arundinacea*) and invasive (*Phalaris arundinacea* subsp.) reed canary grass species have been identified across Ontario, especially southern

Ontario. Although *Phalaris arundinacea* is native to North America, the invasive subspecies, native to Eurasia, is proliferating, aggressively taking over sensitive habitats and excluding native species by establishing dense colonies that compete for space and nutrients (Environment Canada, 1996; Houlahan & Findlay, 2004; Lavergne & Molosky, 2004; Trebitz & Taylor, 2007; Anderson, 2012; Faulkner & Byers, 2019). Reed canary grass is most prevalent in wet soil habitats including wetlands, lake shores, riverbanks and wet meadows. Due to its wide tolerance and ability to survive temporary droughts and frost, it can also be found in dry areas (Lavergne & Molofsky, 2004; Anderson, 2012; Faulkner & Byers, 2019). Unfortunately, the only reliable identification method to distinguish the two species is genetic analysis (Anderson, 2012). As a result, it is uncertain which species was observed during the *in-situ* wetland experiments. The *Typha*, Canada thistle, reed canary grass and tufted vetch observations that were inputted into iNaturalist were added by Invasive Species in Ontario, and the interior sandbar willow observation was added by Ontario Willows.



<u>Figure 15: Common Milkweed (*Asclepias syriaca*)</u>

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

Common milkweed (*Asclepias syriaca*) is a native perennial herb that occurs along the banks of lakes, ponds and waterways. It has broad leaf blades, small flowers that bloom from May to August and spindle-shaped fruits that are coated with soft hairs. Many milkweed species are poisonous to humans and other organisms except monarch butterflies which use the poisonous cardiac glycoside as a chemical defence (USDA, 2006b).

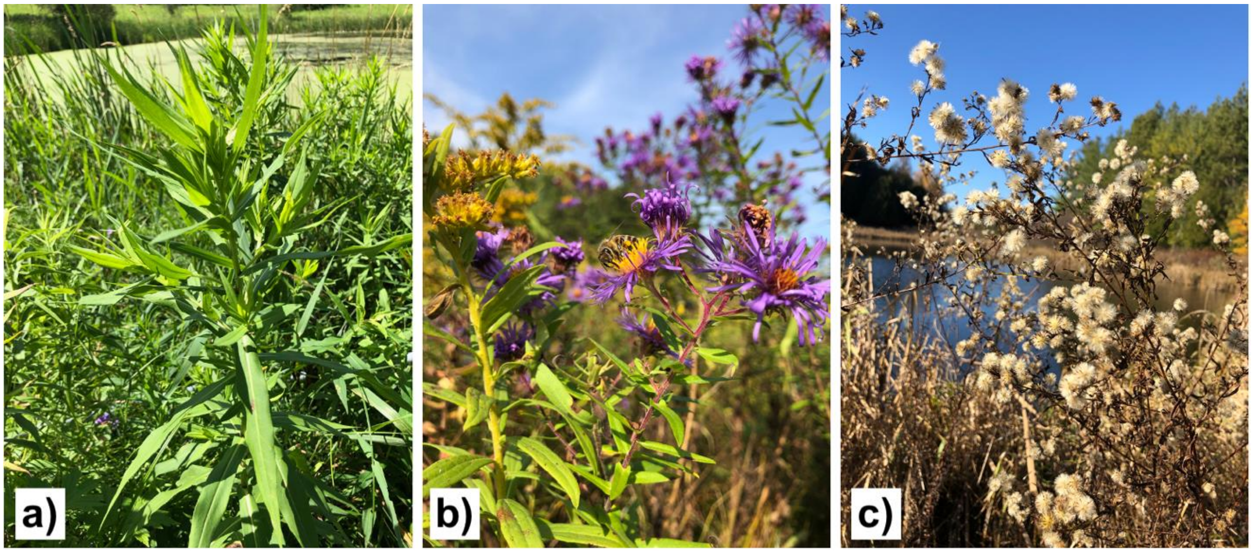


Figure 16: Aster (*Aster sp.*)

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

The aster plants could only be identified to the Genus level (*Aster sp.*). Most members of the aster family (Asteraceae) are considered annual or perennial herbs that grow in open areas and fields containing full sun and can usually occur in wetland areas. They have large flower heads consisting of numerous rays that bloom during summer and autumn and can extend to a height of 150 cm (Muma, 2021b; Native Plant Trust, 2021c).

Figure 17: Spotted Joe-Pye Weed (*Eutrochium maculatum*)

a) August 5th b) October 6th (Photos by Joanna Tucci)

The spotted joe-pye weed (*Eutrochium maculatum*) is another native species that thrives in wet areas with full sun such as marshes, swamps, meadows, fields, and the edges of rivers, lakes and wetlands. It has the widest geographical distribution genus, can grow as tall as 150 cm and has disk flowers that bloom during the growing season (Muma, 2021d; Native Plant Trust, 2021b).

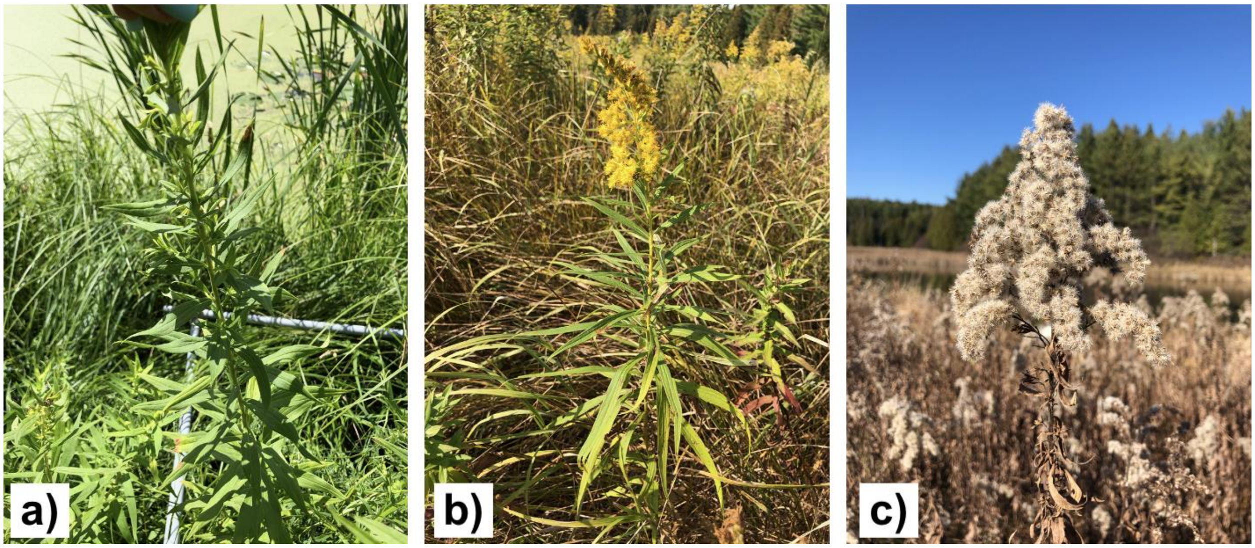


Figure 18: Canadian Goldenrod (*Solidago canadensis*)

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

The Canadian goldenrod (*Solidago canadensis*) was found to be the most predominant species at the experimental site. It is native to North America and is widely spread across Ontario. It is a herbaceous long-lived perennial that often forms dense patches with aboveground biomass reaching up to 2 m in height (Huang *et al*., 2007; Pavek, 2011). *S. canadensis* has adapted to growing in areas of full sun or part shade and can tolerate all soil types but prefers moist soil. Due to this it can establish in various habitats such as damp meadows, waterways and ditches, and may also occupy prairies and deciduous forests. Despite its wide range, S. canadensis is rarely found in waterlogged or extremely dry areas (Huang *et al*., 2007; Sanderson *et al*., Pavek, 2011; USDA, 2012).

<u>Figure 19: White Meadowsweet (*Spiraea alba*) on August 5th</u>

(Photo by Joanna Tucci)

White meadowsweet (*Spiraea alba*) is a native shrub that grows best in swamps, bogs, wet meadows and stream banks. It can grow up to 2 m in height and flowers between June to September (Faulkner & Byers, 2019).

Figure 20: Blue Vervain (*Verbena hastata*) on August 5th

(Photos by Joanna Tucci)

Blue vervain (*Verbena hastata*) is a native perennial wildflower that grows best in moist conditions with full or partial sun such as moist meadows, riversides and marshes. Its hairy stems can grow to 1.5 m with opposite leaves and flowers that bloom from June to September (USDA, 2011; Faulkner & Byers, 2019).

Figure 21: White Water Lily (*Nymphaea alba*) & Cattail (*Typha sp.*)

(Photo by Joanna Tucci)

The white-water lily (*Nymphaea alba*) is a native plant typically found in still or slow-moving waters such as ponds, lakes and wetlands with full sun. Although this species is mainly identified by its leaves and flowers that float on the surface of the water, its shoot system is buried in the sediment (Villani & Etnier, 2008). The cattail (*Typha*) is an emergent, herbaceous, rhizomatous perennial that can obtain very high biomass and grow up to 3 m in height. They are commonly found in tropical and temperate regions worldwide where they grow in or near water in wetland marshes, coastal areas, ponds and lakes (USDA, 2006a; USDA, 2006c; Tiley, 2013).

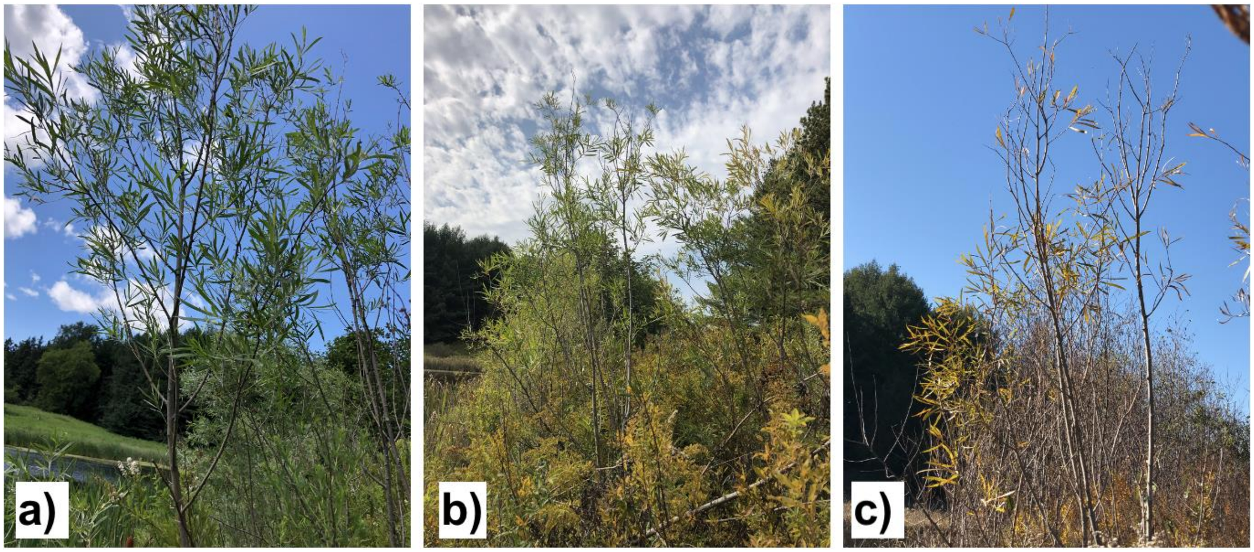


Figure 22: Interior Sandbar Willow (*Salix interior*)

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

The interior sandbar willow (*Salix interior*) was one of the species identified external of the quadrats but was noticed as it was one of the only woody plants in the immediate area. It is a native shrub that is distributed along streams, rivers and shoreline sites that experience frequent flooding. It can grow up to 6 m in height and is commonly used for erosion control, riparian area development and restoration. This species can aggressively spread to other sites and displace other species, causing it to become weedy or invasive in certain habitats (USDA, 2002b).

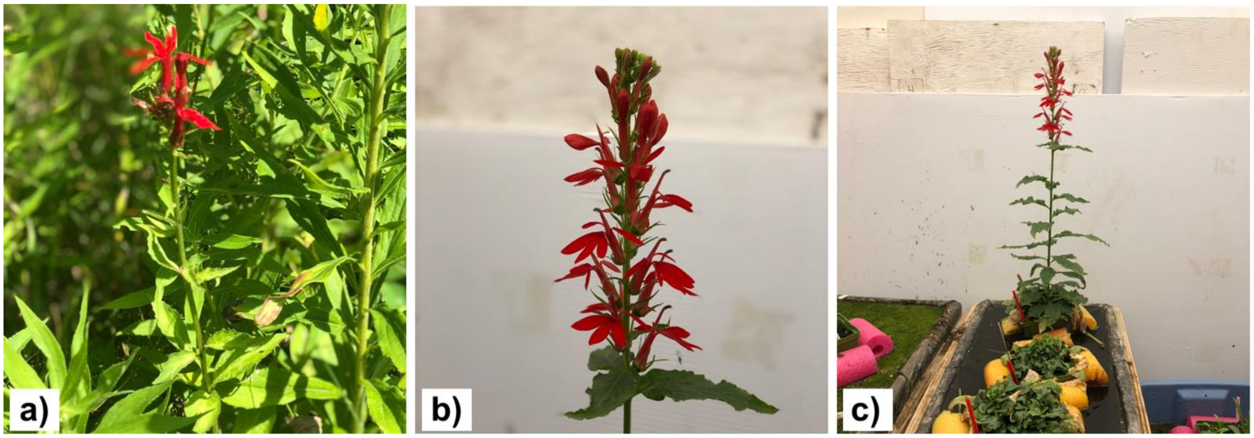


Figure 23: Cardinal Flower (*Lobelia cardinalis*)

a) Palgrave Forest and Wildlife Area on August 5th, 2020 b) and c) in-lab floating engineered wetland on December 18th, 2019 (Photos by Joanna Tucci)

Although the cardinal flower (*Lobelia cardinalis*) was not observed within any of the quadrat regions and only one plant was seen in one area of the wetland during the initial field trip, it was a considerably significant observation since it was one of the species that was not only purchased, but used, during the engineered wetland experiment.

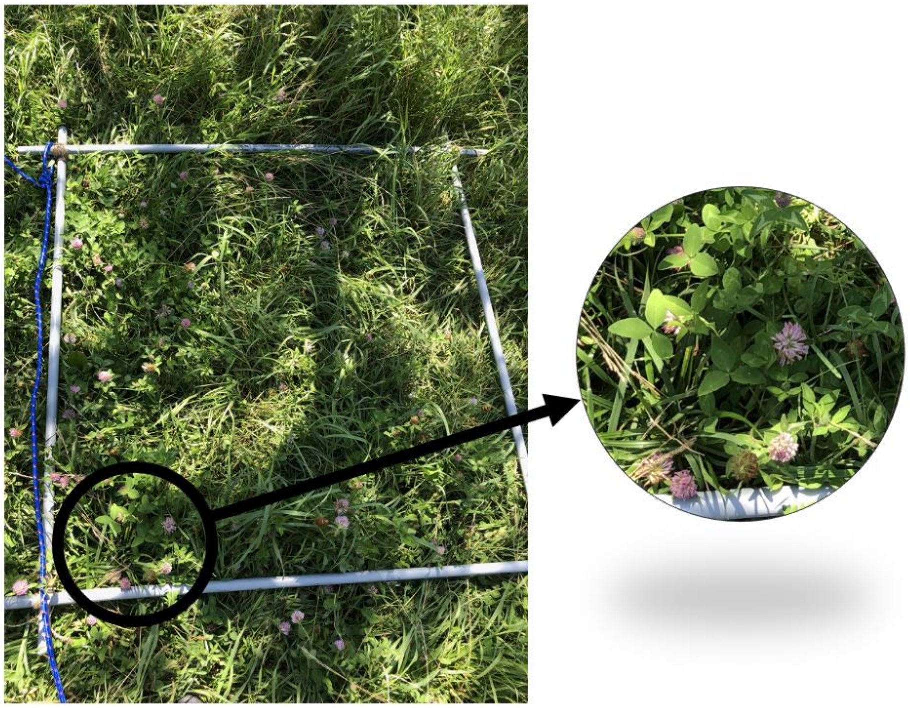


Figure 24: Red Clover (*Trifolium pratense*) on August 5th

(Photos by Joanna Tucci)

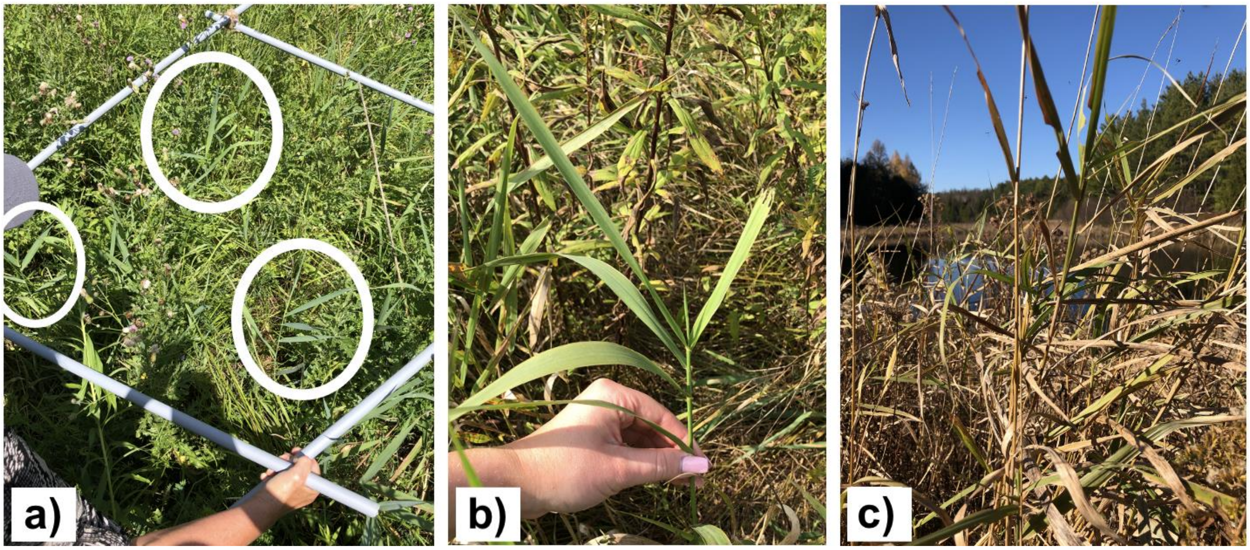
Red clover (*Trifolium pratense*) is an introduced short-lived perennial, native to Asia and Europe that can be considered weedy in some areas. It prefers well-drained and fine textured soils with sufficient moisture (USDA, 2008).

Figure 25: Canada Thistle (*Cirsium arvense*)

a) August 5th b) October 6th

(Photos by Joanna Tucci)

The Canada thistle (*Cirsium arvense*) was one of the exotic species observed at Palgrave Forest and Wildlife Area as it is native to Eurasia. It is one of the most invasive plant species in the world and is even considered as a detrimental weed in areas where it is native. *Cirsium arvense* can be found in fields, meadows and roadsides (Ang *et al*., 1995; Tiley, 2010; Guggisberg, 2012; Nature Conservancy Canada, 2020).



<u>Figure 26: Reed Canary Grass (*Phalaris arundinacea*)</u>

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

Reed canary grass (*Phalaris arundinacea*) is a long-lived perennial sod-forming grass with species native to North America, Europe and Asia. There are both native and non-native reed canary grass species *Phalaris arundinacea* located in Ontario. Although the native species does not cause harm on the environment, the non-native subspecies often dominates wetlands, lake shores, riverbanks and wet meadows where it outcompetes native species (Houlahan & Findlay, 2004; Lavergne & Molosky, 2004; Trebitz & Taylor, 2007; Anderson, 2012; Faulkner & Byers, 2019).

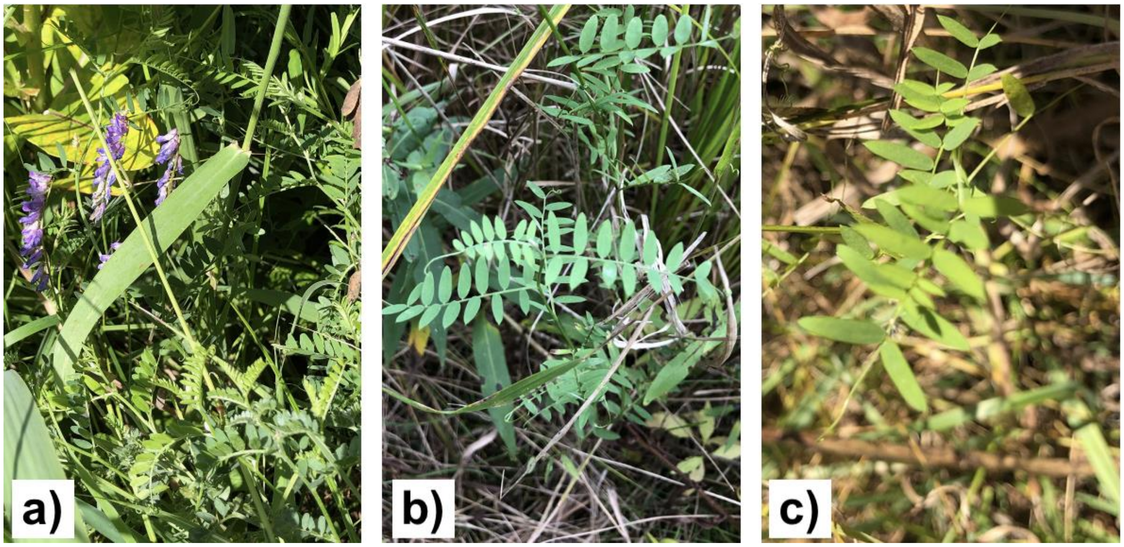


Figure 27: Tufted Vetch (*Vicia cracca*)

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

Tufted vetch (*Vicia cracca*) is another non-native invasive plant species that is native to parts of Europe and Asia and has dominated habitats across Canada where it has caused negative effects on the environment. It typically grows in roadsides, meadows and arable fields (Nordstrom, 1980; Seefeldt *et al*., 2007; Gibson *et al*., 2019).

4.2.2 Seasonal Observations

Between the months of August and November, seasonal changes of the wetland community and its vegetation were also observed.

4.2.2.1 Common Duckweed (*Lemna sp*.)

The first observation was the presence of common duckweed or *Lemna sp*. in the water. During the first field trip on August 5, a great amount of the wetland surface was covered in common duckweed along the shoreline in the littoral zone. There was a significant decrease in the presence of duckweed during the subsequent field trips on October 6 and November 4 as the biomass around the littoral zone of the wetland had reduced to a small portion on the east end of the wetland (Figure 28).

Figure 28: Presence of Common Duckweed (*Lemna sp.*)

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

Duckweeds are small free-floating aquatic plants that can be found of the surface of still or slow-moving bodies of fresh or brackish waters and have a wide geographic range as various species have been found everywhere around the world, excluding the desert and tundra (Vymazal, 2008; Asolekar *et al*., 2014; Verma & Suthar, 2015; Faulkner & Byers, 2019). Duckweed growth is temperature dependent with optimum growth rates between 20-30 °C and serious effects between 35-40 °C. For most duckweed species, growth rates tend to decrease at temperatures below 17 °C, however some species are capable of growing at temperatures as low as 1-3 °C (Vymazal, 2008; Asolekar *et al*., 2014). Under optimal conditions, duckweeds rapidly grow and reproduce as their biomass can double within 2-3 days (Vymazal, 2008; Asolekar *et* *al*., 2014; Verma & Suthar, 2015; Zeshan *et al*., 2016; Faulkner & Byres, 2019). These findings correspond to the observations in this thesis as the duckweed biomass was highest in summer months when temperatures are high, and significantly decreased under colder temperatures.

In addition to temperature, nutrient availability also controls the growth of water plants such as duckweed by causing cycles of senescence and regeneration. Since nutrients are often limiting in freshwater environments, an increase in nutrient concentration causes duckweed to rapidly grow (Leng, 1999; Zeshan *et al*., 2016).

Lemna also have phytoremediation potential. Due to their rapid growth, high reproduction rates and nutrient removal capacity, there is recent interest in duckweed phytoremediation strategies (Leng, 1999; Van der Spiegel *et al*., 2013; Zeshan *et al*., 2016). Studies have found that they are able to absorb mineral nutrients, heavy metals, phenols, pesticides, dioxins and pathogens in their plant tissues via direct uptake from wastewater and/or associated microbes (Leng, 1999; Picard *et al*., 2005; Vymazal, 2008; Van der Spiegel *et al*., 2013; Zeshan *et al*., 2016). However, it is important to keep the population limited during water purification because extensive amounts of duckweed can be detrimental to the system as it can completely cover the surface of the water, blocking sunlight to lower depths, thus, contributing to eutrophication by creating hypoxic environments (Verma & Suthar, 2015). Therefore, duckweed can be an important component of any engineered or constructed wetland used for wastewater treatment.

4.2.2.2 Water lilies (*Nymphaea alba*)

Another observation was the presence of water lilies along the north shoreline of the wetland between quadrat 2 and 3 (Figure 21). The water lilies slightly decreased in biomass over time but were present in that specific area during all field trips.

4.2.2.3 Vegetation and Senescence

There was also a major change in the wetland vegetation over time (Figure 29). In temperate regions, wetland communities get altered as perennials respond to environmental changes, where growth occurs during the warm summer months and senescence occurs in the winter (Kröger *et al*., 2007; Vymazal, 2007).

4.2.2.3.1 Summer

During the first field trip on August 5th, the wetland ecosystem was thriving with macrophyte diversity and biomass. The wetland was surrounded with emergent and terrestrial vegetation from grasses to shrubs and trees. Only a few of the identified species were flowering during this trip such as: red clover, blue vervain, meadowsweet, tufted vetch and spotted Joe-Pye weed. The north and east shorelines of the wetland were mainly occupied by *Typha* whereas the south shoreline comprised mainly of reed canary grass. The Canadian golden rod species was considered to be the most dominant species as it was present in almost every quadrat region.

4.2.2.3.2 Early Autumn

By October 6th there were already a few differences in the wetland community as the temperatures began to decrease, the wetland macrophytes were responding to the environmental changes and preparing for fall senescence. Firstly, Canadian goldenrod and aster plants, which were not flowering previously, had started to bloom. Both of these plants bloom in later summer and fall, which can vary among different regions due to variations in altitude and latitude (Huang, 2007; Pavek, 2011; Muma, 2021b). The Canadian golden rod was still observed to be the most dominant identified species as it was present in every quadrat region except for Q1. This particular species was extremely prevalent on the south end of the wetland in quadrats 7-9. While some species were still flowering and thriving, some of the wetland vegetation was starting to deteriorate. Most of the grasses were starting to brown, leaves were starting to change colour and certain plants were starting to die and disperse their seeds.

4.2.2.3.3 Late Autumn

On November 4th the entire wetland community had shifted into fall senescence and most of the macrophytes were dead. Despite the fact that almost all of the leaves on the trees had fallen, their fruits were still present. The Canadian golden rod was still the most widespread species, dominating the south end of the wetland. Overall, the biomass had significantly decreased compared to the first field trip in August.

Senescence is the final stage of a plants developmental process, also known as plant aging, decomposition or growth arrest. The observations of senescence in the wetland were expected as this process is triggered by various internal and external signals and environmental cues such as changing seasons. It has been regarded as an evolutionary strategy for survival and adaptation, providing the plant with optimal fitness during unfavourable environmental conditions, such as decreased temperatures and sun exposure in fall and winter (Kröger *et al*., 2007; Lim *et al*., 2007; Thomas, 2012; Woo *et al*., 2018). During this period, the overall plant biomass decreases as various species begin to lose leaves, wilt and brown before entering winter dormancy (Lim *et al*., 2007; Vymazal, 2007).

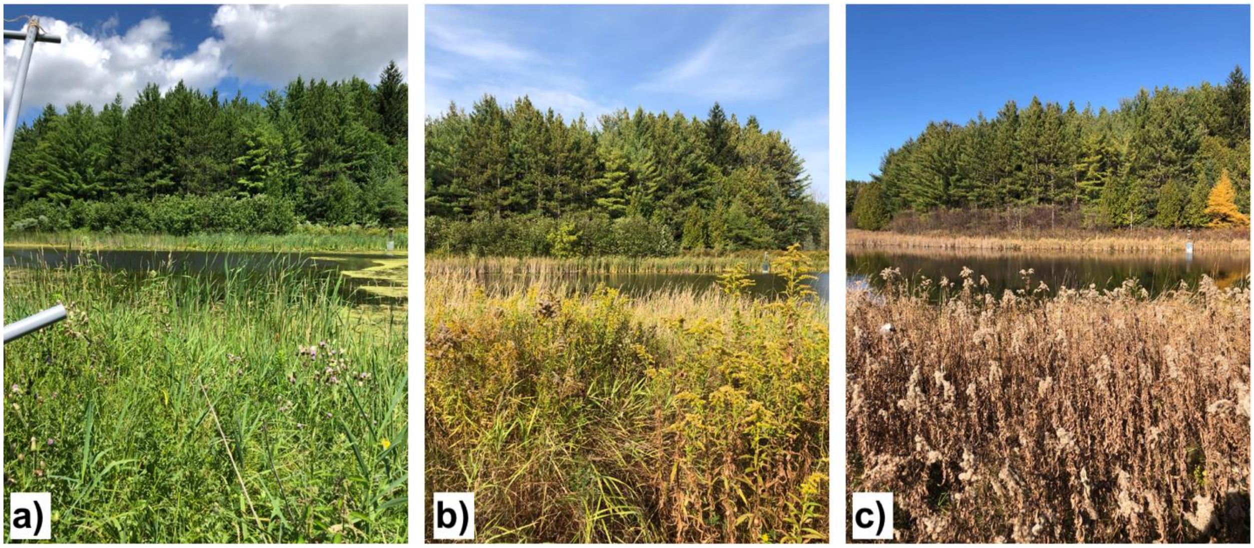


Figure 29: Senescence of Wetland Community

Photos of sample site over time from the same location.

a) August 5th b) October 6th c) November 4th (Photos by Joanna Tucci)

4.2.2.4 Presence of Species

The presence and diversity of the various identified species around the wetland area is outlined in Table 19 and Figure 30.

Table 19: List of Identified Species Present in each Quadrat

Date	**Aug 5**	**Oct 6**	**Nov 4**
Species	**Quadrat #**		
Common Milkweed	4, 5, 7	4, 6, 7, 8	4, 6, 7
Aster	2, 3, 4, 7	2, 3, 4, 5, 7, 8	2, 5
Canada Thistle	8	8, 9	
Spotted Joe-Pye Weed	6	8	
Reed Canary Grass	2, 3, 4, 5, 6, 8, 9	2, 3, 4, 5, 6, 9	2, 3, 4, 5, 6, 9
Canadian Goldenrod	2, 3, 4, 5, 6, 7, 8, 9	2, 3, 4, 5, 6, 7, 8, 9	2, 3, 4, 6, 7, 8, 9
White Meadowsweet	3		
Red Clover	1	1	1
Blue Vervain	2		
Tufted Vetch	2, 4, 5, 6, 8	2, 4, 6, 7, 8, 9	2, 3,

As outlined in Table 19, the wetland within Palgrave Forest and Wildlife Area had high species diversity and richness. Most of the quadrat zones consisted of various species, which were also dispersed in numerous areas along the transect line. Environmental factors such as sun exposure, slope and drainage could play a role in the high local diversity and spatial heterogeneity of different species.

4.2.2.4.1 Canadian Goldenrod

It was clear that the Canadian Goldenrod was the most dominant species present at Palgrave Forest and Wildlife Area as it was found in all of the quadrat regions, except Q1 (Table 19). It was also evidently dominant at the south end of the wetland as it created a "sea of yellow" during the October 6th field trip due to its florescence. Canadian goldenrod species *Solidago canadensis* is native to North America and has a wide range which includes much of Ontario (Huang *et al.*, 2007; Pavek, 2011; Sanderson *et al.*, 2015).

Despite being native, this wetland species can be considered "weedy" due to its ability to easily spread and rapidly colonize but are rarely regarded as problematic within its native range (Huang *et al.*, 2007; Pavek, 2011; USDA, 2012; TRCA, 2020a). However, it is one of the most extensive invasive plant species throughout Europe and east Asia due to its allelopathic characteristics, high densities and tall height (Pavek, 2011; Dudek *et al.*, 2016). These characteristics may explain the overall dominant presence and of high-density clusters of this species around the wetland area at Palgrave Forest and Wildlife Area.

4.2.2.4.2 Other Species

Both blue vervain (*V. hastata*) and white meadowsweet (*S. alba*) were the least common as they were each only observed in one quadrat area on one occasion (Table 19). The red clover (*T. pratense*) was also only observed in Q1 but was present throughout all field trips. It is possible that this species was present in other quadrats around the wetland site but was unnoticed amongst the larger plants. The Spotted Joe-Pye weed (*E. maculatum*) was also very rare. It was only found in a couple of quadrat regions throughout the seasonal assessment, and when it was observed there were only 1-2 species present. Although the Canada thistle (*C. arvense*) was not greatly dispersed throughout the wetland area, it was clearly evident throughout the south side of the wetland, especially during the summer season when growth was optimal. As for the common milkweed (*A. syriaca*), aster (*Aster sp.*), reed canary grass (*P. arundinacea*) and tufted vetch (*V. cracca*); all of these species were well distributed around the wetland as they were observed in many of the quadrat areas. Despite the fact that they were widespread, the Canadian goldenrod (*S. canadensis*) remained to be the most dominant as it was identified in the most quadrats and had the highest richness within those quadrats.

Figure 30: Presence of Species Around the Wetland Sample Site (Google Maps, n.d.)

5.0 CONCLUSIONS AND RECOMMENDATIONS

This thesis attempted to fill gaps in the knowledge and build on previous reports regarding the incorporation of native non-invasive plant species in engineered wetlands, acting as a steppingstone for future related studies. First, 9 native non-invasive plant species were acquired and grown in the Ryerson lab (KHN 302). Of the species purchased, 4 (sneezeweed, blue flag iris, prairie cordgrass and blue vervain) were used in Kao et al (2009) study on constructed wetlands for wastewater treatment and nitrogen removal, adding value to the species used in this thesis. Following this, the species that thrived were used for the development of in-lab floating engineered wetlands and phosphorus analysis. Subsequently, the overall changes in aboveground biomass were documented by measuring plant height and lifecycle progression which were used to assess any effects the phosphorus and controlled laboratory conditions had on plant production. During the second part of this thesis, *in situ* field work was conducted which included observing the senescence cycle of a diverse community, examining the presence, distribution and identification of plant species, as well as determining which plants could possibly be used to further develop lab-based engineered wetlands.

It was found that about half of the species purchased for the study grew well in a laboratory setting. This included the giant ironweed (*V. gigantean*), sneezeweed (*H. autumnale*), blue flag iris (*I. versicolor*) and cardinal flower (*L. cardinalis*). Despite the fact that the ironweed thrived in the lab, it could not be used for the experiments as all plants of this species were infected with a fungal disease. Thus, the sneezeweed, blue flag iris and cardinal flower were used to create mixed culture mesocosms. During the experiments, the control and mixed culture mesocosms were exposed to three different phosphorus treatments (0, 100, 1000 μgPO$_4$-P/L) during a two-week period. The overall concentrations were initially high and remained high throughout the experiment, with the highest absorbance value reaching 2.7713, failing to demonstrate significant nutrient removal rates via plant uptake. Plants continued to grow well under the laboratory conditions, even with the phosphorus inputs, as they experienced increases in height and number of flowers. During the *in situ* field work it was found that the wetland was extremely biodiverse as a total of 15 species were identified, 4 of which (Canadian goldenrod, aster species, milkweed species and duckweed species) are suggested for future engineered wetland studies in combination with the species used in this study. The Canadian goldenrod was the most dominant species at the Palgrave Forest and Wildlife Area as it was found in all quadrat regions except quadrat one. The use of iNaturalist contributed additional knowledge such as providing species clarifications and status with regards to invasiveness in Ontario. Seasonal changes were observed throughout the wetland such as significant biomass decreases as temperatures began to drop, entertaining the idea of manipulating this type of system in a laboratory setting.

5.1Future Directions and Recommendations

The research that took place during this thesis is very preliminary but again, provides a steppingstone for future studies and can be used in on-going research pertaining to native non-invasive wetland species.

5.1.1 Mistakes from this Study

It was evident that more time for these experiments, specifically the in-lab phosphorus analysis, is required and that future studies should exceed two weeks. This research lacks the experimental design to include submersible pumps for increased water circulation to prevent anoxic conditions which cause internal nutrient loading and may reduce plant survival due to lack of oxygen. Likewise, future studies should consider the nutrient contents of their substrate to further avoid the potential for internal nutrient loading. Increased biomass in each rectangular trough, such as four plants as opposed to three, could also increase overall biomass, stabilize the water-to-biomass ratio and reduce evaporation rates, which skewed results by substantially increasing nutrient concentrations. The inability to successfully grow all of the plants purchased for the Ryerson lab study indicates that additional research and protocols for growing native non-invasive species under laboratory conditions are needed.

5.1.2 Suggested Species

There should be further experimentation on the growth and nutrient uptake of native non-invasive wetland macrophytes for lab-based engineered wetland systems. The “suitable 6”, also known as the sneezeweed (*Helenium autumnale*), blue flag iris (*Iris versicolor*), duckweed *(Lemna sp*.), cardinal flower (*Lobelia cardinalis*), Canadian goldenrod (*Solidago canadensis*) and giant ironweed (*Veronia gigantean*) are the species from this thesis that are highly recommended for future studies. This is because during the engineered wetland experiments, these species (sneezeweed, blue flag iris, cardinal flower and giant ironweed) grew very well in the lab-based floating wetlands. During the seasonal field work the Canadian goldenrod was one of the most prevalent plants observed at the Palgrave wetland site that flourished throughout the seasons with a considerable amount of biomass. Although it can be considered weedy, research suggests that it does not possess any other invasive characteristics, thus, further investigating this species is worthwhile as it could do well under laboratory conditions and have the potential to provide enough biomass for nutrient uptake and act as a phosphorus sponge. Duckweed can also be an important component of engineered wetlands due to their phytoremediation potential and ability to easily be grown in the environment or under lab conditions. Experiments including both polycultures and monocultures should also be examined to determine how well these species can be grown together as a diverse community to support the theory that increased species richness and diversity increases nutrient removal rates, while also determining how much phosphorus each of these selected species takes up individually.

Other notable plants include the aster and milkweed species. The aster was also observed around the wetland, had high richness and is native. However, it could not be identified to the species level, therefore a specific species cannot be recommended. Additionally, the common milkweed (*Asclepias syriaca*) was observed at the Palgrave wetland, while swamp milkweed (*Asclepias incarnata*) was used in previous studies, including this thesis. Fernandes (2017) had successfully germinated swamp milkweed in the lab, and although I was not as successful growing it, milkweed species can also be a suggestion for future related studies.

5.1.3 Additional Field Work

It is also recommended that the presence and distribution of wetland plant species in the environment be further investigated in other areas around southern Ontario, such as the Greater Toronto Area (GTA), to determine the natural dispersal of native non-invasive species. Such studies can either follow the structure of this thesis and visit only one site over an extended period of time or can visit multiple wetland sites around the GTA for a comparative study.

Proposed locations include: Rouge National Urban Park, Kortright Centre for Conservation, Coote’s Paradise, High Park and/or Rattray Marsh Conservation Area.

5.1.4 Modifications to KHN 302

There are various modifications to the KHN 302 laboratory that are recommended for future studies. The intention of developing lab-based engineered wetlands is to eventually implement what was achieved in the lab into the environment. The incorporation of a flow through system in the troughs to mimic an inflow would improve water circulation and prevent internal loading. To prevent fungal spores from spreading throughout the confined space and infecting species, a dehumidifier or stronger air circulation system is also suggested. Additionally, during the *in situ* field work the seasonality of the wetland was obvious in the environment, but was not represented in the lab. Thus, allowing the flow through system to mimic fluctuations in water level throughout the seasons (high water in spring from snowmelt/rain, drying in late summer etc.) as well as using the lights to alter temperatures and the inclination of the sun could thoroughly simulate seasonality within the lab. This seasonality could not only accommodate and enhance plant growth but can also be used to examine phosphorus removal by selected species over different seasons. Although it may be difficult to build in the lab of KHN 302, it is highly recommended that it is attempted.

6.0 EPILOGUE

Over the last two and a half years, a great deal of research and work has been put into this thesis to attempt to prove the use of native non-invasive plant species for constructed wetland wastewater treatment. It is known that the main cause of eutrophication is excessive inputs of phosphorus from non-point sources, and while farmers are trying their best to contain this phosphorus, it is important that constructed wetland barriers be implemented along coastlines as a safeguard. *Typha sp*. have proven to be impressive water quality improvers and phosphorus sponges as many studies have demonstrated their ability to treat wastewater. This thesis could have easily incorporated *Typha* into the engineered wetlands, which likely would have resulted in higher phosphorus sequestration via plant uptake, providing more acceptable results. However, previous research by Tiley (2013), as well as personal moral standards, enabled me to recognize that it is morally wrong to consider the use of *Typha* monocultures for open constructed wetland systems in the environment and that it is of value to investigate appropriate alternatives.

As a consequence of the preliminary results gathered from this thesis, the question then becomes, does the end justify the means? Should *Typha sp.*, which clearly display invasive characteristics, continue to be used in constructed wetlands developed along the edges of Lake Erie for wastewater treatment?

Many freshwater ecosystems, such as Lake Erie, are dying due to eutrophication, thus projects pursuing the implementation of environmentally sustainable constructed wetlands around such areas are crucial. It is understandable to use *Typha* from an engineering perspective relating to costs, effectiveness and convenience, however their ability to become highly invasive and negatively impact the entire ecosystem does not satisfy the demands of long-term sustainability. Native non-invasive plant species on the other hand would not pose any threats to the environment and will actually enhance native biodiversity. The strongest suggestion from this thesis would be to incorporate the “suitable 6” (sneezeweed, blue flag iris, cardinal flower, common duckweed, Canadian goldenrod and giant ironweed) in constructed wetlands intended to prevent eutrophication in freshwaters. Throughout this thesis, these native non-invasive species displayed promising potential due to either their successful growth in the lab or high richness in the environment. Since *Typha* are not inherently invasive and are still considered an essential species that provide environmental services, it may be justifiable to incorporate *Typha* with the “suitable 6”. As observed at the *in situ* wetland, the presence of *Typha* did not seem to hinder the growth or presence of any other species and had actually contributed to the diversity of the system. Thus, if *Typha* dominance/competitiveness can be kept under control by the surrounding native biodiversity, it would be interesting to see how well constructed wetlands (in the lab or in the environment) containing a high abundance of diverse native species and a handful of *Typha* can remove nutrients and prevent eutrophication. Overall, reducing the number of invasive species in constructed wetlands and incorporating more native non-invasive species would be a major step in the right direction.

7.0 APPENDICES

Appendix I: 1M HCl Preparation for In-Lab Engineered Wetland Phosphorus Analysis

Water samples from each trough were collected every other day in 50 ml centrifuge tubes over a 14-day period. Following each collection, a drop of 1M HCl was added to each sample for storage purposes until they could be further examined via the ascorbic acid method. To prepare 1M HCl, the 12.1M HCl available in the lab needed to be diluted.

Table 1: Materials for 1M HCl Preparation

• 10 ml graduated cylinder	• Glass jar >100 ml

The following equation was used:

$C_1V_1 = C_2V_2$

Where:

C_1 = initial concentration of solution = 12.1 M
V_1 = initial volume of solution = unknown
C_2 = final concentration of solution = 1 M
V_2 = final volume of solution = 1 L

$(12.1\ M)(V_1) = (1\ M)(1\ L)$
$V_1 = \frac{1.0}{12.1}$
$V_1 = 0.083\ L$
$V_1 = 83\ ml$

83 ml HCl into 917 ml H_2O = 1 L

Therefore, 8.3 ml of HCl was added to 91.7 ml H_2O = 100 ml

The following solution was then stored in a glass jar greater than 100 ml.

Appendix II: Stock Phosphorus Preparation and Addition Calculations for In-Lab Engineered Wetlands

Various treatments of phosphorus were added to each of the troughs to act as a non-point source phosphorus input. Thus, a stock phosphorus solution was required. To prepare the stock phosphorus solution, 0.4393 g of pre-dried (at 105°C for one hour) anhydrous KH_2PO_4 was dissolved in distilled water and diluted to 1000 ml. Where 1.0 ml = 0.1 mg phosphorus. Thus, the stock phosphorus solution is 100 ppm (APHA, 1998).

Table 2: Materials for Stock Phosphorus Solution Preparation

• Beakers/flasks of appropriate size	• Scoopula
• KH_2PO_4	• Weigh boat
• Scale	•

In order to determine the appropriate amount of prepared stock phosphorus solution added to each trough, the volume of each trough was calculated using the following equation:

$$L = \frac{in^3}{61.024}$$

Once the trough volume was calculated, the following equation was used to determine the amount of phosphorus added to each trough:

$$C_1V_1 = C_2V_2$$

Where:
C_1 = initial concentration of solution = 100 ppm
V_1 = initial volume of solution = unknown
C_2 = final concentration of solution = 1 ppm
V_2 = final volume of solution = volume of trough (varied)

Table 3: Measurements for Trough #1 – Mixed Culture 1000 μg PO_4-P/L

Length	Width	Height	Volume
34 inches	11.5 inches	14.5 inches	5669.6 in^3

L = $\frac{in^3}{61.024}$ = $\frac{5669.5}{61.024}$ = 91.4 L

(100 ppm) (V_1) = (1 ppm) (91.4 L)

= 0.914 L

= **914 ml**

Thus, 914 ml of phosphorus solution was added to trough #1.

Table 4: Measurements for Trough #2 – No Plants 0 μg PO_4-P/L

Length	Width	Height	Volume
34 inches	10.5 inches	14.5 inches	5176.5 in^3

L = 83.47 L

No phosphorus was added to trough #2.

Table 5: Measurements for Trough #3 – No Plants 100 μg PO_4-P/L

Length	Width	Height	Volume
34 inches	10.5 inches	14.5 inches	5176.5 in^3

L = 83.47 L

(100 ppm) (V_1) = (0.1 ppm) (83.47 L)

= 0.0835 L

= **83.5 ml**

Thus, 83.5 ml of phosphorus solution was added to trough #3.

Table 6: Measurements for Trough #4 – Mixed Culture 0 μg PO_4-P/L

Length	Width	Height	Volume

34 inches	11.5 inches	14.5 inches	5669.6 in^3

L = 91.4 L

No phosphorus was added to trough #4.

Table 7: Measurements for Trough #5 – Mixed Culture 100 μg PO_4-P/L

Length	**Width**	**Height**	**Volume**
34 inches	11.5 inches	14.5 inches	5669.6 in^3

L = 91.4 L

(100 ppm) (V_1) = (0.1 ppm) (91.4 L)

= 0.0914 L

= **91.4 ml**

Thus, 91.4 ml of phosphorus solution was added to trough #5.

Table 8: Measurements for Trough #6 – No Plants 1000 μg PO_4-P/L

Length	**Width**	**Height**	**Volume**
34 inches	11.5 inches	14.5 inches	5669.6 in^3

L = 91.4 L

(100 ppm) (V_1) = (1 ppm) (91.4 L)

= 0.914 L

= **914 ml**

Thus, 914 ml of phosphorus solution was added to trough #6.

Appendix III: Stock Solution and Reagent Preparation for Ascorbic Acid Method

The ascorbic acid method, a colorimetric test, was used to determine the amount of reactive phosphorus present in the water samples collected from the mesocosms. The materials and steps for preparing the individual stocks for the ascorbic acid method are outlined below (APHA, 1998; Fernandes, 2017).

Table 9: Materials for Preparing Individual Stocks

• Ammonium molybdate	• Sulfuric acid
• Antimonyl potassium tartate	• 100 mL graduated cylinder
• Ascorbic acid	• 500 mL graduated cylinder
• Distilled water	• 250 mL volumetric flasks
• Scale	• 3 1000 mL volumetric flasks
• Scoopula	• 3 weigh boats

Prior to preparing the mixed reagent (described in section 3.1.5.3.1), the individual stocks needed to be prepared. Table 10 outlines the different stock solutions and how to prepare it. The materials from Table 9 were used to do this.

Table 10: Reagent Preparation for Ascorbic Acid Method (Fernandes, 2017)

Reagents	Preparation
1. Sulfuric acid	70 mL H_2SO_4 in 500 mL d.H_2O
2. Antimonyl potassium tartate	1.3715 g $K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O$ in 400 mL d.H_2O
3. Ammonium molybdate	20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL d.H_2O
4. Ascorbic acid	1.76 g $C_6H_8O_6$ in 100 mL d.H_2O

d.H2O = distilled water

Sulfuric acid: A 500 mL graduated cylinder was used to measure 500 mL of d.H_2O. Half of the d.H_2O was added to a 1000 mL volumetric flask labelled “SA”. In a fume hood, a 100 mL graduated cylinder was used to measure 70 mL of sulfuric acid, which was then slowly added to the volumetric flask. The rest of the d.H_2O was added to the sulfuric acid volumetric flask.

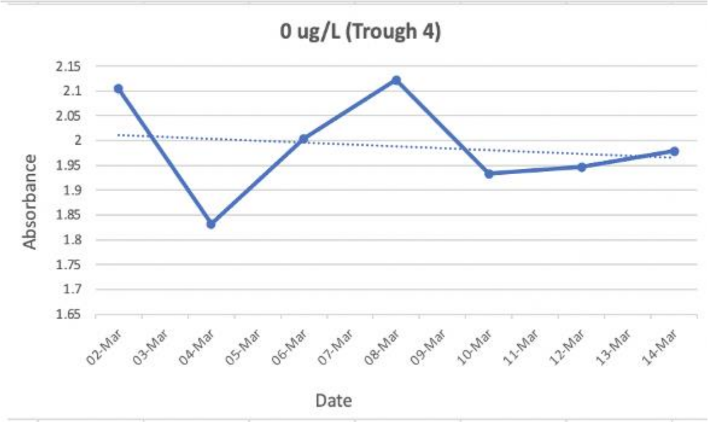
Antimonyl potassium tartate: A weighing boat was placed on a scale and set to 0 g. Using a 500 mL graduated cylinder, 400 mL of d.H_2O was measured and added to a 1000 mL volumetric flask labelled “APT”. Then, 1.3715 g of antimonyl potassium tartate was measured and added to the flask and mixed.

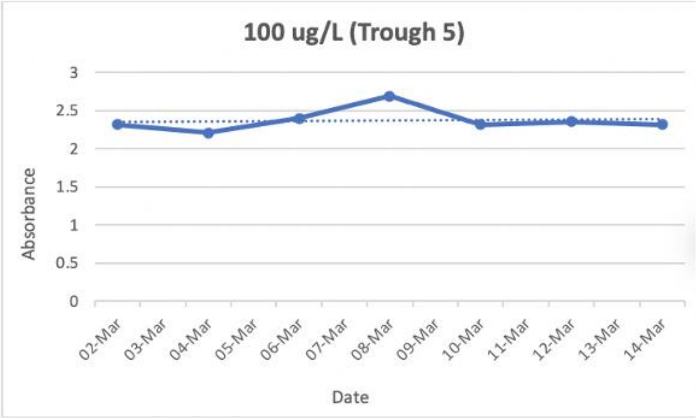
Ammonium molybdate: A new weighing boat was placed on the scale and set to 0 g. A 500 mL graduated cylinder was used to measure 500 mL of d.H_2O, which was added to a 1000 mL volumetric flask labelled “AM”. Then, 20 g of ammonium molybdate was measured and added to the flask and mixed.

Ascorbic acid: A new weighing boat was placed on the scale and set to 0 g. A 100 mL graduated cylinder was used to measure 100 mL d.H_2O, which was added to a 250 mL volumetric flask labelled “AA”. Then, 1.76 g of ascorbic acid was measured and added to the flask and mixed.

Appendix IV: In-Lab Engineered Wetland Phosphorus Analysis Absorbance Readings

The following figures are visual representations of the absorbance values obtained from the phosphorus analysis.





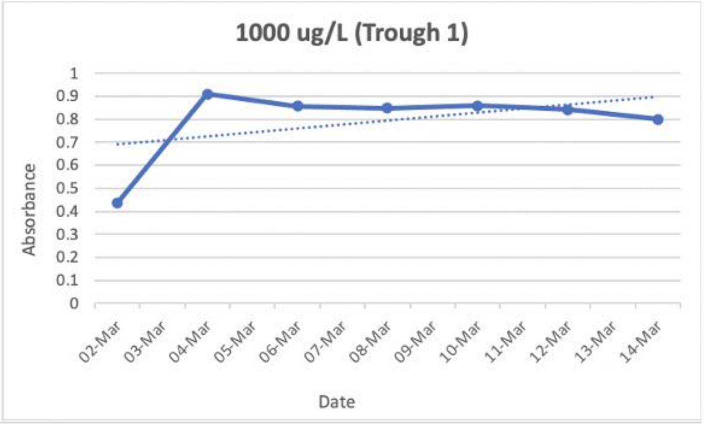


Figure 1: Absorbance Readings Using UV/Vis Spectrophotometer for all Mixed Cultures

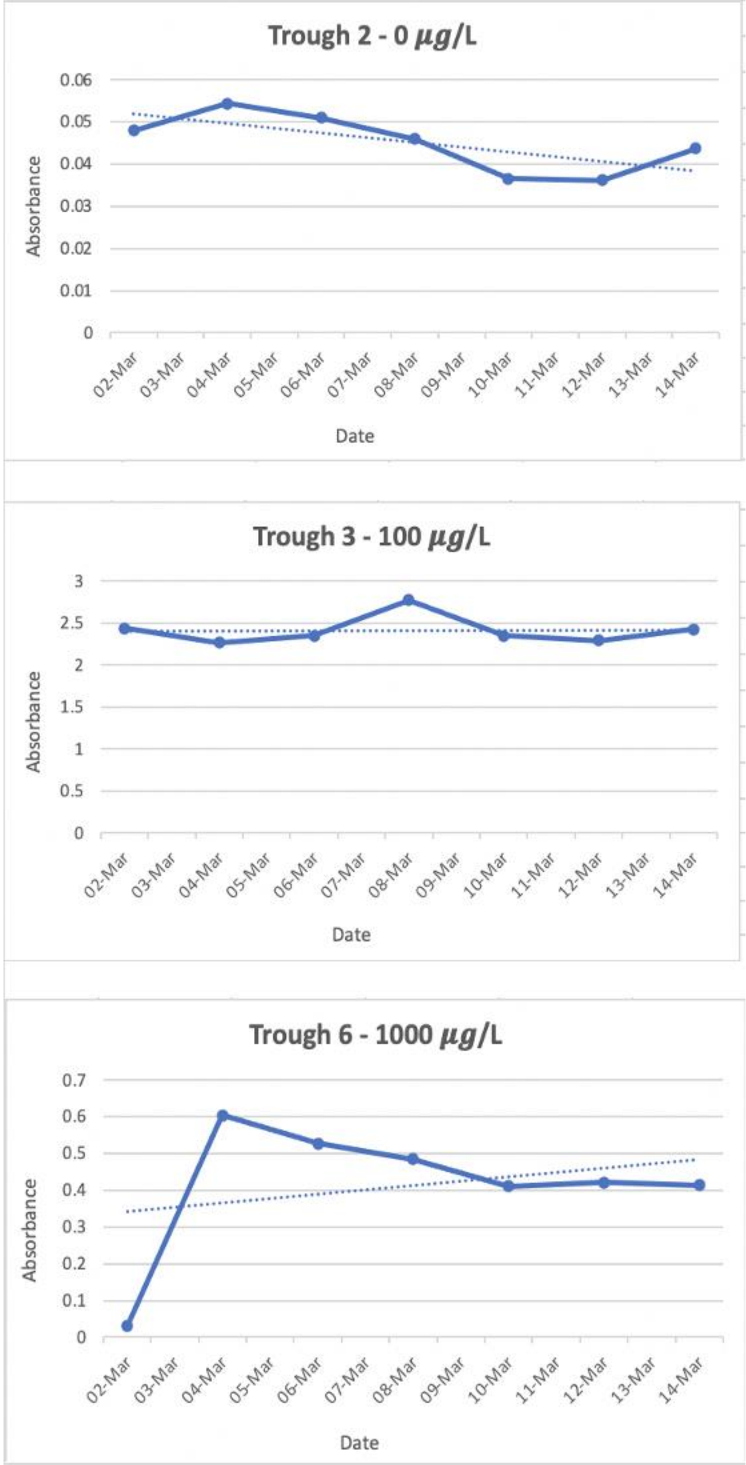


Figure 2: Absorbance Readings Using UV/Vis Spectrophotometer for all Controls

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