INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning 300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA 800-521-0600

UM®

CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT IN THE SUBARCTIC

A THESIS

Presented to the Faculty

Of the University of Alaska Fairbanks

In Partial fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

David Charles Maddux, B.S.

Fairbanks, Alaska

May 2002

UMI Number: 3053230

UMI®

UMI Microform 3053230

Copyright 2002 by ProQuest Information and Learning Company. All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

> ProQuest Information and Learning Company 300 North Zeeb Road P.O. Box 1346 Ann Arbor, MI 48106-1346

CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT

IN THE SUBARCTIC

By

David Charles Maddux

RECOMMENDED

antino

Co

Advisory Committee Co-Chair

Department Head. Plant, Animal. and Soil Sciences

APPROVED

Dean. School of Agriculture and Land Resource Management

Dean of the Graduate School

4-15-12

Date

ABSTRACT

This research had two basic objectives: to assess the capability of macrophytes indigenous to the subarctic in removal of heavy metals from wastewater and to determine the feasibility of using constructed wetlands for sewage wastewater treatment in a subarctic environment with a focus on rural application. The research consisted of two parts: a greenhouse study in which indigenous macrophytes were subjected to heavy metal pollutants similar to those found in roadway runoff and a constructed wetland built to treat sewage wastewater. Five species of plants were tested in both projects: Arctophila fulva, Carex rhynchophysa, Menyanthes trifoliata, Scirpus validus and Typha latifolia. In the greenhouse study, the plants were exposed to four heavy metals: cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) over a 68-day period. The plants were grown under a photoperiod of 20 hours light: 4 hours dark. There were significant differences in metal uptake among species and more metals were stored in below-ground plant parts than in above-ground plant parts. In separate experiments, plants took up zinc in greater quantities than the other metals except A. fulva which took up copper in the greatest quantity. Effects of phytotoxicity from the metal concentrations were apparent only in M. trifoliata. The constructed wetland study consisted of a five-cell system. Biological oxygen demand (BOD), total suspended solids (TSS), fecal coliforms (FC), total phosphorus (TP), total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH 1) were measured bi-weekly during each growing season over a three-year period. Reduction efficiencies, averaged over the ice-free season, ranged from 24-67% for BOD; 38-62% for TSS: 93-99% for FC: 21-60% for TP; 43-76% for TKN; and 50-92% for NH₄⁺. The reduction of pollutants indicated the ability of constructed wetlands to work well in the subarctic. Vegetation colonized the constructed wetland rapidly, with a complex community structure emerging over the study period. Pollutant reduction appeared to be limited by the size of the constructed wetland and not by the extreme climatic conditions.

TABLE OF CONTENTS

ABSTRACT	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	vii
LIST OF TABLES	x
ACKNOWLEDGEMENTS	xi

CHAPTER 1	INTRODUCTION	1
Background	d	1
Heavy meta	al removal	2
Wastewater	r treatment	5
Types o	f constructed wetlands	6
Treatm	ent comparisons	6
Functio	ons of wetlands	7
Literature c	cited	

CHAPTER 2 UPTAKE OF HEAVY METALS BY SUBARCTIC PLANTS: A GREENHOUSE STUDY

Abstract	
Introduction	23
Sources of heavy metals	23
Macrophytes as accumulators of metals	23
Justification for this study	24
Questions and hypotheses	25
Methods and Materials	25
Plants used as experimental subjects	25
Metals used in experiment	26
Experimental design	26

Planting procedures	. 27
Dosing procedure	.27
Sample preparation	. 28
Statistics	. 29
Results	. 29
Discussion	. 30
Literature Cited	.35

CHAPTER 3 PERFORMANCE OF A CONSTRUCTED WETLAND FOR

WASTEWATER TREATMENT IN SUBARCTIC ALASKA

Abstract	48
Introduction	49
Constructed wetlands for sewage treatment in cold regions	49
Functions of wetlands	49
Project Description	50
Research questions	51
Methods and materials	52
Plant species	53
Experimental design. planting procedures	53
Statistical analysis	53
Treatment procedure	54
Sampling procedure	54
Results	55
Discussion	58
Literature Cited	66

CHAPTER 4 A VEGETATIVE STUDY OF A CONSTRUCTED WETLAND TREATING SWINE WASTEWATER

Abstract	
Introduction	
Methods and Materials	
Plant species	
Experimental design, planting procedures	
Sampling methods	
Results	
Discussion	
Literature Cited	

CHAPTER 5 SUMMARY

Summary	
Future Study	ł

APPENDIX1	2	8
-----------	---	---

LIST OF FIGURES

Figure 2-1	Average dry biomass of plants treated with heavy metals	
	compared with control plants grown without addition of metals	41
Figure 2-2	Metal concentrations by metal and plant	42
Figure 2-3	Average percent of metal storage above and below ground	
	in plant tissue	43
Figure 2-4	Metal distribution among plant, soil and water at conclusion	
	of the experiment	44
Figure 2-5	Metal storage by plant part	45
Figure 3-1	Plan view of the constructed wetland built on the University of	
	Alaska Fairbanks Campus	72
Figure 3-2	Dissolved oxygen concentrations of water in constructed	
	wetland cells	73
Figure 3-3	pH readings in surface waters of constructed wetland cells	74
Figure 3-4	Dissolved carbon dioxide concentrations of water in constructed	
	wetland cells for 1999	75
Figure 3-5	Air, soil and water temperature readings for constructed	
	wetland cells	76
Figure 3-6	Biological oxygen demand concentration ratios for vegetated	
	cells in 1998 by station and date	77
Figure 3-7	Biological oxygen demand concentration ratios for vegetated	
	cells in 1999 by station and date	78
Figure 3-8	Biological oxygen demand concentration ratios for vegetated	
	cells in 2000 by station and date	79
Figure 3-9	Total suspended solids concentration ratios for vegetated cells	
	in 1998 by station and date	8 0
Figure 3-10	Total suspended solids concentration ratios for vegetated cells	
	in 1999 by station and date	81

Total suspended solids concentration ratios for vegetated cells	
in 2000 by station and date	82
Fecal coliforms concentration ratios for vegetated cells	
in 1998 by station and date	83
Fecal coliforms concentration ratios for vegetated cells	
in 1999 by station and date	84
Fecal coliforms concentration ratios for vegetated cells	
in 2000 by station and date	85
Total phosphorus concentration ratios for vegetated cells	
in 1998 by station and date	
Total phosphorus concentration ratios for vegetated cells	
in 1999 by station and date	
Total phosphorus concentration ratios for vegetated cells	
in 2000 by station and date	
Total Kjeldahl nitrogen concentration ratios for vegetated	
cells in 1998 by station and date	89
Total Kjeldahl nitrogen concentration ratios for vegetated	
cells in 1999 by station and date	90
Total Kjeldahl nitrogen concentration ratios for vegetated	
cells in 2000 by station and date	91
Ammonium nitrogen concentration ratios for vegetated	
cells in 1998 by station and date	92
Ammonium nitrogen concentration ratios for vegetated	
cells in 1999 by station and date	93
Ammonium nitrogen concentration ratios for vegetated	
cells in 2000 by station and date	94
Plan view of the constructed wetland built on the University of	
Alaska Fairbanks campus	110
	Total suspended solids concentration ratios for vegetated cells in 2000 by station and date

Figure 4-2	Cell unit planting schematic for the constructed wetland built
	on the University of Alaska Fairbanks campus 111
Figure 4-3	Cell 1 Vegetation Succession
Figure 4-4	Vegetation density for Arctophila fulva by year and station 113
Figure 4-5	Vegetation density for Carex rhynchophysa by year and station
Figure 4-6	Vegetation density for Menyanthes trifoliata by year and station
Figure 4-7	Vegetation density for Scirpus validus by year and station116
Figure 4-8	Vegetation density for Typha latifolia by year and station 117
Figure 4-9	Water temperatures for the vegetated and unvegetated
	cells for the year 2000 118
Figure A-1	Stem lengths for metal treated and non-treated (control) plants
Figure A-2	Stem count for metal treated and non-treated (control) plants 129
Figure A-3	Nitrogen given to cadmium-treated plants
Figure A-4	Nitrogen given to copper-treated plants
Figure A-5	Nitrogen given to lead-treated plants
Figure A-6	Nitrogen given to zinc-treated plants
Figure A-7	Example data sheet for Table 3-2: 1998 total phosphorus
Figure A-8	Example for calculating the uptake rate constant k for
	total phosphorus in Table 3-1
Figure A-9	Side-by-side comparison of vegetation coverage to pollutant
	reduction in 1998136
Figure A-10	Side-by-side comparison of vegetation coverage to pollutant
	reduction in 1999 137
Figure A-11	Side-by-side comparison of vegetation coverage to pollutant
	reduction in 2000 138
Figure A-12	Average redox potentials for vegetated and unvegetated cells
	for 1997
Figure A-13	Average redox potentials for vegetated and unvegetated cells
	for 1998

Figure A-14	Average redox potentials for vegetated and unvegetated cells	
	for 1999	141
Figure A-15	Data set for Arctophila fulva treated with cadmium	142
Figure A-16	Data set for Carex rhynchophysa treated with cadmium	143
Figure A-17	Data set for Menyanthes trifoliata treated with cadmium	144
Figure A-18	Data set for Scirpus validus treated with cadmium	145
Figure A-19	Data set for Typha latifolia treated with cadmium	146

LIST OF TABLES

Table 2-1	Average amount of metal taken up by plant and residual in pot	
	at end of experiment	46
Table 2-2	Comparison of accumulation of metals among plant parts	47
Table 3-1	Mean pollutant concentrations by station and year	95
Table 3-2	Uptake rate constants for <i>k</i>	96
Table 3-3	Vegetation and open water percent presence by year	97
Table 4-1	Vegetation and open water percent presence by year	119
Table 4-2	Vegetation and open water percent presence by station and year	120

Acknowledgements

This work was financially supported by the Fitzgerald Family Foundation, the Murdock Charitable Trust Foundation and the Philip Morris Corporation Environmental Program. Rich Hughes from the Ryan Lode Mine and Royal Bidwell from Alaska Pipe and Supply both supplied materials for the constructed wetland project. I thank my committee, Dr. Charles Knight, Dr. Stephen Sparrow, Dr. Mark Oswood, Dr. Patricia Holloway and Dr. Daniel Hawkins for giving me the time and space to pursue this research. Without their combined skills and efforts these projects would not have been possible. I would be remiss if I did not thank Darlene Masiak and Bob Van Veldhuizen for giving me a hard time about my project whenever they saw me. Their good-natured ribbing helped to keep me from getting too wrapped up in the wetland I was building. I owe many thanks to the efforts of Lola Oliver and Tim Quintel in the Forest Soils Lab who kept me from blowing the place up or flooding it, depending on how my day was going. I thank Yvonne Nieman for her diligent and proficient editing skills in the crafting of this thesis. My friends and family have my heartfelt thanks for believing in me. I thank Pint who always stood by me, extending a helping hand when I was down and ready to help me get back on track whenever I went off the deep end. Thank you one and all.

For those who follow in the endeavor of higher academic achievement I offer the following:

PRESS ON

Nothing in the world can take the place of persistence. Talent will not: nothing is more common than unsuccessful men with talent. Genius will not: unrewarded genius is almost a proverb. Education alone will not: the world is full of educated derelicts. Persistence and determination alone are omnipotent.

- Franklin Roosevelt

CHAPTER 1 – INTRODUCTION

Background

Wetlands have been used as wastewater discharge points in the U.S. for as long as sewage has been collected (Powicki, 1987; and Smardon, 1989). However, it was not until the mid-1960's that water quality monitoring of these wetland discharges was initiated and the scientific basis of water purification was realized (Kadlec and Knight, 1995). Since that time, wetland science has grown and been applied in construction of wetlands for a variety of wastewater treatments.

Water pollutants have a variety of sources, both natural and anthropogenic. The human sources include roadway runoff, human sewage, and agricultural and industrial wastewater. Many authors have referred to wetlands as "the kidneys of the landscape" (Mitsch and Gosselink, 1993; and Kadlec and Knight, 1995). Wetlands filter a variety of pollutants including nutrients, sediments and heavy metals (Wetzel, 1993) providing a critical step in cleansing the freshwater that flows across the landscape. Removal of pollutants within a wetland depends on a combination of processes including sedimentation, filtration, adsorption, complexation, precipitation, plant uptake and microbially-mediated reactions (Watson et al., 1989; and Kadlec et al., 2000).

A constructed wetland is a human-made wetland meant to reproduce many of the processes of a natural wetland such as nitrification-denitrification, sedimentation, chemical precipitation, gasification and uptake of nutrients by plants (Brix, 1993). Constructed wetlands have been used to successfully treat sewage effluent (Green et al., 1998), agricultural effluent (Schaafsma et al., 1999), stormwater runoff (Shutes et al., 1993) and landfill leachates (Mæhlum and Stålnacke, 1999). In temperate climates, constructed wetlands or natural wetlands have been used to treat wastewater for the past 30 years (Livingston, 1989). However, little or no data exist for such systems in the subarctic such as Fairbanks, Alaska (Jenssen et al., 1994). Most other cold climate regions where constructed wetlands have been used are either located at lower latitudes

or near coastlines influenced by maritime climates (Jenssen, et al., 1996; and Mæhlum and Stålnacke, 1999) and are therefore not comparable to the cold climate of interior Alaska. Thus it was unknown how well a constructed wetland or the subarctic plants used in these constructed wetlands would function in a more northern climate.

Heavy metal removal

This research investigated the tolerance of wetland plants indigenous to the subarctic to high heavy metal concentration, how much metal the plants removed from the wastewater, and where the metals were stored within the plant.

Although metallic elements are often essential micronutrients, high levels of exposure to metals are toxic to humans and other organisms (Manahan, 1992; and Beltman, et al. 1999). Metals occur naturally as a result of weathering and other biogeochemical sources but human activities, such as mining and smelting industries, fossil fuel combustion and the use of corrosive metal can greatly increase inputs of metals to freshwater systems (Wetzel, 1983; Sansalone and Buchberger, 1997; and Paschke et al. 2000). Pollutants can be categorized as point source or nonpoint source. Point source pollutants are those that have a known, controlled outlet, such as a sewage discharge line, whereas nonpoint source pollutants have an uncontrolled discharge point, such as roadway or parking lot runoff. The sources of heavy metal pollution usually place them in the category of nonpoint source pollution (Davies, 1995).

Macrophytes respond to exposure to heavy metals in a variety of ways, with considerable genetic variation among species (Chigbo et al., 1982; Lombi et al. 1999; and Hutchinson et al. 2000). There is some evidence that metal accumulation and nonspecific metal detoxification are constitutive characteristics within individual species (Reeves and Baker, 1984; Shen et al. 1997; and Lombi et al. 1999) and macrophyte responses typically fall into three areas: exclusion, tolerance, and accumulation (Woolhouse, 1983; and Baker and Brooks, 1989). Mechanisms for exclusion include metal binding with soil organic matter (Alloway, 1990), the binding of metals to root-cell walls (Santa Maria and Cogliatti, 1988; and Marschner, 1995) and detoxification by rhizosphere organisms such as mycorrhizae (Bradley et al. 1982; and Jones and Hutchinson, 1986). The rhizosphere microbial community can also play a significant role in allowing some macrophytes to act as accumulators of metals, especially when that microbe community is predominantly bacteria rather than fungi (de Souza et al., 1999). de Souza found that plants with a rhizosphere dominated by bacteria have a compound in their root exudate that increases metal uptake five-fold over plants without a bacterial rhizosphere. However other researchers have found that the presence of mycorrhizal fungi on the root or root system (Baker 1981; and Joner et al., 2000) provides an external protection (Levitt, 1980). As cited in Kadlec et al. (2000) Ye, et al. (1994) found that Typha latifolia avoids accumulation of heavy metals to a toxic level by the presence of an iron coating on the plant root system that acts to decrease the uptake of metals by the root hairs. Most wetland plants adept at heavy metal removal translocate the stored metals to the root, then the rhizome, with varying lesser amounts to the stem, leaf or flower (Hansen et al., 1998).

Tolerance and accumulation of metals by plants are currently undergoing intense scrutiny by researchers world-wide. Some of the mechanisms being investigated are the use of transporter systems to cross the root-cell plasma membrane and enter into the root symplasm (Lasat et al. 1996) and an increase of specific transporter sites on root cells by specific plants (Lasat et al. 1996; and Lombi et al. 1999, 2001). The effect of metal-solubilizing root exudates (Grinsted et al. 1982) and the altering of the rhizosphere pH through the production of H⁻ ions by the root is another area of investigation (Burnal et al., 1994; Knight et al. 1997; and Whiting et al. 1999). The results of these studies vary, with some species producing protons and organic acids in the rhizosphere and others not. Another important area of research is the mechanism used by plants exhibiting tolerance and accumulation of heavy metals by detoxification with phytochelatins (Steffens, 1990; Rauser, 1990; and Ortiz et al., 1995). Numerous species of plants are able to produce

metal-binding polypeptides but it appears that phytochelatins are only found when toxic amounts of a metal are present. Plants produce these substances even for micronutrient metals such as zinc and copper when they are present in toxic concentrations. Phytochelatins may be involved in the transfer of metals from the cytoplasm to the vacuole (Steffens, 1990). More research needs to be completed, especially in the field of molecular biology and biochemistry, before the mechanisms that drive exclusion, tolerance and accumulation will be fully understood (Ernst, 1996; and McGrath et al. 2001).

The term phytoremediation typically refers to terrestrial plants that hyperaccumulate heavy metals such as zinc, cadmium and nickel and have the aboveground plant tissues harvested to remove the metals from the soil permanently. Constructed wetlands used for heavy metal removal are not meant to be harvested nor to act as hyperaccumulators of metals; they function to reduce metal concentrations in wastewater by storing them in below-ground plant tissue and in the substrate of the wetland. Typical metal concentrations flowing into a constructed wetland are not usually at the high concentrations found in areas where hyperaccumlator plants will grow.

The primary goal of a constructed wetland built to ameliorate heavy metals from wastewater is to remove the metals from the water column before their discharge to a receiving body of water. The metal that is taken up by a plant and translocated to aboveground tissues will be released back into the water column during senescence and subsequent decomposition (Kadlec, et al., 2000). The metal that is translocated to the root or rhizome will be buried and when these plant tissues die the metals should be much less mobile and thus not be bioavailable or at least be much more resistant to being transported downstream.

Wastewater treatment

Sewage wastewater disposal in the subarctic has always created problems for communities. large and small, urban and rural. However, the rural communities are most burdened with disposal costs, both in real dollars and in negative impacts to the environment. The second objective of this research was to determine if constructed wetlands could provide rural communities in the subarctic with a low technology, reasonably inexpensive and safe method of treating wastewater. Constructed wetlands built to treat sewage wastewater have several advantages over conventional sewage treatment plants. They are inexpensive to build compared with a conventional sewage treatment plant that typically costs between two and ten times more than a constructed wetland system (Litchfield and Schatz, 1989). For example, a conventional sewage treatment plant proposed for an oil refinery in North Dakota was estimated to cost between \$1 million and \$3 million dollars. The constructed wetland system that was built cost \$250.000.

Constructed wetland systems have low energy requirements and corresponding low operating and maintenance costs (Hunt and Poach, 2001; Ayaz and Akca, 2000). Because they are low technology systems. an operator needs only limited training to successfully operate the system. The systems are less susceptible to variations in loading rates compared to a conventional sewage treatment plant system (Gopal, 1999; Brix and Schierup, 1993). For example, most wetland plants and associated organisms can tolerate fluctuating water levels for short periods of time without impacting treatment capabilities, whereas a sewage treatment plant is designed for optimum operation at a continuous flow. A constructed wetland system also produces little or no sludge, can achieve a high degree of nutrient reduction, and is capable of removing heavy metals and pathogens (Kadlec and Alvord, 1989; Lan et al., 1992).

Types of constructed wetlands

There are two types of constructed wetlands that are used for pollutant removal: surface flow and subsurface flow. Surface flow wetlands look like a typical wetland, with effluent flow on top of the soil (Kadlec and Knight, 1995). In contrast, vegetated subsurface-flow wetlands have a subsurface flow of effluent (Kruzic, 1994) through a constructed medium of gravel or sand (Brix, 1993). Where annual frost is driven deep into the ground each winter, as it is in northern latitudes, these submerged systems are not practical. A subsurface-flow wetland would work longer into the winter months but would take longer to thaw in the spring, negating any extra treatment time gained as the winter season commenced.

Both systems have relative advantages and disadvantages. The vegetated subsurface flow system has very few odor or insect problems and requires less space to operate (Steiner and Freeman, 1989). However, these systems require more resources to construct and maintain and offer little in the way of wildlife habitat or recreational opportunities. The surface flow wetland is cheaper to construct and maintain, offers a wider range of wildlife habitat and recreational opportunities (Lofgren, 1993) and the treatment is not impacted by deep frost. For the purposes of this project, a surface flow wetland was constructed.

Treatment comparisons

The fundamental difference between a sewage treatment plant and a constructed wetland is that in sewage treatment plant systems, wastewater is treated rapidly in a highly managed, energy intensive environment, usually requiring inputs of fossil fuels (Knight, 1973). In macrophyte-based systems (constructed wetlands) treatment occurs at a comparatively slow rate in essentially unmanaged "natural" environments with very little or no input of fossil fuel.

6

Major drawbacks of constructed wetland systems include the requirements of larger land areas to operate, more time to complete the purification process and the possibility of initially negative public perceptions of this relatively new technology (Smardon, 1989). Public perceptions usually change as the constructed wetland matures into an attractive greenspace and provides habitat for birds and other wildlife. Potential flooding from an outside source can be a problem, but with proper engineering this can be prevented. Droughts typically are not a problem because a constructed wetland has its own source of water in the form of the effluent flow. In times of drought, a constructed wetland can be a wildlife haven in an otherwise dry landscape.

Functions of wetlands

The macrophyte-microbe complex in wetlands is responsible for many functions (Kadlec and Knight. 1995; and Portier and Palmer. 1989). The microbe community found growing on the submerged plants and on the detrital mat of the substrate is collectively called periphyton. This community of microbes is made up mostly of algae, bacteria and fungi (Lock, 1981: and Vymazal, 1994) residing in a polysaccharide "slime" matrix which is attached to the substrate (Craigie, 1974). This matrix helps to capture plant exudates and organic matter suspended in the water column (Moeller et al., 1988; and Fletcher and Marshall, 1982) which the microbes then transform (Kadlec, 1999). The plant rhizosphere also supports large microbial populations that transform metallic ions, nutrients and other compounds (Hammer and Bastian, 1989). Thus, two important roles that macrophytes play in constructed wetlands do not depend on their uptake of nutrients, but on the surface area they provide on their submerged stems for periphyton attachment (Hammer, 1994, and Soto et al., 1999) and as a detrital carbon source for microbes upon senescence (Brix, 1997, and Chappell and Goulder, 1994).

Wetlands are the only landscape form that has a reducing environment as a major component of their ecosystem (Kadlec et al., 2000; and Hammer and Bastian, 1989). This reducing environment is coupled in close proximity with the oxidizing environment which allows chemical transformations requiring aerobic and anaerobic microbes to occur. The boundary layer between the aerobic zone of the rhizosphere and the surrounding anaerobic zone of the sediments is a good example. Nitrogenous products can be transformed back and forth between organic and inorganic forms as well as reduced and oxidized forms and can be released to the atmosphere as gases such as NH₃, N_2O or N_2 (Kadlec and Knight, 1995). Water saturation of soils in a wetland results in the sediments becoming largely anaerobic (Faulkner and Richardson, 1989) which reduces gas exchange rates between sediments and the atmosphere (Mitch and Gosselink, 1993). Rates of decomposition and mineralization of large quantities of organic matter made by primary producers are significantly reduced under these conditions so that particulate organic matter accumulates (Kadlec, 1989). Moreover, water flows are restricted by plant growth which leads to physical sedimentation (Nichols, 1983). However, dissolved and particulate organic carbon is transformed by microbial decomposition to CO_2 and CH_4 which are lost to the atmosphere, thus permanently removing this material from the constructed wetland (Kadlec and Knight, 1995). In this way, carbon in a constructed wetland is constantly being turned over and accretion of new soil is slowed to an average of approximately 2-5 mm/year in temperate climates (Richardson and Craft, 1993; and Kadlec et al., 2000).

When a constructed wetland is initially placed into operation, it is an incomplete treatment system. If the cells were constructed using upland soils, the redox state of the newly submerged soil will be in transition and the soil will probably be lacking in density and diversity of anaerobic microbes during the first growing season. A constructed wetland in its first year has a lot of open water and little vegetative biomass until the plants start to colonize the open areas. Data collected from constructed wetlands over the past 20 years in temperate climates indicate that a minimum of one to four years of operation are needed before a constructed wetland system approaches a steady-state in treatment capability (Kadlec and Knight, 1995).

8

Constructed wetlands receive external inputs (e.g., sewage effluent) containing constituents such as inorganic nutrients, bacteria and organic matter that are measured as water quality parameters. However, some of these constituents can also be generated internally by such wetland processes as nutrient regeneration from sedimentary storage and seasonal blooms of algae. Upon discharge into receiving waters, these nutrients and other constituents are described as pollutants once a certain concentration is reached. It is important to assess the source of the pollutant when determining the efficiency of a constructed wetland in removing it. Although degradable carbon compounds in the effluent are rapidly sequestered or respired by the biota in constructed wetlands, decomposition processes produce available carbon in other forms (Kadlec and Knight. 1995). This internal processing can yield a background of biological oxygen demand (BOD) up to 6 mg L^{-1} in constructed wetlands, with one study showing a background level near 10 mg L^{-1} (Lakshman, 1982). For example, if a die-off of algae occurs, algal cells will flood the water column, particularly increasing total suspended solids (TSS) and BOD, and lead to an increase in these pollutants (Vvmazal, 1994). Other mechanisms, such as senescing plant matter, fecal deposition by waterfowl (Scherer et al., 1995) and bioturbation of the substrate within the constructed wetland can add to the total pollutant load (Kadlec et al., 2000; and Gersberg et al., 1986). In this way, fluctuating reduction rates can be experienced from point to point and in some instances a pollutant does not diminish as the effluent moves from inlet to outlet. TSS and BOD discharged from a constructed wetland are often zooplankton and phytoplankton, which are not normally considered ecologically damaging to the receiving waters. The difficulty of separating innocuous components of wastewater discharges such as phytoplankton and zooplankton from truly hazardous components such as pathogenic microbes can make test results of discharge standards for constructed wetlands used in wastewater treatment misleading (Miller, 1989).

Constructed wetlands can remove phosphorus from wastewater, both on a shortterm and long-term basis (Kadlec et al., 2000). Uptake by plants and other biota represents the short-term removal and incorporation within the sediments represents the long-term removal (Kadlec, 1996). Phosphorus, a macronutrient required for plant growth, is often the limiting nutrient in natural wetlands (Vymazal, 1994) and when added to a natural wetland in sewage wastewater it can have a profound effect on the structure of the aquatic ecosystem (Kadlec and Knight, 1995). Constructed wetlands are not efficient in phosphorus reduction on a per unit area basis, but with enough hydraulic retention time and area they can effectively remove phosphorus from wastewater (Watson et al., 1989).

Constructed wetlands work because of the biological processes that naturally occur in wetland systems and do not depend on inputs of external energy sources except for the sun. Northern latitudes receive a lot of sunlight during the summer months and have an abundance of wetland habitats, and thus all the macrophytes and microbes required for wetland treatment. This would appear to make the subarctic an ideal place for constructed wetlands, as long as climatic and latitudinal factors are taken into consideration.

Literature cited

- Alloway, B.J. (1990). Soil Processes and the Behavior of Metals. In. Heavy Metals in Soils, B.J. Alloway, (Ed.). John Wiley & Sons, New York., 7.
- Ayaz, S.C., and Akca, I. (2000). Treatment of Wastewater by Constructed Wetland in Small Settlements. Wat. Sci. Tech., 41, 69.
- Baker, A.J.M., and Brooks, R.R. (1989). Terrestrial Higher Plants which Accumulate Metallic Elements – A Review of their Distribution, Ecology and Phytochemistry. *Biorecovery* 1, 81.
- Baker, A.J.M. (1981). Accumulators and Excluders- Strategies in the Response of Plants to Heavy Metals. J. Plant Nutr., 3, 643.
- Beltman, D.J.; Clements, W.H.; and Lipton, C. D. (1999). Benthic Invertebrate Metals Exposure Accumulation, and Community-Level Effects Downstream from a Hard-Rock Mine Site. *Environ. Toxicol. Chem.* 18, 299.
- Bradley, R.; Burt, A.J.; and Read, D.J. (1982). The Biology of Mycorrhiza in the Ericaceae. VIII. The Role of Mycorrhizal Infection in Heavy Metal Resistance. New Phytol 91, 197.
- Brix, H. (1993). Wastewater Treatment in Constructed Wetlands: System Design,
 Reduction Processes, and Treatment Performance. In Constructed Wetlands for
 Water Quality Improvement. G.A. Moshiri (Ed.), Lewis Publishers, 9.
- Brix, H., and Schierup, H.H. (1993). The Use of Macrophytes in Water Pollution Control. Ambio, 18, 100.

- Brix, H. (1997). Do Macrophytes Play a Role in Constructed Wetlands? Wat. Sci. Technol. 35, 11.
- Burnal, M.P.; McGrath, S.P.; Miller, A.J.; and Baker, A.J.M. (1994). Comparison of the Chemical Changes in the Rhizosphere of the Nickel Hyperacccumulator *Alyssum murale* with the Non-Accumulator *Raphanus sativus*. *Plant and soil* 164, 251.
- Chappell, K.R., and Goulder, R. (1994). Seasonal Variation of Epiphytic Extracellular Enzyme Activity on Two Freshwater Plants, *Phragmites australis* and *Elodea* canadensis. Arch. Hydrobiol. 132, 237.
- Chigbo, F.E.; Smith, R.W.; and Shore, F.L. (1982). Uptake of Arsenic, Cadmium, Lead and Mercury from Polluted Waters by the Water Hyacinth Eichhornia crassipes, Environ. Pollut. 27, 31.
- Craigie, J.S. (1974). Storage Products. In, *Algal Physiology and Biochemistry*. W.D.P. Stewart, (Ed.), University of California Press, Berkeley, CA., 206.
- Davies, P. H. (1995). Factors in Controlling Nonpoint Source Impacts. In Stormwater Runoff and Receiving Systems, E.E. Herricks, (Ed.), Lewis Publishers. Boca Raton FL., 83
- de Souza, M. P.; Chu, D.; Zhao, M.; Zayed, A.M.; Ruzin, S.E.; Schichnes, D.; and Terry, N. (1999). Rhizosphere Bacteria Enhance Selenium Accumulation and Volatilization by Indian Mustard. *Plant Physiol.*, **119**, 565.
- Ernst, W.H.O. (1996). Bioavailability of Heavy Metals and Decontamination of Soils by Plants. Applied Geochemistry 11, 163.

- Faulkner, S.P., and Richardson, C.J. (1989). Physical and Chemical Characteristics of Freshwater Wetland Soils. In *Constructed Wetlands for Wastewater Treatment*.
 D.A. (Ed.), Lewis Publishers, Chelsea, MI., 41.
- Fletcher, M., and Marshall, K.C. (1982). Are Solid Surfaces of Ecological Significance to Aquatic Bacteria? Adv. Microb. Ecol. 6, 199.
- Gersberg, R.M.; Elkins, B.V.; Lyon, S. R.; and Goldman, C.R. (1986). Role of Aquatic Plants in Wastewater Treatment Using Artificial Wetlands. *Wat. Res.*, **20**, 363.
- Gopal, B. (1999). Natural and Constructed Wetlands for Wastewater Treatment: Potential and Problems. *Wat. Sci. Tech.* **40**, 27.
- Green, M.H.; O'connell, P.J.; and Griffin, P. (1998). Uprating and Rescuing Small Wastewater Treatment Facilities by Adding Tertiary Vegetated Reed Beds. *Water Environ. Res.*, **70**, 1307
- Grinsted, M.J.; Hedley, M.J.; White, R.E.; and Nye, P.H. (1982). Plant Induced Changes in the Rhizosphere of Rape (*Brassica napus* var. Emerald) Seedlings. I. Change and the Increase in P Concentration in the Soil Solution. *New Phytol.*, **91**, 19.
- Hammer, D.A., and Bastian, R.K. (1989). Wetland Ecosystems: Natural Water Purifiers?
 In Constructed Wetlands for Wastewater Treatment. D.A. Hammer (Ed.), Lewis
 Publishers, Chelsea, MI., 5.
- Hammer, D.A., and Knight, R.L. (1994). Designing Constructed Wetlands for Nitrogen Removal. Wat. Sci. Tech., 29, 15.

- Hansen, D.; Duda, P.J.; Zayed, A.; and Terry, N. (1998). Selenium Removal by Constructed Wetlands: Role of Biological Volatilization. *Environ. Sci. Technol.*, 32, 591.
- Hunt, P.G., and Poach, M.E. (2001). State of the Art for Animal Wastewater Treatment in Constructed Wetlands. *Wat. Sci. Tech.* 44, 19.
- Hutchinson, J.J.; Young, S.P.M.; West, H.M.; Black, C.R.; and Baker, A.J.M. (2000).
 Determining Uptake on 'Non-Labile' Soil Cadmium by *Thlaspi caerulescens* Using Isotopic Dilution Techniques. *New Phytol.* 146, 453.
- Jenssen, P.D.; Mæhlum, T.; and Krogstad, T. (1994). Adapting Constructed Wetlands for Wastewater Treatment to Northern Environments. In, *Global wetlands: Old world* and new. W.J Mitsch (Ed.), Elsevier Science, Amsterdam.
- Jenssen, P.D.; Mæhlum, T.; and Zhu, T. (1996). Design and Performance of Subsurface Flow Constructed Wetlands in Norway. In: Proceedings of the Symposium on Constructed Wetlands in Cold Climates. Lemon, E. (Ed.), Ontario, Canada, 100.
- Joner, E.J.; Briones, R.; and Leyval, C. (2000). Metal-Binding Capacity of Arbuscular Mycorrhizal Mycelium. *Plant and Soil*, **226**, 227.
- Jones, M.D., and Hutchinson, T.C. (1986). The Effect of Mycorrhizal Infection on the Response of *Betula papyrifera* to Nickel and Copper. *New Phytol.*, **102**, 429.
- Kadlec, R.H. (1989). Dynamics of Inorganic and Organic Materials in Wetlands
 Ecosystems Decomposition in Wastewater Wetlands. In Constructed Wetlands for
 Wastewater Treatment, Municipal, Industrial and Aagricultural. D.A. Hammer
 (Ed.), Lewis Publishers, Chelsea, MI., 459.

Kadlec, R.H. (1996). An Autobiotic Wetland Phosphorus Model. Ecol. Eng. 8, 145.

- Kadlec, R.H. (1999). Chemical, Physical and Biological Cycles in Treatment Wetlands. Wat. Sci. Tech. 40, 37.
- Kadlec, R.H., and Alvord Jr. H. (1989). Mechanisms for Water Quality Improvement in Wetland Treatment Systems. American Water Resources Association.
- Kadlec, R.H., and Knight, R.L. (1995). Treatment Wetlands. Lewis Publishers, Boca Raton, FL.
- Kadlec, R.H.; Knight, R.L.; Vymazal, J.; Brix, H.; Cooper, P.; and Haberl, R. (2000).
 Constructed Wetlands for Pollution Control: Processes. Performance, Design and Operation. Scientific and Technical Report No. 8. IWA specialist group on use of macrophytes in water pollution control. IWA Publishing, London, 1.
- Knight, R.L. (1973). Operating Experience with Constructed Wetlands for Wastewater Treatment. *Tappi Journal.*, **76**, 67.
- Knight, B.P.; Zhao, F.J; McGrath, S.P.; and Shen, Z.G. (1997). Zinc and Cadmium Uptake by the Hyperacccumulator *Thlaspi caerulescens* in Contaminated Soils and its Effects on the Concentration and Chemical Speciation of Metals in Soil Solution. *Plant and soil* 197, 71.

Kruzic, A.P. (1994). Natural Treatment Systems. Water Environ. Res., 66, 357.

Lakshman, G. (1982). A Demonstration Project at Humboldt to Provide Tertiary Treatment to the Municipal Effluent using Aquatic Plants. SRC Tech Report No. 129. Saskatchewan Research Council.

- Lan, C.; Chen, G; and Wong, M.H. (1992). Use of Cattails in Treating Wastewater from a Pb/Zn Mine. *Environmental Management*, 16, 75.
- Lasat, M.M.; Baker, A.J.M.; and Kochian, L.V. (1996). Physiological Characterization of Root Zn⁺² Absorption and Translocation to Shoots in Zn Hyperaccumulator and Nonaccumulator Species of *Thlaspi. Plant Physiol.*, **112**, 1715.
- Levitt, J. (1980). Responses of Plants to Environmental Stresses, 2nd Ed., vol. 2. Academic Press, New York
- Litchfield, D.K., and Schatz, D.D. (1989). Constructed Wetlands for Wastewater Treatment at Amoco Oil Company's Mandan, North Dakota Refinery. In Constructed Wetlands for Wastewater Treatment. D.A. Hammer, (Ed.), Lewis Publishers, FL., 233.
- Livingston, E.H. (1989). Use of Wetlands for Urban Stormwater Management. In Constructed Wetlands for Wastewater Treatment. D.A.Hammer, (Ed.). Lewis Publishers, Chelsea, MI, 253.
- Lock, M.A. (1981). River Epilithon a Light and Organic Energy Transducer. In Perspectives in Running Water Ecology. M.A. Lock, and D.D. Williams, (Eds.), Plenum Press, New York, 3.
- Lofgren, R. (1993). Creating a Wetlands Wildlife Refuge from a Sewage Lagoon. In Constructed Wetlands for Water Quality Improvement. G.A. Moshiri (Ed.), Lewis Publishers, Boca Raton, FL., 585.

- Lombi, E.; Zhao, F.J.; Dunham, S.J.; and McGrath, S.P. (1999). Cadmium Accumulation in Populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. New Phytol., 145, 11.
- Lombi, E.; Zhao, F.J.; McGrath, S.P.; Young, S.D.; and Sacchi, G.A. (2001).
 Physiological Evidence for a High-Affinity Cadmium Transporter Highly
 Expressed in a *Thlaspi caerulescens* Ecotype. New Phytol., 149, 53.
- Mæhlum, T.; and Stålnacke, P. (1999). Removal Efficiency of Three Cold-Climate Constructed Wetlands Treating Domestic Wastewater: Effects of Temperature, Seasons, Loading Rates and Input Concentrations. *Wat. Sci. Tech.*, 40, 273.

Manahan, S. (1992). Toxicological Chemistry. Lewis Publishers, Florida.

- Marschner, H. (1995). *Mineral Nutrition of Higher Plants, Ed. 2.* Academic Press, London.
- McGrath, S.P.; Lombi, E.; and Zhao, F.J. (2001). What's New about Cadmium Hyperaccumulation? *New Phytol.*, **149**, 2.
- Miller, G. (1989). Use of Artificial Cattail Marshes to Treat Sewage in Northern Ontario. Canada. In *Constructed Wetlands for Wastewater Treatment*. D.A. Hammer (Ed.), Lewis Publishers, Chelsea, MI., 636

Mitch, W.J., and Gosselink, J.G. (1993). Wetlands. Van Nostrand Reinhold, New York

Moeller, R.E.: Burkholder, J.M.; and Wetzel, R.G. (1988). Significance of Sedimentary Phosphorus to a Rooted Submersed Macrophyte (<u>Najas flexilis</u>) and its Algal Epiphytes. *Aquat. Bot.*, **32**, 261.

- Nichols, D.S. (1983). Capacity of Natural Wetlands to Remove Nutrients from Wastewater. J. Water Pollut., Control Fed., 55, 495.
- Ortiz, D.F.: Ruscitti, T.: McCue, K.F.; and Ow, D.W. (1995). Transport of Metal-Binding Peptides by HMT1, a Fission Yeast ABC-Type Vacuolar Membrane Protein. J. Biol. Chem., 270, 4721.
- Paschke, M.W.; Redente, E.F.; and Levy, D.B. (2000). Zinc Toxicity Thresholds for Important Reclamation Grass Species of the Western United States. *Environ. Toxicol. Chem.*, 19, 2751.
- Portier, R.J., and Palmer, S.J. (1989). Wetlands Microbiology: Form, Function,
 Processes. In Constructed Wetlands for Wastewater Treatment. Municipal,
 Industrial and Agricultural. D.A. Hammer (Ed.), Lewis Publishers, Chelsea, MI.,
 89.

Powicki, C.R. (1987). Wetlands: Treat Wastewater Naturally. EPRI Journal, 22, 17.

Rauser, W.E. (1990). Phytochelatins. Annu. Rev. Biochem., 59, 61.

- Reeves, R.D., and Baker, A.J.M. (1984). Studies on Metal Uptake by Plants from Serpentine and Non-serpentine Population of *Thlaspi goesingense* Halacsy (Cruciferae). *New Phytol.*, 98, 191.
- Richardson, C.J., and Craft, C.B. (1993). Effective Phosphorus Retention in Wetlands:
 Fact or Fiction? In Constructed Wetlands for Water Quality Improvement. G.A.
 Moshiri (Ed.), Lewis Publishers, Boca Raton, FL., 271.

- Sansalone, J.J., and Buchberger, S.G. (1997). Partitioning and First Flush of Metals in Urban Roadway Storm Water. J. Environ. Eng., 123, 134.
- Santa Maria, G.E., and Cogliatti, D.H. (1988). Bidirectional Zn-Fluxes and Compartmentation in Wheat Seedling Roots. J. Plant Physiol., 132, 312.
- Schaafsma, J.A.; Baldwin, A.H.; and Stregb, C.A. (1999). An Evaluation of a Constructed Wetland to Treat Wastewater from a Dairy Farm in Maryland, USA. *Eco. Eng.*, 14, 199.
- Scherer, N.M.; Gibbons, H.L.; Stoops, K.B; and Muller, M. (1995). Phosphorus Loading of an Urban Lake by Bird Droppings. *Lake and Reserv. Manage.*, **11**, 317.
- Shen, Z.G.: Zhao, F.J.: and McGrath, S.P. (1997). Uptake and Transport of Zinc in the Hyperaccumulator *Thlaspi caerulescens* and the Non-Hyperaccumulator *Thlaspi* ochroleucum. Plant, Cell and Environment, 20, 898.
- Shutes, R.B.; Ellis, J.B; Revitt, D.M.; and Zhang, T. T. (1993). The Use of Typha latifolia for Heavy Metal Pollution Control in Urban Wetlands. In Constructed Wetlands for Water Quality Improvement. G.A. Moshiri (Ed.). Lewis Publishers, Boca Ration, FL., 407
- Smardon, R.C. (1989). Human Perception of Utilization of Wetlands for Waste Assimilation. or How Do You Make a Silk Purse Out of a Sow's Ear?. In Constructed Wetlands for Wastewater Treatment. D.A.Hammer, (Ed.). Lewis Publishers. Boca Raton, FL., 287
- Soto, F: García, E.: de Luís, E: and Bécares, E. (1999). Role of Scirpus Lacustris in Bacterial and Nutrient Removal from Wastewater. *Wat. Sci. Tech.* **40**, 241.

- Steffens, J.C. (1990). The Heavy Metal-Binding Peptides of Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 41, 553.
- Steiner, G.R., and Freeman Jr. R. J. (1989). Configuration and Substrate Design Considerations for Constructed Wetlands Wastewater Treatment. In Constructed Wetlands for Wastewater Treatment. D .A. Hammer (Ed.), Lewis Publishers, Boca Raton, FL., 363.
- Vymazal, J. (1994). Algae and Element Cycling in Wetlands. Lewis Publishers, Boca Raton, FL.
- Watson, J.T.; Reed, S.C.; Kadlec, R.H.; Knight, R.L.; and Whitehouse, A.E. (1989). Performance Expectations and Loading Rates for Constructed Wetlands. In Constructed Wetlands for Wastewater Treatment. D.A. Hammer (Ed.), Lewis Publishers, Boca Raton, FL., 319.

Wetzel, R.G. (1983). Limnology. Saunders College Publishing, Texas.

- Wetzel, R.G. (1993). Constructed Wetlands: Scientific Foundations are Critical. In: Constructed Wetlands for Water Quality Improvement. G. A. Moshiri, (Ed.), Lewis Publishers, Boca Raton, FL., 3.
- Whiting, S.N.: Leake, J.R: McGrath, S.P.: and Baker, A.J.M. (1999). Positive Responses to Zn and Cd by Roots of the Zn and Cd Hyperacccumulator *Thlaspi caerulescens*. *New Phytol.* 145, 199

- Woolhouse, H.W. (1983). Toxicity and Tolerance in the Responses of Plants to Metals.
 In Encyclopedia of Plant Physiology, New Series, Vol. 12C, Physiological Plant
 Ecology III, Responses to the Chemical and Biological Environment. O.L. Lange,
 P.S. Nobel, C.B. Osmond, and H. Ziegler (Eds.), Springer-Verlag, Berlin, 245.
- Ye, Z.H., Baker, A.J.M., Wong, M.H. (1994). Heavy Metal Tolerance, Uptake and Accumulation in Populations of *T. latifolia* L. and *Phragmites australis* (Cav.) Trin. ex Steudel. In: Proceedings of the 4th International Conference on Wetland Systems for Water Pollution Control. Guangzhou, China. ICWS '94 Secretariat, Guangzhou, China.

CHAPTER 2 – UPTAKE OF HEAVY METALS BY SUBARCTIC PLANTS: A GREENHOUSE STUDY

Abstract

The purpose of this experiment was to investigate the ability of subarctic wetland plants to remove heavy metals from wastewater such as roadway runoff, drainage from mining operations or municipal waste effluents. Five species, Arctophila fulva, Carex rhynchophysa, Menyanthes trifoliata, Scirpus validus and Typha latifolia were watered with four metals: cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). This mesocosm experiment was conducted in a greenhouse over 68 days under a 20-hour photoperiod. Nitrate salts of the metals were added to the water and provided to plants on a regular schedule at a concentration of 10 mg L^{-1} . After harvest, each plant was separated into five parts: stem. leaf. flower/seed, root and rhizome. Each part was dried, ground and analyzed in a direct coupled plasma spectrophotometer to determine heavy metal concentration. There were significant differences in uptake capabilities among species by metal and metals were differentially sequestered in below-ground plant structures compared to above-ground structures. In separate experiments, plants took up zinc in greater quantities than the other metals except for A. fulva, which took up copper in the greatest quantity. All of the plants survived and grew while receiving this high concentration of heavy metals. However, T. latifolia and S. validus outperformed others in sequestering metals and would make good selections for constructed wetlands used in treating metal-laden effluent waters.

Key words: <u>Arctophila</u>; <u>Carex</u>; <u>Menyanthes</u>; <u>Scirpus</u>; <u>Typha</u>; Aquatic macrophyte; Constructed wetland: Heavy metal; Subarctic; Cadmium; Copper; Lead; Zinc.

Prepared for submission to *Aquatic Botany* as Maddux, D.C., Uptake of heavy metals by subarctic plants - a greenhouse study.
Introduction

Sources of heavy metals

Although metallic elements are often essential micronutrients for plants, high levels of exposure to many metals are toxic to humans and other organisms (Manahan, 1992; Beltman, et al., 1999). Metals occur naturally in biological systems as a result of weathering and other biogeochemical processes but human activities can greatly increase inputs of metals to freshwater systems (Wetzel, 1983; Sansalone and Buchberger, 1997). The anthropogenic constituents in roadway runoff derive mostly from vehicular wear (deterioration of the brakes, tires, frame and body) and are discharged onto roadway surfaces and ultimately into the surrounding environment. Typical metals found in roadway runoff in both particulate and soluble forms include cadmium, copper, lead, zinc, chromium and iron (Sansalone and Buchberger, 1997). Exposure of mine tailing piles to the atmosphere can release toxic amounts of metals to the environment through oxidation and microbial processes (Wildeman and Laudon, 1989; Macur et al., 2001). Metals commonly found in effluents originating from mining activities include nickel, copper, cobalt and zinc (Eger et al., 1993). Municipal waste streams include household sewage. storm drain effluent and discharges from industrial applications. The heavy metal inputs from these sources are varied. typically including the metals found in roadway runoff and mining operations as well as other metals (Kleiman and Cogliatti, 1998).

Macrophytes as accumulators of metals

Toxic metal ions are thought to enter plant cells via the same uptake pathways as micronutrient metals such as zinc and copper (Silver, 1983; Korshunova et al., 1999). Macrophytes respond to exposure to heavy metals in several ways, with considerable genetic variation among species (Chigbo et al., 1982; Lambers et al., 1998). Macrophyte responses typically fall into three broad categories: avoidance (exclusion), tolerance, and accumulation (Woolhouse, 1983). Plants that tolerate or accumulate metals do not avoid

23

uptake but rather depend on internal processes for protection, such as detoxifying metals with cell-wall binding and/or pumping of ions into vacuoles (Baker, 1987).

One of the purposes of tracking the translocation of heavy metals by plant part in this study was to determine which plants took up the metals in such a way as to remove them from the active biological cycle of the wetland. The primary goal of a constructed wetland built to ameliorate heavy metals from wastewater is to remove the metals from the water column before discharge to a receiving body of water. This is important when considering the question "what happens to the heavy metal once it enters the constructed wetland?" Metals that are taken up by a plant and translocated to above ground tissues will be released back into the water column during senescence and subsequent decomposition (Kadlec, et al., 2000). Metals that are translocated to the root or rhizome will be buried and when the root or rhizome dies the metals should be much less mobile and thus not be bioavailable or at least be much more resistant to being transported downstream. Harvesting wetland plants to recover metals taken up by above-ground tissues is not practical due to the inherent difficulties of harvesting plants in a wetland (Rahmani and Sternberg, 1999). In contrast, particular terrestrial plants that are hyperaccumulators, such as Thlaspi caerulscens, are planted to specifically remove heavy metals from polluted soils and harvesting the plant is part of the treatment process (Lasat, et al., 1996).

Justification for this study

In temperate climates, constructed wetlands or natural wetlands have been engineered to treat wastewater for the past 30 years (Livingston, 1989). However, little or no data exist for such systems in subarctic regions, such as Fairbanks, Alaska. Most constructed wetlands in cold climate regions are either located at much lower latitudes or near coastlines influenced by maritime climates (Jennsen et al., 1996; Mæhlum and Stålnacke, 1999) and therefore not comparable to the colder climate of interior Alaska.

24

The plants used in this study are common species found throughout the subarctic where climatic factors are similar. In this study, the growth, mortality, uptake and sequestration of heavy metals by subarctic plants were examined.

Questions and hypotheses

This research was undertaken for two purposes: first, to determine if macrophytes indigenous to the subarctic are comparable to plants of the same genera in temperate climates in their abilities to take up heavy metals and withstand the toxic effects of these metals; and second, to determine where the metals are translocated within the plants (stem, leaf, flower/seed, root or rhizome) and how this compares to similar studies conducted on plants from temperate climates. The following hypotheses were tested: 1) growth and survival of plants exposed to metals differ when compared to controls not receiving metals; 2) uptake of a given metal differs among plant species; 3) for a given plant species and metal, sequestration differs among plant tissues.

Methods and Materials

Plants used as experimental subjects

The plants used in this study consisted of five genera of macrophytes: <u>Arctophila</u> <u>fulva</u> (Trin.), Anderss. (pendant grass), <u>Carex rhynchophysa</u> C.A. Mey (sedge), <u>Menvanthes trifoliata</u> L. (buckbean), <u>Scirpus validus</u> M. Vahl. (softstem bulrush) and <u>Typha latifolia</u> L. (broad-leaf cattail) (Hultén, 1968). All these genera are abundant in Alaska and the circumpolar north in general. With the exception of <u>Arctophila</u> and <u>Menyanthes</u>, all have been used in constructed wetlands in the contiguous United States as biofilters for pollutant removal. No references have been found to show that <u>Arctophila</u> or <u>Menyanthes</u> have been previously examined for metal uptake ability. The species used vary in habitat requirements and morphological structure. <u>A. fulva</u> is a member of the grass family (Gramineae). It grows to near two meters in height and is found in shallow water less than one meter in depth and in moist ground near bodies of water. <u>C. rhynchophysa</u> is a member of the sedge family (Cyperaceae). The species used in this experiment has an upright form, growing in height to just under one meter. The habitat for this species is ideal when the hydraulic regime fluctuates between high in the spring to low in the fall. <u>M. trifoliata</u> is a member of the gentian family (Gentianaceae). It has a submerged, creeping rootstock from which leaves, branches and flower stems emerge amongst other wetland vegetation. This plant is found in standing water with stems either floating on the water or supported by the stems of other macrophytes. <u>S. validus</u> is a member of the sedge family (Cyperaceae). This plant can attain two meters in height and its habitat is usually in standing water less than one meter in depth. <u>T. latifolia</u> is a member of the cattail family (Typhaceae). This species can attain a height of over two meters. Its habitat usually consists of standing water less than one meter in depth and plant growth comes from a starchy rhizome.

Metals used in the experiment

This project tested four heavy metals: cadmium (Cd), copper (Cu). lead (Pb) and zinc (Zn). These metals were identified by the Nationwide Urban Runoff Program (NURP) as the most prevalent heavy metals found nationwide in urban runoff (Athayde, 1983). These metals are also among the most common in mine drainage (Hedin, 1989; Brodie et al., 1990) and in municipal wastewater effluents.

Experimental design

The project consisted of four completely balanced, randomized experiments, with one experiment for each metal. Each experiment included each of the five genera of macrophytes with half of the plants receiving metal and the other half receiving no metalcontaminated water and thus serving as controls. Each experiment was replicated four

26

times. The 40 pots that constituted each experiment were randomly assigned locations in a specified area of the greenhouse. In all, the four experiments consisted of 160 pots of plants.

Planting procedures

The plants were collected during the growing season from four different natural stands near Fairbanks Alaska, and stored at 4 °C in a cold chamber until mid-December and then moved to the greenhouse. The plant rhizomes and roots were washed to remove all native soil that may have contained pollutants. Each plant was divided into rhizomes or roots that would support new growth, repotted in 1.4 L pots with holes in the bottom edge for drainage. The substrate used was a soil-less, sphagnum peat mix. To approximate a saturated substrate similar to that found in a wetland, each potted plant was placed inside another pot, made impermeable to the passage of water by a 3.78 liter plastic bag as a liner. The outer pots were approximately 3.09 L in size. To keep the potted plant from sitting on the bottom of the outer pot, a 3.8-cm high PVC spacer was placed on the bottom of the outer pot. The potted plant was inserted and the outer pot filled to within 1 cm of the top rim with water fertilized with a commercial nutrient solution of 15 mg L⁻¹ N, 7 mg L⁻¹ P and 14 mg L⁻¹ K. The plants were kept at a temperature of 21 °C. Natural sunlight was supplemented by 400-watt sodium vapor high-density lamps suspended one meter above the bench adding to the natural sunlight for a light:dark cycle of 20:4 hours.

Dosing procedure

The heavy metals used were in the nitrate salt form, i.e. cadmium nitrate $Cd(NO_3)_2$. copper (II) nitrate trihydrate $Cu(NO_3)_2$ 3H₂O, lead nitrate Pb(NO₃)₂ and zinc nitrate hexahydrate $Zn(NO_3)_2$ 6H₂O, at a concentration of 10 mg salt L⁻¹. This resulted in actual metal concentrations of 4.754 mg Cd L⁻¹ (0.042 *mM*), 2.630 mg Cu L⁻¹

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(0.041 mM), 6.256 mg Pb L⁻¹ (0.03 mM) and 2.197 mg Zn L⁻¹ (0.034 mM). The trace metals copper and zinc as EDTA complexes found in the fertilizer were negligible compared with what was added. The metal concentrations used in this study were higher than those found in roadway runoff reported by NURP. NURP field data were approximately 93 μ g L⁻¹ for Cu, 350 μ g L⁻¹ for Pb and 500 μ g L⁻¹ for Zn (Athayde, 1983). The concentrations used in this experiment are representative of what might be expected from the "first flush" of a storm event or soil water of sediments with accumulated metals (Laxen and Harrison, 1977; Shutes et al., 1993). This dosage was consistent for the duration of the experiment. For the first watering of heavy metals, all standing water was removed from the pots. The pots were filled to within 1 cm of the top rim of the outer pot with water containing heavy metals, thus fully submerging the inner pot and its substrate surface. Every seven days each pot was refilled to the same level with water containing the heavy metal dosage. Four days following the heavy metal watering, each plant was watered with fertilized water only, again to within 1 cm of the top rim of the outer pot. The control plants were treated with the same procedures except they received fertilized water when the treatment plants received heavy metal and fertilized water.

Sample preparation

After 68 days each plant was measured for stem length and stem count then removed from its pot. A soil sample was collected and the root or rhizome was washed with reverse osmosis water to remove all soil. The plant was separated into five components of stem, leaf, flower, root, and rhizome, then dried to a constant weight at 80°C (Campbell and Plank, 1998). The samples were ground to pass through a 0.5-mm mesh screen. Sample preparation and digestion procedures followed standard methods 3111 B procedures for heavy metal determination by direct coupled plasma (DCP) spectrophotometric methods (American Public Health Association, 1995).

Statistics

The data from each experiment were analyzed using 2-way analysis of variance with a post-hoc Tukey-Kramer HSD test for mean separation when significant effects were found. The level of significance for the analysis was set at p < 0.05 (Taylor, 1990). The statistical software used for analysis was JMP version 3, Statistical Discovery Software (SAS Institute Inc., 1998).

Results

There were no significant differences between the treatment and control plants for stem length (Figure A-1) or stem counts (Figure A-2). There were no obvious differences in plant vitality between the control and treated plants except in the Cd treatment of <u>M. trifoliata</u> where the treated plants appeared to be dying by the end of the experiment. The first noticeable effect of Cd on <u>M. trifoliata</u> was that new leaves emerged with irregular shapes by day 35 and 90% of the leaves were dead by day 42. There were no significant differences in plant biomass between the treatment and control plants with any of the metals except that biomass was lower for <u>M. Trifoliata</u> with Cd, <u>C. rhynchophysa</u> with Cu and <u>A. fulva</u> and <u>T. latifolia</u> with Pb and Zn (Figure 2-1).

There were substantial differences among plant species in tissue concentrations of each metal (Figure 2-2). Plant tissue concentration of Cd was significantly greater for <u>S. validus</u> and <u>T. latifolia</u> compared to the other three species. Cu concentration in <u>S. validus</u> was significantly greater than the other species, except for <u>A. fulva</u>. Pb concentration was significantly greater in <u>S. validus</u> than in <u>A. fulva</u>. Concentration of Zn was significantly greater in <u>S. validus</u> and <u>T. latifolia</u> than in <u>C. rhynchophysa</u> and <u>M. trifoliata</u>. Location of the stored metals between above-ground tissues and below-ground tissues varied among the species for each metal. All plants except <u>M. trifoliata</u> stored more Cd in the below-ground tissues than the above-ground tissues (Figure 2-3). For Cu and Pb, all plants except <u>C. rhynchophysa</u> stored more metal in below-ground

tissue than above-ground. However for Zn, <u>A. fulva</u> stored significantly more metal in the above-ground tissues than in the below ground tissues (Figure 2-3). Both <u>S. validus</u> and <u>T. latifolia</u> always stored the largest proportion of metals taken up in below-ground tissues.

There was no flow of water out of the pots except for evapotranspiration and thus the metal not taken up by the plants remained in the pot, either bound to the soil or dissolved in the water. Thus concentrations increased each week. In general, the amount of metals in the surrounding soil and water of the plant roots were considerably higher than that found in the plant tissue (Table 2-1, Figure 2-4), with some exceptions for Cu and Zn.

There were clear differences in translocation of the metals to different plant parts. (Table 2-2, Figure 2-5). Although not always significant, the roots of every plant except the <u>A. fulva</u> that received Zn treatment sequestered more metal than the other plant parts. <u>M. trifoliata</u> was the only plant to store near equal amounts of Cd in the root and stem. Of the four metals, Zn was the most evenly distributed among the plant parts.

The fertilizer used in these experiments had EDTA in the chemical makeup of the trace micronutrients. The presence of such a strong complexing agent alters the behavior of metals and can change the concentration of metal that would normally be available to the plant. This may have had a confounding effect on the results.

Discussion

All plants were watered on the same day, however, the amount each plant received depended on its evapotranspiration which controlled how much water the plant received. It is doubtful that the added nitrogen from the metal nitrate salt had any biological influence on the growth of the plants (Figures A-3 to A-6).

30

Although this study was done in a greenhouse, the plants were collected from wild populations growing in a subarctic environment. The analysis of the uptake of heavy metals by these subarctic wetland plant species shows that the macrophytes' ability to remove heavy metals is comparable to those from temperate climates. In most instances the results from this research were comparable to previous studies; however there were some departures from previously published results. A project conducted by Meiorin (1989) showed that in general <u>T. latifolia</u> has a greater ability to accumulate heavy metals than <u>S. validus</u> but this study indicated just the opposite, with <u>S. validus</u> have a remarkable ability to withstand and remove heavy metals, including Cd, Cu, Pb, and Zn, from wastewater. Both genera seem to be efficient at removing a variety of metals and have the ability to withstand high concentrations in the effluent (Meiorin, 1989; Daukas et al., 1989; and Crites et al., 1997).

Many experiments conducted with macrophytes and heavy metals have included various species of <u>Carex</u> (Sobolewski, 1996; Sanders et al., 1999) and past results typically have indicated this genus is a desirable plant for metal removal (Howard et al., 1989; Brodie, 1993). However, no reports were found in the literature on heavy metal uptake or tolerance by <u>C. rhynchophysa</u>. In this study, <u>C. rhynchophysa</u> thrived under the high concentration metal regime of the experiment, but in most cases it was the lowest ranked plant for metal removal efficiencies. This species appears to have been an avoider rather than an accumulator of heavy metals and it is possible that under such a heavy effluent concentration <u>C. rhynchophysa</u> has a mechanism to slow uptake simply for self preservation. However, because of its survival potential <u>C. rhynchophysa</u> may be a desirable plant for recolonizing a wetland that has been debilitated by heavy metal pollutants.

Two macrophytes, <u>A. fulva</u> and <u>M. trifoliata</u>, previously unstudied for metal removal, performed well when compared with the other species. <u>A. fulva</u> removed more

31

copper than either <u>S. validus or T. latifolia</u>, and <u>M. trifoliata</u> removed more metal except Zn than <u>C. rhynchophysa</u>. While in general these two species did not outperform <u>S. validus</u> and <u>T. latifolia</u>, they did outperform <u>C. rhynchophysa</u> and showed comparable growth to the other species. thus adding to the selection of potential plants for wetland construction and rehabilitation.

It is apparent that the uptake of the metals differentially affected biomass production among plant species (Figure 2-1) consistent with results obtained from previous experiments (Demchik and Garbutt, 1999). <u>A. fulva</u> was the only plant to consistently show greater growth of the controls over the metal-treated plants. The relatively small differences in biomass production between controls and treated plants show that these species can thrive in heavy metal concentrations much higher than what could be expected at background levels and thus would survive and grow in a wetland fed by storm water runoff.

Metal concentrations in the soil and water of these experiments (Figure 2-4) were high but not unrealistic when compared to environmental field conditions where metalladen water and soils are found (Shutes et al., 1993; Baker and Brooks, 1989). Despite the high concentrations of metals, the plants thrived in all cases but one. However, due to the presence of EDTA in the fertilizer used in this study, the concentrations of metals found in the water was much higher than expected. The effect of EDTA on the metals would be to change the partitioning of the metal among the plant, soil and water, with the concentration of metals in the soil much lower than expected and the metal concentration in the water much higher than expected. This makes the metals more bioavailable to the plant than would be expected in typical roadway runoff. For example, Pb would be strongly partitioned to the soil in the absence of EDTA. In general, it appears that the order of tissue storage (mg metal/kg plant tissue) across all metals by plant was <u>S. validus</u> > <u>T. latifolia</u> > <u>A. fulva</u> > <u>M. trifoliata</u> > <u>C. rhynchophysa</u> (Figure 2-2). <u>S. validus</u> and <u>T. latifolia</u> had the highest tissue concentrations of metals overall; the other species did not show consistency in metal storage capability across all metals.

While not statistically comparable, micronutrient metals (Cu, Zn) usually appeared to be taken up more than non-micronutrient metal (Cd, Pb) uptake, as would be expected. However, this was not the case of <u>T</u>. <u>latifolia</u>, where Cd was taken up in greater quantities than Cu (Figure 2-2). The greater uptake of Zn over Cu was also expected, since Cu is required in such small amounts, compared to Zn, for plant growth (Salisbury and Ross, 1991). Metals were preferentially sequestered in roots as was evident by comparing the percentage of the total amount of metal accumulated in a plant part with the percentage of dry weight by plant part (Table 2-2). This is consistent with the results of studies done on other aquatic plants (Zhu et al. 1999; Qian et al. 1999) where metal concentration differences between root tissue and other plant part tissues ranged from 4-fold to 60-fold. For <u>T</u>. <u>latifolia</u> specifically, Shutes et al. (1993) found that 50-62% of the same metals (Cd. Cu. Pb. Zn) taken up was stored in the rhizome, 30-33% in the leaf and 6-10% in the root. In contrast, results for <u>T</u>. <u>latifolia</u> from this study found 9-29% was stored in the rhizome, 3-15% in the leaf and 48-86% was stored in the root.

Based on the results from this project and on similar studies, a constructed wetland designed to remove heavy metals from wastewater would place <u>S. validus</u> and <u>T. latifolia</u> near the effluent inlet. These two species survived well when subjected to a concentrated application of heavy metals and removed the most heavy metals when compared with the other species. If a particular metal is targeted for removal, for example zinc from mine drainage, the results from this study can help in selection of the macrophyte species most suited for the constructed wetland. Each wetland macrophyte possesses a unique

combination of responses to heavy metals: the ability to survive and grow; the capacity to take up metals; and the ability to sequester metals among various plant parts. Knowledge of the responses of wetland plants to heavy metals can help managers design wetland communities uniquely suited as heavy metal biofilters.

Literature Cited

American Public Health Association, 1995. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington D.C.

Athayde, D.N., 1983. Results of the nationwide urban runoff program. U.S. Environmental Protection Agency, Washington D.C. Accession number: P884-185545.

Baker, A.J.M., 1987. Metal tolerance. New Phytol. 106 (Suppl.), 93-111.

Baker, A.J.M, Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery, Vol. 1, Academic Publishers, Great Britain, 81-126.

Beltman, D.J., Clements, W.H., Lipton, C. D., 1999. Benthic invertebrate metals exposure accumulation, and community-level effects downstream from a hard-rock mine site. Environ. Toxicol. Chem. 18, 299-307.

Brodie, G.A., 1993. Staged, aerobic constructed wetlands to treat acid drainage: case history of Fabius Impoundment 1 and overview of the Tennessee Valley Authority's program. In: Moshiri, G. A. (Ed.), Constructed wetlands for water quality improvement, Lewis Publishers, Louisiana, pp. 157-166.

Brodie, G.A., Britt, R.C., Taylor, T.M., Tomaszewski, D. T., 1990. Passive anoxic limestone drains to increase effectiveness of wetlands acid drainage treatment systems. Proceedings: 12th Annual NAAMLP Conference, Breckinridge, CO. September 16-20, 1990.

Campbell, C.R., Plank, C.O., 1998. Preparation of Plant Tissue for Laboratory analysis. In: Kalra, Y. P., (Ed.), Reference Methods for Plant Analysis. CRC Press, Boca Raton, Florida, 39.

Chigbo, F.E., Smith, R.W., Shore, F.L., 1982. Uptake of arsenic, cadmium, lead and mercury from polluted waters by the water hyacinth *Eichhornia crassipes*, Environ. Pollut. (Ser. A.) 27, 31-39.

Crites, R.W., Combeck, G.D., Watson, R.C., Williams, C.R., 1997. Removal of metals and ammonia in constructed wetlands. Water Environ. Res. 69, 132-135.

Daukas, P., Lowry, D., Walker Jr., W.W., 1989. Design of wet detention basins and constructed wetlands for treatment of stormwater runoff from a regional shopping mall in Massachusetts. In: Hammer, D.A., (Ed.), Constructed wetlands for wastewater treatment. Lewis Publishers, Michigan, pp. 686-694.

Demchik, M., Garbutt, K., 1999. Growth of woolgrass in acid mine drainage. J. Environ. Qual. 28, 243-249.

Eger, P., Melchert, G., Antonson, D., Wagner, J., 1993. The use of wetland treatment to remove trace metals from mine drainage. In: Moshiri, G. A. (Ed.), Constructed wetlands for water quality improvement, Lewis Publishers, Louisiana, pp. 171-178.

Hedin, R.S., 1989. Treatment of coal mine drainage with constructed wetlands. In: Majumdar, S.K., Brooks, R.P., Brenner, F.J., Tiner Jr., R.W. (Eds.), Wetlands ecology and conservation: Emphasis in Pennsylvania. The Pennsylvania academy of Science, pp. 348-362. Howard, E.A., Emerick, J.C., Wildeman, T.R., 1989. Design and construction of a research site for passive mine drainage treatment in Idaho Springs, Colorado. In: Hammer, D.A. (Ed.), Constructed wetlands for wastewater treatment. Lewis Publishers, Michigan, pp. 761-764.

Hultén, E., 1968. Flora of Alaska and neighboring territories: a manual of the vascular plants. Stanford University Press, California.

Jennsen P.D., Mæhlum, T., Zhu, T., 1996. Design and performance of subsurface flow constructed wetlands in Norway. In: Lemon, E. (Ed.), Proceedings of the Symposium on constructed wetlands in cold climates. Ontario, Canada, pp. 100-112.

Kadlec, R.H., Knight, R.L., 1995. Treatment wetlands. Lewis Publishers, Florida.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P., Haberl, R., 2000. Constructed wetlands for pollution control. processes, performance, design and operation. Scientific and Technical Report No. 8. IWA Publishing, London, England.

Kleiman, I.D., Cogliatti, D.H., 1998. Chromium removal from aqueous solutions by different plant species. Environ. Technol. 19, 1127-1132.

Korshunova, Y.O., Eide, D., Clark, W.G., Guerinot, M.L., Pakrasi, H.B., 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. Plant Mol. Biol. 40, 37-44.

Lambers, H., Chapin III, F. S., Pons, T.L., 1998. Plant physiological ecology. Springer-Verlag, New York. Lasat, M.M., Baker, A.J.M., Kochian, L.V., 1996. Physiological characterization of root Zn^{2-} absorption and translocation to shoots in Zn hyperacccumulator and nonaccumulator species of *Thlaspi*. Plant Physiol. 112, 1715-1722.

Laxen, D.P.H., Harrison, R.M., 1977. The highway as a source of water pollution: an appraisal with the heavy metal lead. Water Res. 11, 1-11.

Livingston, E.H., 1989. Use of wetlands for urban stormwater management. In: Hammer, D.A. (Ed.), Constructed wetlands for wastewater treatment. Lewis Publishers, Michigan, pp. 253-262.

Mæhlum, T., Stålnacke, P., 1999. Removal efficiency of three cold-climate constructed wetlands treating domestic wastewater: effects of temperature, seasons, loading rates and input concentrations. Wat. Sci. Tech. 40, 273-281.

Macur, R.E., Wheeler, J.T., McDermott, T.R, Inskeep, W.R., 2001. Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. Environ. Sci. Technol. 35, 3676-3682.

Manahan, S., 1992. Toxicological chemistry. Lewis Publishers, Florida.

Meiorin, E.C., 1989. Urban runoff treatment in a fresh/brackish water marsh in Fremont. California. In: Hammer, D.A. (Ed.), Constructed wetlands for wastewater treatment. Lewis Publishers, Michigan, pp. 677-685.

Mitsch. W.J., Gosselink, J.G., 1992. Wetlands. Van Nostrand Reinhold, New York.

Qian, J.H., Zayed, A., Zhu, Y.L., Yu, M., Terry, N., 1999. Phytoaccumulation of trace elements by wetland plants: III. Uptake and accumulation of ten trace elements by twelve plant species. J. Environ Qual. 28, 1448-1455.

Rahmani, G.N.H., Sternberg, S.P.K., 1999. Bioremoval of lead from water using Lemna minor. Biores. Technol. 70, 225-230.

Salisbury, F.B., Ross, C.W., 1991. Plant Physiology. Wadsworth Publishing Company, California.

Sanders, F., Rahe, J., Pastor, D., Anderson, R., 1999. Wetlands treat mine runoff. Civil Eng. 69, 52-55.

Sansalone, J.J., Buchberger, S.G., 1997. Partitioning and first flush of metals in urban roadway storm water. J. Environ. Eng. 123, 134-143.

SAS Institute Inc., 1998. JMP statistical software, version 3.2. Cary, NC.

Shutes. R.B., Ellis, J.B., Revitt, D.M., Zhang, T.T., 1993. The use of <u>T. latifolia</u> for heavy metal pollution control in urban wetlands. In: Moshiri, G. A. (Ed.), Constructed wetlands for water quality improvement. Lewis Publishers, Louisiana, pp. 407-414.

Silver, S., 1983. Bacterial interactions with mineral cations and anions: good ions and bad. In: Westbroek, P., deJong, E.W. (Eds.), Biomineralization and biological metal ion accumulation. Amsterdam, pp. 439-437.

Sobolewski, A., 1996. Metal species indicate the potential of constructed wetlands for long-term treatment of metal mine drainage. Ecol. Eng. 6, 259-271.

Taylor, J.K., 1990. Statistical techniques for data analysis. Lewis Publishers, Florida.

Watson, J.T., Reed, S.C., Kadlec, R.H., Knight, R.L., Whitehouse, A.E., 1989.
Performance expectations and loading rates for constructed wetlands. In: Hammer, D.A. (Ed.), Constructed wetlands for wastewater treatment, Lewis Publishers, Michigan, pp. 319-351.

Wetzel, R.G., 1983. Limnology. Saunders College Publishing, Texas.

Wetzel, R.G., 1993. Constructed wetlands: scientific foundations are critical. In: Moshiri, G. A. (Ed.). Constructed wetlands for water quality improvement. Lewis Publishers, Louisiana, pp. 3-8

Wildeman, T.R., Laudon, L.S., 1989. Use of wetlands for treatment of environmental problems in mining: non-coal mining applications. In: Hammer, D.A. (Ed.), Constructed wetlands for wastewater treatment, Lewis Publishers, Michigan, pp. 221-231.

Woolhouse, H.W., 1983. Toxicity and tolerance in the responses of plants to metals. In: Lange, O.L, Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Encyclopedia of plant physiology. New Series, Vol. 12C, Physiological plant ecology III, Responses to the chemical and biological environment. Springer-Verlag, Berlin, pp. 245-300.

Zhu, Y.L., Zayed, A.M., Qian, J.H., de Souza, M., Terry, N., 1999. Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J. Environ. Qual. 28, 339-344.



Figure 2-1 Average dry biomass of plants treated with heavy metals compared with control plants grown without addition of metals. These subarctic indigenous plants were grown in a greenhouse and subjected to cadmium, copper, lead and zinc. Ar = Arctophila fulva, Cx = Carex rhynchophysa, My = Menyanthes trifoliata, Sc = Scirpus validus and Ty = Typha latifolia. * indicates significant difference (p < 0.05) between metal treated plants and controls for each species. n = 4 for all histogram bars. Error bars are ± 1 S.E.





Figure 2-2 Metal concentrations by metal and plant. Significant (p < 0.05) pair-wise difference in plant tissue concentration for a given metal indicated by different lower-case letters (a,b,c). Aqueous metal ion concentrations were $Cd = 4.754 \text{ mg L}^{-1}$, $Cu = 2.630 \text{ mg L}^{-1}$, $Pb = 6.256 \text{ mg L}^{-1}$ and $Zn = 2.197 \text{ mg L}^{-1}$. Ar = <u>Arctophila fulva</u>, Cx = Carex rhynchophysa, My = Menyanthes trifoliata, Sc = Scirpus validus, Ty = Typha latifolia. n = 4 for all histogram bars. Error bars are ± 1 S.E.



Figure 2-3 Average percent of metal storage above and below ground in plant tissue. Above ground parts compared to below ground parts for Cadmium, Copper, Lead and Zinc. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus</u> validus and Ty = <u>Typha latifolia</u>. * indicates significant difference (p < 0.05) for each plant. n = 4 for all histogram bars. Error bars are \pm 1 S.E. * above column signifies a significant difference in that pair. Number above each pair is the total amount of metal found in the plant in mg.



Figure 2-4 Metal distribution among plant, soil and water at conclusion of the experiment. Number above each group represents the total amount of metal given during the experiment. Quantities of metal in plant, soil and water sum to the total quantity of metal given during the experiment, for metals cadmium, copper, lead and zinc. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex</u> <u>rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are ± 1 S.E. Note that Y - axis scales vary for each metal.



Figure 2-5 Metal storage by plant part. The total amount of metal taken up was partitioned by plant part for Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha</u> <u>latifolia</u>. Significant differences (p < 0.05) in metal storage among plant parts for a given species and metal, indicated by different letters (a, b, c). n = 4 for all histogram bars. Error bars are ± 1 S.E.

Table 2-1 Average amount of metal taken up by plant and residual in pot at end of experiment.

Pot indicates metal in soil and water; plant indicates metal in plant tissue;

% in plant indicates the percent of total dose of metal that was taken up by the plant.

n = 4 for each metal and species.

	Arctophila fulva			Carex rynchophysa			<u>Menyanthes</u> trifoliata			<u>Scirpus validus</u>			<u>Typha latifolia</u>		
	Pot r	Plant ng	% in Plant	Pot r	Plant ng	% in Plant	Pot 1	Plant mg	% in Plant	Pot	Plant mg	% in Plant	Pot 	Plant mg	% in Plant
Cd	22.6	3.5	13.3	20. I	3.1	13.2	18.8	3.8	16.8	24.2	5.4	18.2	24.8	8.2	24.9
Cu	4.5	9.1	66.7	7.7	0.8	9.8	7.7	2.3	22.7	7. 7	9.0	54.0	14.0	5.0	26.5
Pb	25.9	0.4	1.4	26.9	2.1	7.3	29.2	2.5	7.9	35.6	4.9	12.0	37.3	2.8	6.9
Zn	7.5	4.7	38.6	7.9	1.6	16.5	4.9	5.6	53.3	9.5	11.6	55.0	7.7	12.8	62.3

Table 2-2 Comparison of accumulation of metals among plant parts. Values are the percentage of total uptake of each metal for each plant species and part. Significant pairwise differences (p < 0.05) in % of total metal uptake among plant parts by row are indicated by different lower-case letters (a, b, c). n = 4 for each species and each treatment. Values in parentheses are the percentage (of total dry weight) of each plant part. (-- indicates < 1% uptake of metal).

Metal	Stem	Leaf	Flower	Root	Rhizome	p-value						
Arctophila fulva												
Cd	20 a (31)	18 a (31)	1 a (7)	62 b (31)		<.0001						
Cu	15 a (28)	14 a (29)	4 a (6)	67 b (38)		<.0001						
РЬ	25 a (28)	19 a (35)	(7)	56 b (30)		.0011						
Zn	37 (27)	14 (31)	21 (5)	27 (37)		.1533						
<u>Carex rhynchophysa</u>												
Cd	29 a (29)	3 b (33)	(5)	68 c (33)		<.0001						
Cu	36 a (26)	6 b (33)	6 b (6)	53 c (35)		<.0001						
РЬ	42 a (29)	5 b (32)	(6)	53 a (33)		.0014						
Zn	26 a (23)	27 a (36)	(3)	47 b (39)		.0257						
<u>Menyanthes</u> trifoliata												
Cd	41 a (41)	12 b (31)		47 a (29)		.015						
Cu	17 a (50)	6 b (25)		76 c (26)		<.0001						
РЪ	32 a (50)	3 b (24)		65 c (26)		<.0001						
Zn	28 a (48)	24 a (22)		48 b (30)		.0024						
<u>Scirpus</u> validus												
Cd	10 a (36)		(9)	69 b (17)	21 a (37)	<.0001						
Cu	14 a (41)		3 b (7)	56 c (18)	27 d (34)	<.0001						
РЬ	27 a (38)		2 b (5)	56 c (20)	18 a (37)	<.0001						
Zn	25 a (43)		3 b (6)	45 a (15)	27 a (36)	.0006						
<u>Tvpha</u> Latifolia												
Cd	2 a (21)	3 a (26)		86 b (13)	9 c (37)	<.0001						
Cu	8 a (22)	15 a (27)		54 b (14)	22 a (37)	<.0001						
Pb	9 a (28)	14 a (26)		48 b (16)	29 c (30)	<.0001						
Zn	19 a (27)	14 a (34)		51 b (15)	17 a (24)	<.0001						

CHAPTER 3 – PERFORMANCE OF A CONSTRUCTED WETLAND FOR WASTEWATER TREATMENT IN SUBARCTIC ALASKA

Abstract

This project was undertaken to determine if constructed wetlands in the subarctic can be an effective form of treatment for sewage wastewater, particularly in rural locations. A five-cell system was built in the summer of 1997 at the University of Alaska Fairbanks. Five species of wetland plants, Arctophila fulva, Carex rhynchophysa, Menyanthes trifoliata, Scirpus validus and Typha latifolia were used in the experiment. Swine effluent from a lagoon provided the effluent source at a hydraulic loading rate of 2.17 cm d⁻¹. Biological oxygen demand (BOD), total suspended solids (TSS), Fecal Coliforms (FC), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH₄⁺-N) and total phosphorus (TP) were measured over a three-year period. During the ice-free seasons over the three year period, reduction efficiencies ranged from 24%-67% for BOD; 38-62% for TSS; 93-99% for FC; 43-76% for TKN; 50-92% for NH₄⁺-N and 21-60% for TP. Although effluent exiting the constructed wetland did not consistently meet Alaska Department of Environmental Conservation discharge standards, an effective reduction of pollutants indicates the ability of constructed wetlands to work well in the subarctic. Vegetation colonized rapidly in the constructed wetland. Pollutant reduction appears to be limited by the size of the constructed wetland and not by the extreme climatic conditions.

Keywords: constructed wetland, subarctic, biological oxygen demand, total suspended solids, fecal coliforms, phosphorus, Kjeldahl nitrogen, ammonium-N.

Prepared for submission to *Water Environment Research* as Maddux, D.C., Performance of a constructed wetland used for wastewater treatment in subarctic Alaska.

Introduction

Constructed wetlands for sewage treatment in cold regions

Sewage wastewater disposal in the subarctic has always created problems for communities, large and small, urban and rural. However, it is the rural communities that are most burdened with disposal costs, both in real dollars and in negative impacts to the environment. The goal of this research was to determine if constructed wetlands can provide rural communities with a low technology, reasonably inexpensive and safe method of treating wastewater.

Functions of wetlands

The macrophyte-microbe complex in wetlands is responsible for many functions (Kadlec and Knight, 1995, and Portier and Palmer, 1989). The microbe community found growing on the submerged plants and on the detrital mat of the substrate is collectively called periphyton. The plant rhizosphere also supports large microbial populations that transform metallic ions, nutrients and other compounds (Hammer and Bastian, 1989). Thus, two important roles that macrophytes play in constructed wetlands do not depend on their uptake of nutrients, but on the surface area they provide on their submerged stems for periphyton attachment (Hammer, 1994, and Soto et al., 1999) and as a detrital carbon source for microbes upon senescence (Brix, 1997, and Chappell and Goulder, 1994). Wetlands are the only landscape form that has a reducing environment as a major component of its ecosystem (Kadlec et al., 2000 and Hammer and Bastian, 1989). Rates of decomposition and mineralization of large quantities of organic matter made by primary producers are significantly reduced under these conditions so that particulate organic matter accumulates (Kadlec, 1989). However, dissolved and particulate organic carbon is transformed by microbial decomposition to CO₂ and CH₄ which are lost to the atmosphere, thus permanently removing this material from the constructed wetland (Kadlec and Knight, 1995). In this way, carbon in a constructed wetland is constantly being turned over and accretion of new soil is slowed to an average of approximately 2-5 mm/year in temperate climates (Richardson and Craft, 1993).

In temperate climates constructed wetlands or natural wetlands have been used to treat wastewater for the past 30 years (Livingston, 1989). However, little or no data exist for such systems in the subarctic such as Fairbanks, Alaska. Most other cold climate regions that have used constructed wetlands are either located at lower latitudes or near coastlines influenced by maritime climates (Jennsen et al., 1996, and Mæhlum, 1999) and are therefore not comparable to the cold climate of interior Alaska. Lakshman (1994) developed guidelines for the use of constructed wetlands in cold climates. However the constructed wetlands she studied in Canada were considerably south of Interior Alaska. Thus it was unknown how well a constructed wetland built for treating sewage wastewater would function in a more northern climate.

One of the conditions that make treatment possibilities so different at this location (64.51 ° N Lat. 147.43° W Long.) is the abbreviated growing season and seasonally eccentric photoperiod. In the Interior of Alaska, most wetland plants do not start to sprout until the first week in June and the first killing frost usually occurs by the second week of September, which provides about 100 treatment days per season. June averages 22 hours per day of potential sunlight, July 20 hours. August 16 hours and September 13 hours (American Ephemeris, 1946). This provides a short window for treatment of yearly effluent flow. However, the long day lengths allow for almost continuous photosynthetic production, which in turn drives aerobic microbial transformations of pollutants in the rhizosphere and gaseous exchange between the rhizosphere and the atmosphere.

Project Description

The objective of this project was to determine if a constructed wetland system will successfully treat sewage wastewater in a subarctic environment. The expense of building a research project in a remote location was prohibitive, therefore the project was constructed at the Fairbanks Experiment Farm on the campus of the University of Alaska Fairbanks. Effluent from a swine sewage lagoon was readily available and this effluent is

50

very similar to human sewage lagoon effluent except it is more concentrated in BOD, N, TP and FC (Kadlec and Knight, 1995).

Research Questions

The constructed wetland was comprised of five rectangular cells, four planted with emergent macrophytes indigenous to Alaska and one unvegetated serving as an open-water comparison. The cells were assessed for reduction capability for six components of the swine sewage supplied to the cells: BOD, TSS, FC, TP, TKN and NH₄⁺-N. Performance of the cells was assessed from initial planting over three years of successional development. The purpose of this project was to assess how well a constructed wetland would reduce sewage effluent pollutants at this latitude.

This research focussed on five questions:

- 1. Was the size of the constructed wetland adequate to remove pollutants from the wastewater to background levels?
- 2. What was the spatial pattern in reduction of effluent components? As effluent traveled the length of the cells of the constructed wetlands, what was the balance of reduction, transport and regeneration of pollutants?
- 3. What was the seasonal pattern in reduction of effluent components? How did processing of effluent components respond to changing effluent concentrations and to the seasonal dynamics of wetland processes?
- 4. How did the succession of the constructed wetland affect reduction of effluent components? Over the three years of this study, how did growth and colonization of the macrophytes affect the ecosystem properties of the constructed wetland and its ability to process effluent components?

5. How did the vegetated cells and the unvegetated (open water) cell compare in processing of effluent components? Were there differences in vegetated and open water systems that are important in the function of constructed wetlands?

Methods and materials

This constructed wetland was comprised of five separate cells parallel to each other (Figure 3-1). Each cell was a replicate of the other cells, with four cells being vegetated and one cell unvegetated, acting as an open-water comparison. The dry dimension at the bottom of each cell was one meter wide by 18.3 meters long with side slopes of 2:1. After flooding to a depth of 30 cm, the surface dimension of each cell was 2.4 meters wide by 19.2 meters long. The slope of the cell bottom between the inlet and outlet of each cell was 0.1%. Soil berms separating each cell were one meter higher in elevation than the bottom of the cell with a one-meter wide walkway on top of the berm. This walkway facilitated movement between the cells for inspection and data gathering without disturbing the cell itself. The overall footprint of the constructed wetland was 40 meters by 35 meters.

The pre-existing use of the constructed wetland site was a hay field. Soil from the site was used to build the berms for the cells and provide topsoil. Each cell and berm was covered with an individual sheet of 30 mil high density polyethylene (HDPE) liner material. This liner was then covered with a 30-cm layer of topsoil providing the substrate for the macrophytes and other vegetative growth. A 5-cm diameter PVC pipe carried the effluent to each cell via gravity flow. The inlet to each cell consisted of a 6.4-mm orifice to control the flow rate. At the outlet end of each cell a 5-cm diameter pipe was installed through the end of the berm and connected to a main collection drain line that flowed to a collection sump pit. Inside each cell, the 5-cm diameter pipe was connected to a rotating elbow and a 76-cm tall standpipe that acted as the outlet control structure, providing the swivel action necessary to regulate the effluent level in the cell.

Plant species

The five species of plants used in this project were <u>Arctophila fulva</u> (Trin.). Anderss. (pendant grass), <u>Carex rhynchophysa</u> C.A. Mey (sedge), <u>Menyanthes trifoliata</u> L. (buckbean), <u>Scirpus validus</u> M. Vahl. (softstem bulrush) and <u>Typha latifolia</u> L. (broadleaf cattail) (Hultén, 1968). All are indigenous to the circumpolar north. south of the 68th parallel, with <u>A. fulva and C. rhynchophysa</u> also found north of the 68th parallel. Three genera, <u>Carex</u> <u>Scirpus</u> and <u>Typha</u> have previously been used for pollutant reduction in temperate climates (Burgoon et al., 1999, and Cole and Brooks, 2000). Two other genera, <u>Arctophila</u> and <u>Menyanthes</u>, are abundant in Alaska but no references were found to indicate they had been previously used in wetlands constructed for pollutant reduction.

Experimental design, planting procedures

For the planting design, each cell was divided into three equal lengths of 6.1 meters, demarcated as Station A (Sta. A), Station B (Sta. B) and Station C (Sta. C), with no physical barriers between the stations. Each station was located at the end of the respective 6.1 meter length (Figure 3-1). Plants were randomly assigned a location within one station on a 30 cm by 46 cm pattern. Each station for every cell was a replicate of this planting scheme with the macrophytes planted in exactly the same pattern.

Statistical analysis

The dependent variables in all analyses were the quantities of each effluent pollutant as a proportion of the input values (i.e., the proportion remaining at each station A, B or C of the quantity entering the cell on a sampling date). The significance of the sampling date over each sampling season and the significance of the station within vegetated cells was analyzed with 2-way ANOVA. Pair-wise differences in dates or stations were assessed with Tukey-Kramer's HSD (at $p \le 0.05$ significance level). Statistical analyses were done using JMP version 3 Statistical Discovery Software (SAS Institute, Inc., 1998).

Treatment procedure

Effluent was pumped from the swine lagoon to a 5,678 liter mixing tank. Since previous studies have shown that ammonium-N concentrations > 100 mg L⁻¹ are detrimental to many macrophytes (Hammer, 1994), the effluent was mixed with fresh water in the proportion necessary to bring ammonium levels below 100 mg L⁻¹ before introduction to the cells. The hydraulic loading rate was 2.17 cm d⁻¹ (1136 L d⁻¹) and the cells were pulse loaded once per day. For the 1998 season, flow began on 6-09-98 and ended on 10-06-98; for the 1999 season, flow began on 6-02-99 and ended on 10-04-99; and for the 2000 season, flow began on 6-07-00 and ended on 9-27-00. End of season samples (the last two sampling dates in 1998 and 1999 and the last sampling date in 2000) were taken from under an ice cover.

Sampling procedure

Samples were collected on a bi-weekly basis from the mixing tank and Sta. A, B and C for all five cells for years one and two during the sampling season of mid-May to mid-October. Due to funding restrictions, samples for year three were collected from the mixing tank and Sta. A. B and C from cell 1 (vegetated) and from Sta. C only from all other cells. As a result of the limited data set for year three, statistical analysis was done only for Sta. C. Year three data are included to highlight continuing trends in the functionality of the system. Water quality parameters measured were BOD, TSS, FC, TKN, NH4⁻-N and TP. All water quality analysis followed methods 5210-B, 2540-D, 922-D, 4500-N, 4500-NH, and 4500-P described in Standard Methods (American Public Health Association et al., 1998). Dissolved CO₂ was measured with a Hach DR/2010 Spectrophotometer, method 8205. Dissolved oxygen was measured with YSI dissolved oxygen meter models 54A, 56 and 95 and pH was measured with a Hach pH meter model EC-10. Air, water and soil temperatures were measured with hand held thermometers and temperature probes combined with O₂ meters and automatic data loggers were submerged in the water of cells 1 and 3. Water temperatures were measured at 15 cm below the water surface and 8 cm below the soil surface.

Results

The mean dissolved oxygen for the vegetated cells was at supersaturated levels (> 9.1 mg L⁻¹ at 20 °C) in year one whereas in year two and three O₂ levels were frequently approaching anaerobic conditions (Figure 3-2). The unvegetated cell had the opposite trend in O₂ concentrations with years two and three having supersaturated O₂ concentrations. Seasonal trends did not follow an obvious pattern. CO₂ data was collected in year two (1999) only. The fluctuation in CO₂ concentration seems to follow blooming and senescence of algal populations in the cells (Figure 3-3). Although it was not possible to test statistically, the data for pH levels indicated that the unvegetated cell always maintained a more alkaline environment than the vegetated cells, which were usually between a pH of 7 and 8 (Figure 3-4).

The water and soils warmed rapidly at the beginning of the ice-free season. reaching 10 - 12 °C by May (Figure 3-5). Water and soil temperatures were always lower than air temperatures. Year one had the highest water and soil temperatures, which indicated the effect vegetation had on water and soil temperature. Year one had much less vegetation and much higher soil and water temperatures in general and the temperatures were closer to the air temperature. Years two and three had considerably more vegetation and a much wider difference between the soil and water temperatures compared to air temperature.

BOD inputs to the constructed wetland varied over the three years, with concentrations over the ice-free season increasing each year at the inlet with a corresponding decrease in BOD reduction in the vegetated cells (Table 3-1). Concentration ratios (the percent reduction between the inlet and station) were calculated by subtracting the concentration found at the station from the inlet concentration and dividing that difference by the inlet concentration. There were significant differences in BOD concentration ratios by date and station for all three years (Figures 3-6, 3-7 and 3-8). Concentration ratios were generally lower in year one and year two at Sta. B or C, compared to Sta. A, which indicated a net reduction in BOD over the length of the cells. However, in year three, Sta. C showed net internal regeneration within the cell of BOD for several sampling dates. Variations in concentration ratios among sampling dates were not obviously related to absolute BOD values at the inlet.

TSS inputs to the constructed wetland from the lagoon varied over the three years. with mean values over the ice-free season ranging from a low of 135 mg L^{-1} to a high of 248 mg L^{-1} (Table 3-1). Both vegetated and unvegetated cells internally regenerated TSS at various times either between stations within a cell or from the outlet itself (Sta. C). There were significant differences in the concentration ratios among stations for year two and significant differences among dates for years one and two (Figures 3-9, 3-10 and 3-11). On most sampling dates, concentration ratios were below 1.0, indicating net reduction of TSS from inlet to outlet. The difference among station and dates did not appear to be related to inlet concentrations but rather to growth and senescence of algal populations. Overall reduction of TSS in year one and year two was essentially the same, but in year one most of the TSS was removed by Sta. A. whereas in year two a little less than half was removed by Sta. A. Year two had a more linear distribution in reduction throughout the cell compared to years one and three. In year three TSS was internally regenerated between stations, with approximately one-half of what was removed by the time the effluent had reached Sta. B being added back to the water column before discharge at Sta. C.

Fecal coliform concentrations from the lagoon had a wide range among treatment years, with mean values over the ice-free season ranging from a low of 7,387 cfu/100 ml to a high of 30,531 cfu/100 ml (Table 3-1). Both years one and two had significant differences among the stations and among the dates, but year three did not (Figures 3-12, 3-13 and 3-14). In most cases, fecal coliforms were reduced by at least fifty percent by the time the effluent reached Sta. A when inlet concentrations were above background

levels. Like TSS, most values for the concentration ratio of FC were < 1.0 (net removal) but a few dates showed a net internal regeneration of FC. However, the regeneration occurred only when the FC concentration was extremely low and the apparent regeneration probably represents the background level of FC in the constructed wetland. There was no obvious relationship between FC loading (inlet values) and removal efficiency (concentration ratios) except that regenerations of FC occurred on sampling dates with the lowest FC loading at the inlet on 10-06 for 1998 and 8-02 for 2000.

As shown in Table 3-1 total phosphorus mean values over the ice-free season at the inlet were within 3 mg L⁻¹ for years one and two, but year three had TP concentrations three times higher (Table 3-1). Reduction of TP appeared much lower for years two and three at all stations when compared to year one (Figures 3-15, 3-16 and 3-17). However, on 8-11-98 in year one, which had the lowest inlet values of TP, concentration ratios were high indicating net transport. There were significant differences in TP ratios among dates and among stations in all three years. For year one this may be due to the variation of the inlet concentration over the course of the treatment season, however this variation does not occur as strongly in years two and three.

TKN concentrations at the inlet fluctuated over the three years with mean values over the ice-free season ranging from a low of 30.5 mg L⁻¹ in year two to a high of 82.1 mg L⁻¹ in year three (Table 3-1). There were significant differences among stations and among dates for years one and two and among dates for year three, with less variability among dates in year one than in year two or three (Figures 3-18, 3-19 and 3-20). Concentration ratios were generally < 1.0 (net removal of TKN) except for sampling dates with low inlet values of TKN.

Ammonium inputs to the constructed wetland during the ice-free season were variable over the three year experiment (Table 3-1). There were significant differences in NH₄⁻-N among stations and among dates for all three years (Figures 3-21, 3-22 and

3-23). Overall, NH_4^+ -N was reduced as the effluent flowed through each cell. Individual dates showed a more varied reduction of NH_4^+ -N as the effluent flowed from station to station compared to overall seasonal reductions

Discussion

When sizing a constructed wetland for wastewater treatment purposes the effluent quality parameters are usually known and can be used in calculating the size of the system. The pollutant that is the most difficult to remove becomes the determining size factor, which in the case of sewage wastewater is phosphorus (Bastian and Hammer, 1993; Kadlec and Knight .1995). In this study, the effluent quality parameters were not available before construction of the project began and the size of the constructed wetland was determined by budget and equipment availability.

Using a first order uptake model (k-c* model) developed by Kadlec and Knight (1995).

$$A = \frac{Q}{k} \bullet \ln\left[\frac{C_{in}}{C_{back}}\right]$$

where:

A = wetland area (m²) Q = wastewater flow (m³ year ⁻¹) k = uptake rate constant (m yr ⁻¹) C_{in} = effluent concentration at inlet C_{back} = effluent concentration at outlet

to determine the area required to bring the most difficult pollutant to remove, phosphorus, to background levels, the surface area required for each cell in this project would have been 125 m^2 . The actual surface area of each cell in this project was 46 m^2 and based on the calculation above, the constructed wetland was not large enough for optimum treatment at the concentrations of pollutants in the effluent. Although this
constructed wetland was less than half the optimum size, the average inlet concentrations of all the effluent components measured over the ice-free season were reduced from 21% to 99%, depending on the component, before discharge (Table 3-1).

Due to the pulse loading and daily variation of the input effluent concentration an exponential decrease of pollutants could not be shown, as the k-c* model would indicate. Assuming this constructed wetland would show an exponential decrease nonetheless, k values have been derived. The constant uptake rate k can be thought of as an ecological interest rate, where the higher the k value is the higher the pollutant removal rate is. These k values are specific to this study and the particular conditions it was operated under. They are for the most part considerably lower than the global k values found in the current literature (Table 3-2). Primarily this was due to the undersized treatment area. The shortness of the treatment season and the impact this has on constructed wetland treatment capabilities, e.g. slower movement of pollutants through the microbial cycle and less time for complete decomposition to occur of senesced plant matter, is also a factor. These k values would appear to indicate that design parameters for subarctic constructed wetlands need to take into account the short treatment season and colder temperatures and therefore sizing calculations will be more accurate if local data is used rather than global k values.

The pH values are quite high (Figure 3-3), especially for the unvegetated cell. It appears the increased algal biomass for all cells in year one (1998) and the unvegetated cell in years two and three may have been responsible for the elevated pH, along with high lagoon (inlet) pH values and other unidentified factors. Bavor et al. (1988) found pH levels reaching 9.8 from an unvegetated wetland in their study. Vyzamal (1994) and Cronk and Fennessey (2001) both point out that due to algal photosynthesis, the equilibrium in the CO_2 - HCO_3^2 - CO_3^2 system can increase pH 3 to 4 units.

Overall, reduction of pollutants showed that a substantial portion of reduction occurred between the inlet and Sta. A, with two exceptions. Both BOD and TP were reduced in a more linear fashion as effluent flowed from station to station through the cell. At some point during the sampling season output from cells exceeded inputs i.e., there was a net regeneration for all pollutants studied except FC and NH₄⁺-N. Kadlec et al. (2000) point out that with the increased input of nutrients and the warmer summer temperatures, plant productivity increases as does the decomposition of organic matter. However, averaged over each of the study years, the vegetated cells did not regenerate pollutants. In contrast, the unvegetated cell regenerated BOD and TSS in 1998 and TSS in 1999.

Seasonal changes in algal populations appear to have influenced concentrations of pollutants between stations (Borum, 1983). In particular, Hurse and Conner (1999) point out that algal concentrations influence nitrifier population development, although their paper did not resolve the correlation. In year one, the cells had approximately 95% open water at the beginning of the treatment season (Table 3-3), which provided optimum algal growth conditions of high light availability and abundant nutrients in the effluent. A large bloom of algae occurred in every cell during the warmest period of the summer and a massive die off occurred in the second half of July. The addition of the dead algal cells to the effluent undoubtedly added to the BOD load. In July, the constructed wetland internally regenerated more BOD than the cells received with an average input of 65 mg L⁻¹ and an average discharge of 106 mg L⁻¹. Whenever a large death of algae occurred, usually due to heavy rain during the summer or colder temperatures in the fall, increases in BOD. TSS and TP were evident in effluent discharge concentrations.

The reduction of pollutants typically decreased when an ice cover formed over the cells which agrees with previous studies (Hershkowitz, 1986; Miller, 1989). For example, in the last sampling period of year one, which occurred under a continuous sheet of ice in

October, most cells internally regenerated TP at Sta. A and B but only cell 2 internally regenerated TP between Sta. B and Sta. C.

Pollutant reduction between stations was not the same among years. For example, in the first year the constructed wetland achieved a 53% reduction of TP of the inlet concentration by the time the effluent reached Sta. A. In the second year Sta. A internally regenerated TP and in the third year Sta. A achieved a 52% reduction of TP. The fluctuation of TP as effluent flows through a cell over a period of time is not uncommon and the results of this study follow a similar trend of TP removal shown in a study conducted by Geary and Moore (1999). As discussed by Reddy et al., (1998) the ability of a wetland to retain phosphorus depends on the phosphorus sorption capacity and the physicochemical properties of the sediments.

Out of the five pollutants under study. BOD and TP were the least reduced each year, while FC was ranked the highest. Phosphorus is typically the most difficult nutrient to remove therefore it is not surprising that its reduction efficiency is lowest (Kämppä-Usi et al., 2000; Perdomo et al., 1999) nor is it unusual that FC had the highest reduction rates (Lau and Chu, 2000; Decamp and Warren, 2000). The BOD result is surprising, however, as the overall results from this study are not in agreement with previous studies done by Knight et al., (1996) which showed reductions of 52% to 68% in BOD with similar loading rates. Similar reduction levels in this study were reached in the first year, but years two and three had a steady decline in reduction of BOD, to a low of 24%, which is well below what was found by Knight et al. A study conducted in England (Cooper et al., 1996) shows that typically more BOD leaves a surface flow wetland in summer than at any other time of the year. The seasonal difference is probably due to higher decomposition rates and increased plant productivity during the warmer months of the summer which can offset a higher rate of BOD degradation.

Over the three years of data collection no clear pattern emerged connecting inlet concentrations to outlet concentrations for any of the pollutants. For example, in year one (Figure 3-14) the outlet concentrations (relative to concentrations at the inlet) for TP were the lowest when the inlet concentrations (absolute concentration in mg L^{-1}) were the highest which is similar to a study conducted by McCaskey et al. (1994) yet for years two and three this trend was not repeated. TSS followed a similar trend for year three where the highest inlet concentration had the lowest outlet concentration but this trend was not observed in years one or two. There was also no clear seasonal trend in reduction capabilities for the constructed wetland. As discussed by Kadlec et al. (2000) the stochastic variability of the data make it difficult to determine seasonal trends without a substantial number of years of data.

The beginning of year one was the only time that all vegetated cells had the same density, location and number of plant species (Table 3-3). At that time, plant cover in the vegetated cells occupied approximately 5% of the cell water surface. Once summer growth and effluent flow started, vegetation changes occurred within each cell although vegetation densities remained similar among cells. There did not appear to be any relationship between increasing macrophyte densities and reduction rates of effluent components as each season progressed although a study conducted in Canada by Lakshman (1994) suggests there is a correlation between vegetation density and phosphorus reduction. Burgoon et al. (1999) found that as the macrophyte and litter layer matures. TSS concentration decreases, which was not the case in this study. In general, the ability of the constructed wetland to remove pollutants decreased each year in succession while macrophyte density increased from year one to yeat two but decreased during years two and three.

The most obvious visual difference between the vegetated cells and the unvegetated cell was the difference in the density of algae in the water column. As the vegetation colonized open spaces, available niches for algae growth diminished. Macrophyte coverage of the vegetated cells increased from year one to year two but decreased in year three (Table 3-3). The increased vegetation reduced the amount of open water exposed to the atmosphere which reduced transfer of atmospheric oxygen to the water (Williams et al., 1999). O₂ concentrations in the vegetated cells appeared higher than the unvegetated cell in year one (Figure 3-2), but for years two and three the O₂ levels in the vegetated cells appeared much lower than the unvegetated cell at the same time were consistently at a supersaturated level during daylight hours. High O₂ levels were probably due to photosynthetic oxygen production in the water (Brix, 1993) by the large algal populations present, providing a potentially better environment for aerobic reduction in concentration of pollutants.

Because of the higher O₂ concentration, it was thought that BOD. TKN and NH₄⁺-N concentrations would decrease in the unvegetated cell due to an increase in density of aerobic microbes (Brix, 1987; Bavor et al., 1989) but this did not occur. As previous research has indicated (Winkler, 1981; Cottingham et al., 1999) high concentrations of BOD and TKN can quickly limit oxygen availability due to the demand of heterotrophic bacteria metabolizing organic carbon which can limit the density of the slower growing nitrifying bacteria (Halling-Sørensen and Jørgensen, 1993). The heterotrophic bacteria may have a competitive advantage in acquiring the available O₂. However, given the high O₂ concentrations in the water it would appear another mechanism is involved. Weather conditions also must be considered in the seasonal dynamics of algae and their effect on O₂ concentrations in the water and internally generated pollutant loads. When growing conditions are sunny and warm, algal blooms are prolific and when these conditions deteriorate the same populations die off, adding dead cells to the water along with the concomitant nutrients and organic matter that make up the algal cells.

Results from years one and two are similar for each pollutant with vegetated cells appearing to remove more of the pollutants than the unvegetated cell. TP and NH_4 ⁻-N were the only pollutants to be removed in greater quantities by the unvegetated cell. This occurred in years two and three for TP and year three for NH_4 ⁺-N (Table 3-1). Initially, the vegetated cells removed more TP and NH_4 ⁺-N than the unvegetated cell, but at the conclusion of the study the unvegetated cell was removing more. It appears the increased reduction of TP in the unvegetated cell was facilitated by algal uptake (Richardson and Craft, 1993) rather than precipitation and subsequent incorporation into the substrate. This is supported by the timing of the decrease in reduction of TP with the death of the algae population in the unvegetated cell. As the algal population senesced and died, oxygen levels decreased (Figure 3-2), CO₂ levels increased (Figure 3-4) and pH decreased (Figure 3-3), all pointing towards a positive correlation between the amount of living algae present and TP removed from the water column.

The Alaska Department of Environmental Conservation (DEC) sets the water quality standards for discharge from secondary wastewater treatment systems in Alaska. The standards used for this study were 30 mg L⁻¹ for BOD and TSS and 200 cfu/100 ml for FC. There were no DEC secondary wastewater discharge standards for N or P at the time of this study. The water quality data from this study indicated that the discharge from the constructed wetland did not meet these standards consistently for years two and three. Several possibilities may have contributed to this: primary treatment in the lagoon was not fully complete; the hydraulic loading rate (HLR) was too high; or the constructed wetland was not adequately sized to treat effluent at the inlet concentration levels. A constructed wetland built within five miles of this project containing three of the five plant species tested is surpassing all DEC discharge standards (Maddux, 2001 unpublished data). The HLR of this constructed wetland is twice as high but it has better primary treatment and it is adequately sized to accommodate the effluent concentration.

The size of the constructed wetland was not adequate to remove the water pollutants to the background level desired but it did provide valuable information, for example, the determination of the k values, necessary to design constructed wetland systems that will meet such criteria. Pollutants in general were reduced as the effluent flowed the length of the cell in varying degrees, with pathogen reduction (FC) the highest and TP the lowest. However biotic process within the constructed wetland occasionally generated higher levels of TSS and BOD independently of TSS and BOD inputs. Inlet effluent concentrations did not appear to influence the reduction capabilities of the constructed wetland nor did seasonal patterns seem to have an effect other than the seasonal blooms of algae and their impact on TSS and BOD. Succession and competition by the macrophytes within the cells did not appear to respond to the gradients in effluent concentration, but rather to colonization patterns particular to each species when in a nutrient rich environment. The vegetated cells did improve reduction of pollutants over the unvegetated cell. although not to as great a degree as expected. Data gathered from both systems will help in designing better constructed wetland systems that combine features of vegetated and unvegetated cells.

As more constructed wetlands are built for wastewater treatment in the subarctic, the parameters that work best for this environment will become better established. The results from this study will help bring about a quicker understanding of the most important criteria for sewage wastewater treatment in cold climates.

Literature Cited

American Ephemeris, (1946). *Tables of Sunrise and Sunset and Twilight*. United States Naval Observatory. Washington D.C.

American Public Health Association; American Water Works Association; and Water Pollution Control Federation (1998). *Standard Methods for the Examination of Water and Wastewater* 20th Ed., Washington, D.C

Bastian. R.K., and Hammer, D.A. (1993). The Use of Constructed Wetlands for Wastewater Treatment and Recycling. In *Constructed Wetlands for Water Quality Improvement*. G.A. Moshiri (Ed.), Lewis Publishers, Boca Raton, FL., 68.

Bavor, H.J.; Roser, D.J.; Fisher, P.J.; and Smalls, I.C. (1989). Performance of Solid-Matrix Wetland Systems Viewed as Fixed Film Bioreactors. In *Constructed Wetlands for Wastewater Treatment*. D.A. Hammer (Ed.), Lewis Publishers, Chelsea, ML, 646.

Bavor, H.J.; Roser, D.J.; McKersie, S.A.; and Breen, P. (1988). Treatment of Secondary Effluent. Report to Sydney Water Board, Sydney, NSW, Australia.

Borum, J. (1983). The Quantitative Role of Macrophytes, Epiphytes, and Phytoplankton Under Different Nutrient Conditions in Roskilde Fjord, Denmark. *Proc. Int. Symp. On Aquatic Macrophytes*, Nijmegen, The Netherlands, 35.

Brix, H. (1987). Treatment of Wastewater in the Rhizosphere of Wetland Plants. The Root-Zone Method. *Wat. Sci. Tech.*, **19**, 108.

Brix, H. (1993). Wastewater Treatment in Constructed Wetlands: System Design, Reduction Processes, and Treatment Performance. In *Constructed Wetlands for Water Quality Improvement*. G.A. Moshiri (Ed.), Lewis Publishers, 9. Burgoon, P.S.: Kadlec, R.H.: and Henderson, M. (1999). Treatment of Potato Processing Wastewater with Engineered Natural Systems. *Wat. Sci. Tech.*, **40**, 211.

Chappell. K.R., and Goulder, R. (1994). Seasonal Variation of Epiphytic Extracellular Enzyme Activity on Two Freshwater Plants. *Phragmites australis* and *Elodea canadensis*. *Arch. Hydrobiol.* **132**, 237.

Cole, C.A., and Brooks, R.P. (2000). A Comparison of the Hydrologic Characteristics of Natural and Created Mainstream Floodplain Wetlands in Pennsylvania. *Ecol. Eng.*, 14, 221.

Cooper, P.F.: Job, G.D.: Green, M.B.; and Shutes, R.B.E. (1996). Reed Beds and Constructed Wetlands for Wastewater Treatment. WRc Publications. Medmenham, Marlow, U.K.

Cottingham. P.D.; Davies, T.H.; and Hart, B.T. (1999). Aeration to Promote Nitrification in Constructed Wetlands. *Environ. Tech.*, **20**, 69.

Cronk, J.K. and Fennessy, M.S. (2001). Wetland Plants, Biology and Ecology. Lewis Publishers, Boca Raton, FL.

Decamp. O., and Warren, A. (2000). Investigation of *Escherichia coli* Removal in Various Designs of Subsurface Flow Wetlands used for Wastewater Treatment. *Ecol. Eng.* 14, 293.

Geary, P.M., and Moore, J.A. (1999). Suitability of a Treatment Wetland for Dairy Wastewaters. *Wat. Sci. Tech.*, **40**, 179.

Halling-Sørensen, B., and Jørgensen, S.E. (1993). The Removal of Nitrogen Compounds from Wastewater. *Studies in Environmental Science*. Elsevier, Amsterdam, 54.

Hammer, D.A., (1994). Guidelines for Design, Construction and Operation of Constructed Wetlands for Livestock Wastewater Treatment. In. *Constructed Wetlands for Animal Waste Management*, P.J. Dubois and R.P. Reaves (Eds.), Purdue University, Lafayette, Indiana, 185.

Hammer, D.A., and Bastian, R.K. (1989). Wetland Ecosystems: Natural Water Purifiers? In Constructed Wetlands for Wastewater Treatment. D.A. Hammer (Ed.), Lewis Publishers, Chelsea, MI., 5.

Hershkowitz, J. (1986). Listowel Artificial Marsh Project Report. Ontario Ministry of the Environment, Water Resources Branch, Toronto, ON, Canada.

Hultén, E., 1968. Flora of Alaska and Neighboring Territories: a Manual of the Vascular Plants. Stanford University Press, California.

Hurse, T.J., and Conner, M.A. (1999). Nitrogen Removal from Wastewater Treatment Lagoons. *Wat. Sci. Tech.*, **39**, 191.

Jenssen P.D.; Mæhlum, T.; and Zhu, T. (1996). Design and Performance of Subsurface Flow Constructed Wetlands in Norway. In *Proceedings of the Symposium on Constructed Wetlands in Cold Climates.* E. Lemon, (Ed.), Ontario, Canada, 100.

Kadlec, R.H. (1989). Dynamics of Inorganic and Organic Materials in Wetlands Ecosystems – Decomposition in Wastewater Wetlands. In *Constructed Wetlands for Wastewater Treatment. Municipal. Industrial and Aagricultural.* D.A. Hammer (Ed.), Lewis Publishers, Chelsea, MI., 459. Kadlec R.H., and Knight, R.L. (1995). *Treatment Wetlands*. Lewis Publishers, Boca Raton, FL.

Kadlec, R.H.; Knight, R.L.; Vymazal, J.; Brix, H.; Cooper, P.; and Haberl, R. (2000). Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation. Scientific and Technical Report No. 8. IWA specialist group on use of macrophytes in water pollution control. IWA Publishing, London, 1.

Kämppä-Usi, J.; Braskeruj, B.; Jansson, H.; Syversen, N.; and Uusitalo, R. (2000). Buffer Zones and Constructed Wetlands as Filters for Agricultural Phosphorus. *J. Environ. Qual.*, **29** 151.

Knight, R.L.; Payne, V.; Borer, R.E.; Clarke, R.A; and Pries, J.H. (1996). Final Draft, *Livestock Wastewater Treatment Database*, prepared for the EPA Gulf of Mexico Program. Washington D.C.

Lakshman, G. (1994). Design and Operational Limitations of Engineered Wetlands in Cold Climates – Canadian Experience. In *Global Wetlands, Old World and New.* W. Mitsch (Ed.), Elsevier, Amsterdam, 349.

Lau, S.S.S. and Chu, L.M. (2000). Nutrient and Faecal Contamination and Retention in Wetland Enclosures (gei wais) in the Mai Po Marshes, Hong Kong. *Hydrobiologia*. **431**. 81.

Livingston, E.H. (1989). Use of Wetlands for Urban Stormwater Management. In Constructed Wetlands for Wastewater Treatment. D.A.Hammer, (Ed.), Lewis Publishers, MI., 253. McCaskey, T.A.; Britt, S.N.; Hannah T.C.; Eason, J.T.; Payne, V.W.E.; and Hammer, D.A. (1994). Treatment of Swine Lagoon Effluent by Constructed Wetlands Operated at Three Loading Rates. In *Constructed Wetlands for Animal Waste Management*. P.J. Dubois and R.P. Reaves (Eds.), Purdue University, Lafayette, IN., 23.

Mæhlum, T. (1999). Wetlands for Treatment of Landfill Leachates in Cold Climates. In Constructed Wetlands for the Treatment of Landfill Leachates. G. Mulamoottil; E.A. McBean, and F. Rovers (Eds.), Lewis Publishers, Chelsea, MI., 33.

Miller, G. (1989). Use of Artificial Cattail Marshes to Treat Sewage in Northern Ontario, Canada. In *Constructed Wetlands for Wastewater Treatment*. D.A. Hammer (Ed.), Lewis Publishers, Chelsea, MI., 636.

Perdomo, S.; Bangueses, C.; and Fuentes, J. (1999). Potential Use of Aquatic Macrophytes to Enhance the Treatment of Septic Tank Liquids. *Wat. Sci. Tech.*, **40**, 225.

Portier, R.J., and Palmer, S.J. (1989). Wetlands Microbiology: Form, Function, Processes. In *Constructed Wetlands for Wastewater Treatment, Municipal . Industrial and Agricultural.* D.A. Hammer (Ed.), Lewis Publishers, Chelsea, ML, 89.

Reddy, K. R.: O'Conner, G.A.; and Gale, P.M. (1998). Phosphorus Sorption Capacities of Wetland Soils and Stream Sediments Impacted by Dairy Effluent. J. Environ. Qual., 27, 438.

Richardson, C.J., and Craft, C.B. (1993). Effective Phosphorus Retention in Wetlands: Fact or Fiction? In *Constructed Wetlands for Water Quality Improvement*. G.A. Moshiri (Ed.), Lewis Publishers, Boca Raton, FL., 271.

SAS Institute Inc. (1998). JMP Statistical Software, version 3.2. Cary, NC.

Soto, F.:García, E. de Luís; and Bécares, E. (1999). Role of Scirpus lacustris in Bacterial and Nutrient Removal from Wastewater. *Wat. Sci. Tech.*, **40**, 241.

Vymazal, J. (1994). Algae and Element Cycling in Wetlands. Lewis Publishers, Boca Raton, FL.

Williams, C.; Perry, M.W.; Post, R.; and Dombeck, G. (1999) Plant Talk. Wat. Environ. & Tech., 11, 55.

Winkler, M. (1981). Biological Treatment of Wastewater. Ellis Horwood Publishers, New York, NY.









Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Figure 3-3 pH readings in surface waters of constructed wetland cells. 1998, 1999 and 2000 values were taken 12.2 m from inlet. All readings after 9-27 were taken from beneath an ice cover. n = 4 for vegetated cells and n = 1 for unvegetated cell for each sample date. Error bars are ± 1 S.E.



Figure 3-4 Dissolved carbon dioxide concentrations of water in constructed wetland cells for 1999. Samples were taken 12.2 m downstream from the inlet. Samples taken after 9-27 were taken beneath an ice cover. n = 4 for vegetated cells and n = 1 for unvegetated cells.



Figure 3-5 Air, soil and water temperature readings for constructed wetland cells. 1998, 1999 and 2000 temperature readings were taken with a hand-held thermometer for ambient air temperature, at 15 cm below water surface and at 8 cm below soil surface. n = 1 for each water and soil histogram bar. Temperature readings were taken at cell 10nly.



Figure 3-6 Biological oxygen demand concentration ratios for vegetated cells in 1998 by station and date.

Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are \pm 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-7 Biological oxygen demand concentration ratios for vegetated cells in 1999 by station and date.

Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-8 Biological oxygen demand concentration ratios for vegetated cells in 2000 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters below dates indicate a significant difference (p < 0.05) between dates for Sta. C. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C. Error bars are ± 1 S.E.



Figure 3-9 Total suspended solids concentration ratios for vegetated cells in 1998 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters below dates indicate a significant difference (p < 0.05) between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date. Error bars are ± 1 S.E.



Figure 3-10 Total suspended solids concentration ratios for vegetated cells in 1999 by station and date.

Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are \pm 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-11 Total suspended solids concentration ratios for vegetated cells in 2000 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. There were no significant differences (p < 0.05) between dates for Sta. C. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C. Error bars are ± 1 S.E.





Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-13 Fecal coliforms concentration ratios for vegetated cells in 1999 by station and date. Number above each histogram is the inlet concentration in cfu/100 ml. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.

%



Figure 3-14 Fecal coliforms concentration ratios for vegetated cells in 2000 by station and date.

Number above each histogram is the inlet concentration in cfu/100 ml. There were no significant differences among dates in fecal coliform concentrations. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹.



Figure 3-15 Total phosphorus concentration ratios for vegetated cells in 1998 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ±1 S.E. Concentration ratio = (inlet concentration - station concentration//inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-16 Total phosphorus concentration ratios for vegetated cells in 1999 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Sample after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-17 Total phosphorus concentration ratios for vegetated cells in 2000 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters below dates indicate a significant difference (p < 0.05) between dates for Sta. C. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C. Error bars are ± 1 S.E.



Figure 3-18 Total Kjeldahl nitrogen concentration ratios for vegetated cells in 1998 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-19 Total Kjeldahl nitrogen concentration ratios for vegetated cells in 1999 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-20 Total Kjeldahl nitrogen concentration ratios for vegetated cells in 2000 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C. Error bars are ± 1 S.E.



Figure 3-21 Ammonium nitrogen concentration ratios for vegetated cells in 1998 by station and date. Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations and different letters below dates indicate a significant difference between dates for Sta. C. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date. When input concentrations are low, as in 10-6, cell effluent is at or below background levels and little reduction occurs.



Figure 3-22 Ammonium nitrogen concentration ratios for vegetated cells in 1999 by station and date.

Number above each histogram is the inlet concentration in mg L⁻¹. Different letters above histogram indicate a significant difference (p < 0.05) between stations. Samples after 9-27 were taken beneath an ice cover and were not included in the statistical analysis. Error bars are ± 1 S.E. Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 4 for each station and each date.



Figure 3-23 Ammonium nitrogen concentration ratios for vegetated cells in 2000 by station and date.

Number above each histogram is the inlet concentration in mg L^{-1} . Different letters below dates indicate a significant difference (p < 0.05) between dates for Sta. C. Error bars are ± 1 S.E.

Concentration ratio = (inlet concentration - station concentration)(inlet concentration)⁻¹. n = 1 for Sta. A and Sta. B; n = 4 for Sta.C.
Table 3-1 Mean pollutant concentrations by station and year. Data for 1998 and 1999 were collected from Stations A, B and C for all cells. Data for 2000 were collected from cell 1 Stations A, B, C. All other cells had data collected for Station C only. BOD (biological oxygen demand), TSS (total suspended solids), TP (total phosphorus), TKN (total Kjeldahl nitrogen), NH₄⁺ (ammonium), FC (fecal coliforms). Measurements in mg L⁻¹, except for FC in cfu/100 mł (colony forming units). n = number of samples in that row used to calculate the overall yearly average. ADEC discharge standards for secondary treated wastewater is 30 mg L⁻¹ for BOD and TSS and 200 cfu/100 ml for FC.

		Vegetated Cells				Unvegetated Cells						
		Inlet	<u>Sta. A</u>	<u>Sta. B</u>	<u>Sta. C</u>	<u>(n)</u>	<u>% Removed</u>	<u>Sta. A</u>	<u>Sta. B</u>	<u>Sta. C</u>	<u>(n)</u>	<u>% Removed</u>
<u>1998</u>	BOD	54	40	23	18	20	67%	58	39	99	5	-83%
-	TSS	248	112	100	94	20	62%	112	100	252	5	-2%
	FC	7,387	901	322	48	20	99%	548	245	127	5	98%
	ТР	9.8	6.7	5.1	3.9	16	60%	6.0	5.8	6.0	4	39%
	TKN	55.8	22.6	19.1	13.4	16	76%	26.4	27.4	25	4	55%
	NH4 ⁺	31.8	7.7	4.6	2.7	16	92%	5.8	9.8	4.2	4	87%
1999	BOD	104	83	67	54	28	48%	98	74	78	7	25%
	TSS	135	96	49	53	28	61%	150	155	151	7	-12%
	FC	30,531	13,169	9,660	742	24	98%	13,076	11,603	3,387	6	89%
	ТР	7.1	7.5	6.9	5.6	20	21%	5.7	5.4	5.1	5	28%
	TKN	30.5	20	14.9	13.6	20	55%	20.7	21.4	22.2	5	27%
	NH4'	23.5	12.8	9.5	7.8	20	67%	12.2	12.4	13.2	5	42%
<u>2000</u>	BOD	119	66	63	91	36	24%			114	9	4%
	TSS	196	99	74	121	36	38%			159	9	19%
	FC	16,567	3,538	1,589	1,091	36	93%			1,172	9	93%
	ТР	29.6	25.7	23.6	22.1	36	25%			18.1	9	39%
	TKN	82.1	55.8	52.4	46.5	36	43%			48.4	9	41%
	NH4 ⁺	57.2	46	40	28.7	36	50%			19.8	9	65%

Table 3-2. Uptake rate constants for k.

Comparing global k values to actual k values derived from the constructed wetland built at the University of Alaska Fairbanks campus. Global k values are taken from Kadlec and Knight (1996) and Kadlec et al. (2001). N \approx 38 for global k values. The area of a cell is (A) = 46.08 m2; volume (Q) for 1998 was 136 m³, 1999 142 m³ and 2000 128 m³.

	Global $k \mathrm{m}\mathrm{yr}^{-1}$	<u>UAF k m vr⁻¹</u> Vegetated	<u>UAF k m yr⁻¹</u> <u>Unvegetated</u>
Biological oxygen demand	2 to 34	2	-0.2
Total suspended solids	1 to 36	3	0.1
Fecal coliforms	60 to 72	11	9
Total phosphorus	8 to 10	1	1
Total Kjeldahl nitrogen	14 to 17	3	2
Ammonium	14 to 18	4	3

To calculate the uptake rate constant k, use the equation

$A = \frac{Q}{k} \bullet \ln k$	$\left[\frac{C_{in}}{C_{back}}\right]$
---------------------------------	--

where:

A = wetland area (m²) Q = wastewater flow (m³ year ⁻¹) C_{in} = effluent concentration at inlet C_{back} = effluent concentration at outlet

k = uptake rate constant (m/yr)

Table 3-3. Vegetation and open water percent presence by year.

The five species listed below were planted in a constructed wetland on the University of Alaska Fairbanks campus. Values are the percent presence of each species and open water that were found in the total space available.

Species	<u>1997</u>	<u>1998</u>	<u>1999</u>	<u>2000</u>
Arctophila fulva	1 %	46 %	71 %	46 %
Carex rhynchophysa	1 %	3 %	5 %	5 %
Menyanthes trifoliata	1 %	7 %	7 %	9%
Scirpus validus	1 %	3 %	1 %	1 %
Typha latifolia	1 %	8%	9 %	9%
Open Water	95%	33%	7%	30%

CHAPTER 4 – A VEGETATIVE STUDY OF A CONSTRUCTED WETLAND TREATING SWINE WASTEWATER

Abstract

A vegetation study was conducted over a three-year period to investigate the survival and colonization of five different species of plants. <u>Arctophila fulva, Carex</u> <u>rhynchophysa</u> <u>Menyanthes trifoliata</u>, <u>Scirpus validus</u> and <u>Typha latifolia</u> were used in a constructed wetland designed to treat sewage wastewater. Swine effluent from a sewage lagoon comprised the pollutant source. The constructed wetland was designed in three equal sections with no physical barriers between sections and the initial placement of plants was replicated in each section. Changes in vegetation were not controlled and over the three-year period the natural vegetative pattern altered substantially. All species maintained a viable population which altered in coverage over the study period. Only one species. <u>A. fulva</u> fluctuated widely in coverage during this time and this probably impacted the colonization of the other species. Plant community stability had apparently not been reached by the conclusion of this study.

Introduction

Constructed wetlands are increasingly recognized for providing a variety of ecosystem benefits such as wastewater treatment, wildlife habitat and recreational opportunities (Kadlec and Knight, 1995; Smardon, 1989). Selection of species, spacing of plants, initial hydraulic regime and fertilization are all important considerations in wetland design. Macrophyte selection is not only critical to a specific wetland use, it can represent a considerable cost for any wetland project (George Waller, 1998, per. comm.). Local climate and plant availability will often drive macrophyte selection, necessitating familiarity with local ecosystems.

Plant communities in natural wetlands grow in a non-predictable cyclic pattern of vegetation change (Niering, 1989) rather than in a unidirectional pattern of succession. As Odum (1971) pointed out, wetlands are pulsed systems that are subjected to regular but acute physical disturbances from allochthonous sources, such as precipitation events. This tends to keep wetlands in an intermediate state of development in the sense of the traditional paradigm of succession (Morin, 1999). Steady-state water levels are not the rule in natural wetlands and wetland vegetation communities reflect this (Van der Valk, 1981; Mitsch and Gosselink, 1993). A constructed wetland used to treat sewage wastewater usually has a very steady and predictable hydraulic regime which damps fluctuations in water and nutrient levels, in turn altering the vegetation community (Weiher and Keddy, 1995). The competitive balance between species can shift due to the input of nutrients found in sewage wastewater (Twolan-Strutt and Keddy, 1996). A wetland community found to be very successful in surrounding natural wetlands may not thrive when subjected to elevated nutrient inputs and may change to an unplanned and undesirable community of plants.

The introduction of volunteer or exotic species is another consideration when planning the vegetation needs of a constructed wetland. The term exotic often refers to invasive species that are aggressive and usually undesirable in a wetland, natural or constructed (Wilcove et al., 1998). Two prominent wetland examples of invasive species are <u>Eichhornia crassipes</u> (water hyacinth) and <u>Lythrum salicaria</u> (purple loosestrife). Plant species indigenous to local wetlands may be transported to constructed wetlands via a soil seed bank, the wind, water or waterfowl (Cronk and Fennessy, 2001). In most cases, the volunteer indigenous plants are not considered a nuisance and do not vegetatively dominate a constructed wetland.

Methods and Materials

This vegetation study was part of a larger five-cell constructed wetland project, near Fairbanks. Alaska, with four cells vegetated and one cell left unvegetated. This study was done on one cell designed in three equal sections. Station A (Sta. A), Station B (Sta. B) and Station C (Sta. C) with no physical barriers between the stations (Figure 4-1). The dry dimension at the bottom of the cell was one meter wide by 18.3 meters long with side slopes of 2:1. After flooding to a depth of 30 cm, the surface dimension of the cell was 2.4 meters wide by 19.2 meters long. The slope of the cell bottom between the inlet and outlet was 0.1%. A soil berm surrounding the cell was one meter higher in elevation than the bottom of the cell with a one-meter wide walkway on top of the berm.

Soil from the site was used to build the berm and the cell and provide topsoil. The cell and berm were covered with a single, continuous sheet of 30 mil high density polyethylene (HDPE) liner material. This liner was then covered with a 30-cm layer of topsoil which provided the substrate for the macrophytes and other vegetative growth. A 5-cm diameter PVC pipe carried the effluent to the cell via gravity flow and a 6.4-mm orifice at the cell inlet controlled the flow rate. At the outlet end of the cell a 5-cm diameter pipe was connected to a rotating elbow and a 76-cm tall standpipe that acted as the outlet control structure, providing the swivel action necessary to regulate the effluent level in the cell. This pipe carried effluent through the end of the berm and connected to a main collection drain line that flowed to a sump pit.

Water temperatures were collected daily with hand held thermometers and temperature probes combined with O_2 meters at 15 cm below the water surface and automatic data loggers submerged at 30 cm below the water surface.

Plant species

The five species of plants used in this project were <u>Arctophila fulva</u> (Trin.), Anderss. (pendant grass), <u>Carex rhynchophysa</u> C.A. Mey (sedge), <u>Menyanthes trifoliata</u> L. (buckbean), <u>Scirpus validus</u> M. Vahl. (softstem bulrush) and <u>Typha latifolia</u> L. (cattail) (Hultén, 1968). All are indigenous to the circumpolar north, south of the 68th parallel, with <u>A. fulva</u> and <u>C. rhynchophysa</u> also found north of the 68th parallel. Three genera, <u>Carex</u>, <u>Scirpus</u> and <u>Typha</u> have previously been used for pollutant reduction in temperate climates (Burgoon et al., 1999, and Cole and Brooks, 2000). Two other genera, <u>Arctophila</u> and <u>Menyanthes</u>, are abundant in Alaska but no references were found to indicate they had been previously used in wetlands constructed for pollutant reduction.

<u>A. fulva</u> is a grass that grows upright with numerous leaves and multiple stems per plant. Colonization occurs through stolon extension from the parent root and the ideal habitat is on the edge of standing water but not in standing water. <u>C. rhynchophysa</u> is a sedge that has the ability to colonize rapidly and depends on stolon extension for colonization. <u>M. trifoliata</u> has a thick, creeping rootstock with floating stems that hold the leaves above the water. It is one of the first plants to emerge in a wetland in the spring where it flowers and sets seed before being overtopped in growth as other plants emerge. Its preferred habitat is the understory of the wetland vegetation. This plant has numerous adventitious roots growing from the stem which help to support the plant and supply nourishment. <u>S. validus</u> is a bulrush that prefers growing in standing water under a constant flooding regime. This plant is one of two used in this study that has a rhizome. with smaller rhizomes extending out from the parent rhizome to achieve colonization. <u>T. latifolia</u> is a cattail found to grow in standing water, one to two meters tall, that uses starchy, rhizomous extensions to colonize new areas.

Experimental design, planting procedures

The vegetated cell was planted on a 30 cm by 46 cm pattern (Figure 4-2). The cell was divided into three equal units 6.1 meters in length, demarcated as Station A (Sta. A), Station B (Sta. B) and Station C (Sta. C) with no physical barriers between stations. Plants were randomly assigned a location within one unit in equal numbers. Each unit was a replicate of this planting scheme with the macrophytes planted in exactly the same pattern for each unit. Thus three units end-to-end made up the cell as Sta. A, Sta. B and Sta. C. The plants were harvested from local wild sources and planted by hand the same day of harvest. Immediately following planting the cells were flooded with well water to a depth of 15 cm for 14 days. After that, the water depth was raised to 30 cm and that depth was maintained until freeze-up. Planting was completed by mid-August, the cell water surface froze by the third week of September, and the constructed wetland was

completely frozen by the fourth week of November. Approximately 60 cm of snow cover blanketed the cell for the duration of the first winter after planting.

Effluent flow from the sewage lagoon of the University of Alaska Fairbanks Experiment Farm began the following spring at a hydraulic loading rate of 2.17 cm d⁻¹ (1,136 L d⁻¹) and the cell was pulse loaded once per day. The hydraulic retention time was a theoretical 9 days. For year one, flow began 6-09-98 and ended 10-06-98; for year two, flow began 6-02-99 and ended 10-04-99; and for year three, flow began 6-07-00 and ended 9-27-00. There were no destructive sampling procedures used during the course of this study nor were there any anthropogenic disturbances of the cell substrate or vegetation at any time.

Sampling methods

In the third week of August of each year, vegetation coverage was estimated with a modified quadrat-charting method (Cain and Castro, 1959; Phillips, 1959; and Greig-Smith, 1952). The entire cell (cell 1 in Figure 4-1) was partitioned into a sampling grid of 10-cm square quadrats. Scaffolding was erected over the cell and the vegetative cover was determined by visually inspecting each square from directly above and cataloging by presence of a species (the area within each grid square occupied by each plant species). Vegetation density was determined on a rank scale of 1 (low), 2 (medium-low), 3 (medium), 4 (medium-high) and 5 (high). A percent of coverage (presence/absence) by each species of the total space available in the cell was calculated based on a visual count of the number of times the species occurred within each grid throughout the cell.

Results

Although it was not possible to statistically analyze the plant coverage data due to lack of replication, differences were rather dramatic (Figure 4-3) and are believed to be due to natural effects, such as competition, rather than anomalous effects, such as anthropogenic influences. <u>A. fulva</u> exhibited the widest range of variation in coverage

among the five species planted over the study period, fluctuating from a low of 1% in 1997 to a high of 71% in 1999 (Table 4-1). <u>T. latifolia</u> was the only plant that remained at a near constant coverage level among all three treatment years. There were apparent differences in individual species coverage from station to station but the differences were not consistent from year to year (Table 4-2).

At the time of planting each species occupied approximately the same amount of the cell surface area in each section. By the end of year one (1998) <u>A. fulva</u> had colonized considerably more of the available space than all the other species' colonization combined (Table 4-1). For year one, both <u>A. fulva</u> (Figure 4-4) and <u>T. latifolia</u> (Figure 4-8) diminished in coverage from the inlet to the outlet, whereas the other three species (Figures 4-5 to 4-7) increased in coverage or remained the same as the effluent flowed through the cell and the pollutant concentrations were reduced. By the end of year one, <u>A. fulva</u> had colonized from bank to bank in a somewhat even manner (Figure 4-4).

Year two (1999) did not follow the same pattern. <u>A. fulva</u> increased in coverage from the inlet to the outlet (Figure 4-4) and occupied 71% of the available surface area (Table 4-1): however the other species had variable densities between stations. Overall, <u>A. fulva</u> increased in coverage from inlet to outlet and <u>T. latifolia</u> increased in coverage overall (Figure 4-8) but had a decrease in coverage between Sta. A and Sta. B. <u>M. trifoliata</u> remained almost the same as the previous year (Figure 4-6) but <u>C. rhynchophysa</u> (Figure 4-5) and <u>S. validus</u> (Figure 4-7) decreased in coverage. Both <u>C. rhynchophysa</u> and <u>S. validus</u> increased in coverage within Sta. B before decreasing in coverage by Sta. C (Table 4-2).

Year three was different again with <u>A. fulva</u> decreasing in coverage to 46%. similar to year one. However, the location of the plants within the cell changed. By year

three <u>A. fulva</u> had not regrown in the center of the cell and occupied the edges almost exclusively (Figure 4-4). The decrease in <u>A. fulva</u> corresponded with an increase in <u>M. trifoliata</u> (Figure 4-6) although the growth patterns may not be a case of cause and effect. <u>C. rhynchophysa</u> also decreased a small amount in year three (Figure 4-5). <u>S. validus</u> and <u>T. latifolia</u> had the same coverage for years two and three (Figures 4-7 and 4-8). It appears that water column temperatures (Figure 4-9) were affected by the change in <u>A. fulva</u> coverage due to the thick mat that resulted from the previous year's senesced <u>A. fulva</u>.

Volunteer species that were cataloged over the three-year study were <u>Sparganium</u> <u>multipedunculatum</u>. <u>Equisetum fluviatile</u>, <u>Equisetum palustre</u>, <u>Eleocharis palustris</u>, <u>Beckmannia erucaeformis</u>, <u>Lemna minor</u> and <u>Senecio congestus</u>. None of the volunteer species were able to establish more than a very small niche within the constructed wetland and did not appear to impact the colonization of the studied species.

Discussion

The morphology of the plants had a bearing on the development of this constructed wetland ecosystem. Overall. <u>A. fulva</u> was the quickest to colonize open areas and <u>C. rhynchophysa</u> was the slowest. This attribute of <u>A. fulva</u> was not necessarily positive for the constructed wetland. When <u>A. fulva</u> senesces in the fall it tends to lodge rather quickly when the plant falls over due to stem breakage, especially if there is an early snow. Conversely. <u>T. latifolia</u> and <u>S. validus</u> can have standing dead plants two to six months after senescence (Sojda and Solberg, 1993; Linde et al., 1976). <u>A. fulva</u> can take from two to six weeks to fall back into the water column and <u>M. trifoliata</u> falls back into the water column immediately upon senescence. The lodged <u>A. fulva</u> formed a dense mat of dead vegetation on the water surface which became covered with snow and was soon frozen into the ice matrix. Little decomposition occurs during the winter when the water column and substrate are frozen solid (Lambers et al., 1998). Therefore, when the snow and ice melted in the spring a thick mat of decomposing <u>A. fulva</u> vegetation was

left on the water surface. This mat slowed the heating of the water column by the sun (Figure 4-9), similar to results found by Hill and Payton (2000) and hindered the sprouting of emerging shoots which in essence deprived the vegetated cells from becoming fully vegetated in the center. Vegetative cover in the center of the cell could only be accomplished by new colonization of adjacent species or by delayed sprouting of previously existing species. The unvegetated cell typically had higher water column temperatures that encouraged rapid algal growth in the early part of the treatment season. The temperature differences between the vegetated and unvegetated cells followed similar patterns to those in a study done by Tanner et al. (1994) which found water temperatures to be 2-4 °C cooler in the vegetated cells.

<u>A. fulva</u> rapidly colonized the open cell area in year one even when it was constantly flooded. However, it died back after one year and resprouted with vigor on the edge of the cell in shallow water or moist soil on the banks, not regrowing in the deeper water it had previously colonized. <u>M. trifoliata</u>, on the other hand, colonized large areas where it was not crowded out by other rapidly colonizing vegetation, in this instance by <u>A. fulva</u>. The only plant that decreased in overall coverage during the study was <u>S. validus</u>. Unlike the other plants, <u>C. rhynchophysa</u> and <u>S. validus</u> did not colonize large areas with a few stems but tended to colonize smaller areas in thick clumps, giving the species the appearance of less vigor as a colonizer.

Of the five species in this study, <u>A. fulva</u> was the only one to have an observable negative effect on the growth of macrophytes, including itself. Without a longer study period it cannot be determined if the long term effect of <u>A. fulva</u> is deleterious to the constructed wetland. Since this species did not repopulate the deeper water portions of the cell by the third year, more area was made available for colonization by the other species the following year. Visual inspection of the cells in 2001 (post effluent flow) revealed that <u>C. rhynchophysa</u> and <u>M. trifoliata</u> had colonized more of the areas previously occupied by <u>A. fulva</u> than either <u>S. validus</u> or <u>T. latifolia</u>.

Colonization results from this study indicated that it is unrealistic to expect initial planting patterns to remain intact or that volunteer species will not become a constant part of the plant ecosystem. Since a constructed wetland built to treat sewage wastewater is a eutrophied ecosystem by design, blooms of algae can be expected to occur and their effects should be factored into the operational plan. Plants able to take advantage of the increased nutrient levels will define the plant community dynamics. In natural systems, plants are distributed in response to environmental gradients such as water depth and nutrient availability and constructed wetland designs need to incorporate competition as a major force in community succession.

Planting can be one of the major costs of developing a constructed wetland, both in the plant costs and labor. This study indicated that a dense planting pattern is not required for rapid colonization to occur. The colonization strategies employed by different species are important to consider when selecting and spacing plants in a constructed wetland.

Literature cited

- Burgoon, P.S.; Kadlec, R.H.; and Henderson, M. (1999). Treatment of Potato Processing Wastewater with Engineered Natural Systems. *Wat. Sci. Tech.*, **40**, 211.
- Cain, S.A., and Castro de Oliveira, G.M. (1959). *Manual of Vegetation Analysis*. Harper & Brothers, Publishers, New York, NY.
- Cole, C.A., and Brooks, R.P. (2000). A Comparison of the Hydrologic Characteristics of Natural and Created Mainstream Floodplain Wetlands in Pennsylvania. *Ecol. Eng.*, 14, 221.
- Cronk, J.K., and Fennessy, M.S. (2001). Wetland Plants, Biology and Ecology. Lewis Publishers, Boca Raton, FL.
- Greig-Smith, P. (1952). The Use of Random and Contiguous Quadrats in the Study of the Structure of Plant Communities. *Ann. Bot. NS*, 16, 293.
- Hill, D.T., and Payton, J.D. (2000). Effect of Plant Fill Ratio on Water Temperature in Constructed Wetlands. *Bioresource Technology*, **71**, 283.
- Kadlec R.H., and Knight, R.L. (1995). *Treatment Wetlands*. CRC/Lewis Publishers, Boca Raton, FL.
- Lambers. H.; Chapin III, F.S.; and Pons, T.L. (1998). Plant Physiological Ecology. Springer-Verlag, New York, NY.

- Linde, A.F.; Janisch, T.; and Smith, D. (1976). Cattail The Significance of its Growth, Phenology and Carbohydrate Storage to its Control and Management. Wisconsin Department of Natural Resources Technical Bulletin 94.
- Mitsch, W.J., and Gosselink, J.G. (1993). Wetlands. Van Nostrand Reinhold, New York, NY.

Morin, P.J. (1999). Community Ecology. Oxford. Blackwell Scientific.

Niering, W.A. (1989). Vegetation Dynamics in Relation to Wetland Creation. In Wetland Creation and Restoration: The status of the Science. Vol.II: Perspectives. J.A Kusler, M.E. Kentula, (Eds.), EPA/600/3-89/038 a, b. Environmental Research laboratory, Corvallis, OR.

Odum, E. P. (1971). Fundamentals of Ecology. W.B. Saunders Co., Philadelphia, PA

- Phillips, E.A. (1959). *Methods of Vegetation Study*. Henry Holt and Company, Inc. NY 84.
- Smardon, R.C. (1989). Human Perception of Utilization of Wetlands for Waste Assimilation, or How do You Make a Silk Purse Out of a Sow's Ear? In *Constructed Wetlands for Wastewater Treatment*. D.A. Hammer (Ed.), Lewis Publishers, FL. 287.
- Sojda, R.S., and Solberg, K.L. (1993). *Management and Control of Cattails*. Fish and Wildlife leaflet 13.4.13.

- Tanner, C.C.; Clayton, J.S.; and Upsdell, M.P. (1994). Effect of Loading Rate and Planting on Treatment of Dairy Farm Wastewaters in Constructed Wetlands – I. Removal of Oxygen Demand. Suspended Solids and Fecal Coliforms. *Wat. Res.*, 29, 27.
- Twolan-Strutt, L; and Keddy, P.A. (1996). Above and Below ground Competition Intensity in Two Contrasting Wetland Plant Communities. *Ecology* 77, 259.
- Van der Valk, A.G. (1981). Succession in Wetlands: a Gleasonian Approach. *Ecology*, **62**, 688.
- Weiher, E. and Keddy, P.A. (1995). The Assembly of Experimental Wetland Plant Communities. *Oikos*. **73**, 323.
- Wilcove, D.S.: Rothstein, D.: Dubow, J.: Phillips, A.: and Losos, E. (1998). Quantifying Threats to Imperiled Species in the Unites States. *Bioscience*. **48**, 607.





S	A	M	A	т	S	A	м	т	м	м	С	С
T	S	A	с	S	С	т	A	м	т	С	A	A
S	Т	S	S	S	A	С	т	С	M	М	т	M

Direction of flow

Figure 4-2 Cell unit planting schematic for the constructed wetland built on the University of Alaska Fairbanks campus. A = Arctophila fulva, C = Carex rhynchophysa, M = Menyanthes trifoliata, S = Scirpus validus, T = Typha latifolia. The planting schematic is on a 30 cm x 46 cm grid. Each plant was randomly assigned a planting location, with one plant at each location. The dry dimension of each unit within the cell is 1 m x 6.1 m. This planting schematic was used in each unit of each cell. This schematic is not to scale.



Cell 1 - 1997: initial planting



Cell 1 - 1998: strong colonization



Cell 1 - 1999: A. fulva dominant



Cell 1 - 2000: open water evident in center of cell





Figure 4-4 Vegetation Density for <u>Arctophila fulva</u> by year and station. Each block indicates colonization and growth of a single species. Stipled area includes entire cell surface and different patterns indicate different plant densities. Percent coverage is indicated beside each station



Figure 4-5 Vegetation Density for <u>Carex</u> <u>rhynchophysa</u> by year and station. Each block indicates colonization and growth of a single species. Stipled area includes entire cell surface and different patterns indicate different plant densities. Percent coverage is indicated beside each station



Figure 4-6 Vegetation Density for <u>Menyanthes</u> <u>trifoliata</u> by year and station. Each block indicates colonization and growth of a single species. Stipled area includes entire cell surface and different patterns indicate different plant densities. Percent coverage is indicated beside each station



Figure 4-7 Vegetation Density for <u>Scirpus validus</u> by year and station. Each block indicates colonization and growth of a single species. Stipled area includes entire cell surface and different patterns indicate different plant densities. Percent coverage is indicated beside each station



Figure 4-8 Vegetation Density for <u>Typha latifolia</u> by year and station. Each block indicates colonization and growth of a single species. Stipled area includes entire cell surface and different patterns indicate different plant densities. Percent coverage is indicated beside each station.



Figure 4-9 Water temperatures for the vegetated and unvegetated cells for the year 2000. Water temperature was collected daily by automatic insitu data loggers at a 30 cm depth and weekly with a hand-held thermometer at a 15 cm depth.

Table 4-1. Vegetation and open water percent presence by year.

The five species listed below were planted in a constructed wetland on the University of Alaska Fairbanks campus. Values are the percent presence of each species and open water that were found in the total space available at the end of the growing season.

Species	<u>1997</u>	<u>1998</u>	<u>1999</u>	<u>2000</u>
Arctophila fulva	1 %	46 %	71 %	46 %
Carex rhynchophysa	1 %	3 %	5 %	5 %
Menyanthes trifoliata	1 %	7 %	7 %	9%
Scirpus validus	1 %	3 %	1%	1 %
Typha latifolia	1 %	8%	9 %	9%
Open Water	95%	33%	7%	30%

Table 4-2 Vegetation and open water percent presence by station and year. The percentage value represents the percent of each station occupied by the indicated species or by open water.

<u>Species</u>					<u>Rank</u>	
	<u>1997</u>	<u>1998</u>	<u>1999</u>	<u>2000</u>	<u>Average</u>	
Arctophila fulva						
Station A	1 %	52 %	65 %	45 %	4	
Station B	1 %	44 %	68 %	42 %	4	
Station C	1 %	39 %	83 %	53 %	4	
Carex rhynchophysa						
Station A	1%	2 %	3 %	3 %	3	
Station B	1%	3 %	8 %	8 %	3	
Station C	1 %	4 %	1 %	3 %	3	
Menyanthes trifoliata						
Station A	1%	7 %	7 %	7 %	3	
Station B	1%	7 %	7 %	12 %	3	
Station C	1 %	7 %	6 %	7 %	3	
Scirpus validus						
Station A	1%	1 %	<1 %	<1%	2	
Station B	1 %	3 %	1 %	1 %	3	
Station C	1 %	4 %	<1 %	<1 %	2	
<u>Typha</u> <u>latifolia</u>						
Station A	1 %	10 %	10 %	14 %	3	
Station B	1%	7 %	5 %	5 %	4	
Station C	1 %	8 %	10 %	6 %	4	
Open Water						
Station A	95%	28%	15%	31%		
Station B	95%	36%	11%	32%		
Station C	95%	38%	0%	31%		

CHAPTER 5 – SUMMARY

The results of this research support the hypotheses that constructed wetlands can be used as an effective treatment process for removing heavy metals, pollutants from roadway runoff and pathogens in sewage wastewater in the circumpolar north. The data collected from the heavy metal experiment shows that the subarctic plants selected for study can thrive in high concentrations of heavy metals and will store the majority of the heavy metals they take up in below-ground tissues. Unlike temperate climates, roadway runoff is not a problem during the winter months in the subarctic and storage of roadway effluent during the long winter is not required. There are applications for this research in treating parking lot and roadway runoff as well as runoff from mining operations.

Based on the results from this project and on similar studies, a constructed wetland designed to remove heavy metals from wastewater would place <u>S. validus</u> and <u>T. latifolia</u> near the effluent inlet. These two species survived well when subjected to a concentrated application of heavy metals and removed the most heavy metals when compared with the other species. If a particular metal is targeted for removal, for example zinc from mine drainage, the results from this study can help in selection of the macrophyte species most suited for the constructed wetland. There was little difference in stem counts or plant biomass (dry weight) between plants that received heavy metals and those that did not. There were significant differences in uptake of metals among plant species for each metal and sequestration of metals taken up by plants were usually stored in greater quantities in the root. Each wetland macrophyte possesses a unique combination of responses to heavy metals: the ability to survive and grow; the capacity to take up metals: and the ability to sequester metals among various plant parts. Knowledge of the responses of wetland plants to heavy metals can help managers design wetland communities uniquely suited as heavy metal biofilters.

Although the constructed wetland used for this sewage wastewater treatment project was undersized to consistently meet the ADEC discharge standards for biological oxygen demand (BOD) and total suspended solids (TSS), an appreciable amount of reduction occurred and this research showed that constructed wetlands can be a viable alternative for wastewater treatment in

the subarctic. The simplicity of the operating system is suitable to a rural village lifestyle, both in economics and in ease of operation. This research indicated that constructed wetlands can work in a subarctic climate but fell short of determining the appropriate size needed to meet specific wastewater discharge standards.

A primary focus of further studies in using constructed wetlands for sewage wastewater treatment could include a determination of the optimum size of a constructed wetland under subarctic conditions to meet the Alaska Department of Environmental (ADEC) secondary sewage wastewater discharge standards. Constructed wetlands are not mechanical filters but are biological systems which can sometimes generate what appears to be a net excess of pollutants, such as BOD. TSS, nitrogen (N) and phosphorus (P). This internal regeneration is biologically benign because it is mostly composed of algal cells and zooplankton which are not health hazards. Often times what appears to be high levels of BOD and TSS are bioseston in nature, i.e., non-pathogenic microbes and zooplankton, not pathogenic bacteria. The addition of zooplankton and low levels of N and P can act as a nutrient subsidy to otherwise oligotrophic waters thus benefiting fish populations, especially juveniles, such as grayling young, which swim and feed in slow moving waters.

The k values derived from this study are specific to the climatic conditions of day length, temperature, and precipitation of the Interior of Alaska. The evapotranspiration, colonization and competitive nature of the macrophyte species used under these conditions contribute to the overall k values. For this study, the k values are an indication of either the flow volume needs to be reduced, the inlet effluent concentration needs to be reduced (dilution) or the treatment area needs to be increased for better treatment. For most practical applications, the volume of effluent to be treated will be fixed, e.g. a full lagoon, and the concentration of the effluent will be fixed because it will be impractical to dilute the wastewater before discharge to a constructed wetland. Therefore increasing the surface treatment area, not the volume capacity of the treatment area, is the most practical way to improve treatment capability of a constructed wetland system.

It may be that ADEC water quality standards need to be reconsidered for the assessment of constructed wetlands for sewage wastewater processing in the subarctic. The key elements that constructed wetlands provide in sewage pollutant reduction is the removal of pathogens, the reduction of BOD to alleviate O₂ depletion in receiving waters and the reduction of plant nutrients such as N and P, which helps to control a shift from oligotrophic to eutrophic conditions in the receiving waters.

It appeared that over the three years since its inception, the constructed wetland was losing its ability to remove pollutants from the wastewater and that a longer residence time would help to remove more of the pollutants. This would most logically be accomplished by increasing the cell surface area of the constructed wetland. The results from this study were not in general agreement with other studies using constructed wetlands for wastewater treatment. Most studies indicate that pollutant reduction performance increased after the initial few years, while this study indicated just the opposite. Possibly, the eccentric climatic factors that contribute to a high photosynthetic rate encouraged a high biomass production over a short season. Coupled with a slower decomposition rate due to colder water temperatures and a shorter season, this may have resulted in a slower response time in the ability of the wetland to remove or store the pollutants than is the case in more temperate climates. However, based on the current reduction in pollutant concentration despite the size limitation in this project, it appears that constructed wetlands will work as an alternative choice in treating wastewater in rural locations. Size constraints are not usually an issue in such locales, making these natural systems more attractive than the energy intensive alternatives. Further study with a constructed wetland sized appropriately for the expected effluent concentration needs to be completed to better understand the efficacy of constructed wetland use in the subarctic. Performance over time of such systems has not been recorded but current projects are being developed that will eventually answer this question.

The planting pattern used in this study masked the ability of a particular species to tolerate differing levels of pollutant concentrations. It was very difficult to determine if species colonization levels were controlled by pollutant concentrations, water depth or by competition

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

effects from the other species. Another project using <u>A. fulva</u>, <u>S. validus</u> and <u>T. latifolia</u> within five miles of this study showed that when these species were planted as a monotypic stand, colonization was rapid, with 85% of the cell surface area covered with the respective vegetation within the second year of planting (Maddux, 2001, unpublished data). In this study, <u>A. fulva</u> quickly added a carbon source in the form of decomposing plant material to the microbial community each fall due to the rapid and near total collapse of the standing biomass into the water during senescence. Because of this <u>A. fulva</u> may fulfill the requirement of a pioneer species in the building of a wetland ecosystem. Visual inspection of the cells in 2001 (post effluent flow) revealed that <u>C. rhynchophysa</u> and <u>M. trifoliata</u> had colonized more of the areas previously occupied by <u>A. fulva</u> than either <u>S. validus</u> or <u>T. latifolia</u>.

The vegetation study indicated that the initial planting of a constructed wetland used for sewage wastewater treatment does not require a high planting density to achieve a high vegetation density over a short period of time. Given the abundant nutrient availability in the effluent discharge, macrophyte colonization of the open spaces within the constructed wetland was rapid and complete within two years of operation. Natural colonization soon obliterated the initial planting patterns.

Future study

Future studies for heavy metal uptake by subarctic macrophytes should include a constructed wetland designed to treat anthropogenic heavy metals under real-world conditions, both in climate and in runoff concentrations. A variety of substrate types should be evaluated to determine if organic or mineral soil is the best for metal removal and storage within a constructed wetland and the ability of macrophytes to reproduce by seed after metal contamination should also be investigated. The use of constructed wetlands to treat stockpiled snow as it melts during spring break-up may be beneficial. Since the snow melts before wetland plants emerge in the late spring, an investigation into microbial activity in the thin layer of thawed soil and detritus within a constructed wetland would indicate the efficacy of building constructed wetlands near major snow dumps.

Further research questions to be considered include:

- What happens to the vegetation over an extended period of time in such a system?
 Knowing this will help in planting designs that lead to successful constructed wetland projects, wastewater treatment or habitat restoration.
- What happens to the wetland ecology as the plants, microbes and aquatic macroinvertebrates acclimate to the nutrient loads and hydraulic regimes?
 As wetland biota changes to fill the niches available, the ability of the wetland to remove pollutants may change.
- Does the efficiency of the constructed wetland to remove pollutants continue to show high year-to-year variability?

The capability to forecast efficiency of a constructed wetland in removing pollutants year after year would be desirable.

- Is there a long-term trend in the ability to process pollutants effectively?
 It will be important to know if a particular pollutant is reduced less efficiently either seasonally or over a period of time so the design of a constructed wetland can be modified to improve specific reduction efficiencies.
- What are the sestonic and dissolved components of the discharge waters from a constructed wetland?

The answers to this question can help to determine if the standard methods of water quality testing are valid for a constructed wetland as a water pollution control device, for instance the total suspended solids criteria and how it pertains to algae and zooplankton in the water column. • What is the conversion of the dissolved inorganic and organic matter to living and detrital particulate matter?

Total dissolved solids can have a negative impact on receiving waters and thus reduction and/or conversion to particulate matter that remains in the wetland is important.

- What species of plants invade a constructed wetland, how quickly do they spread, how quickly does an unvegetated cell become vegetated and what species are the colonizers? The macrophyte selection of a constructed wetland can be a major construction cost. Knowing how the selected macrophytes respond to transplanting and colonization and to the invasion of volunteer species can lower installation costs and prevent unforeseen vegetation problems.
- Will a combination of vegetated and non-vegetated cells be more efficient traps for pollutants?

Pollutant reduction processes are varied, depending on the pollutant being reduced. A pollutant such as nitrogen, which requires both aerobic and anaerobic environments for removal, may be optimized by incorporating both features into a single constructed wetland design.

Results from this project show that constructed wetlands can be a best management practice (BMP) and are a viable alternative to current wastewater treatment practices in subarctic environments, particularly in rural locations. A key to the successful implementation of constructed wetlands to treat sewage wastewater effectively in the subarctic is to incorporate an adequately sized lagoon into the design. The lagoon will serve the function of primary treatment and effluent storage. Effluent stored over the winter in the lagoon would be released to a constructed wetland sized to bring the lagoon to near empty by the end of the treatment season, which is the period of time between plants sprouting in the spring and freeze up in the fall. Thus no treatment by the constructed wetland needs to occur during the winter months. This is an important element in using constructed wetlands in rural locations and in keeping operation and maintenance costs low. Equally important, as illuminated by the k values resulting from this project, are the use of adequate sizing parameters in designing a constructed wetland. These parameters will include the volume of effluent to be treated, the inlet water quality, the required outlet water quality and local climate factors.

This body of research demonstrates that with adequate planning, constructed wetlands can be used successfully in subarctic climates to treat a variety of wastewater including heavy metals from roadway runoff and mine drainage, sewage effluent and agricultural wastes.



Figure A-1 Stem lengths for metal treated and non-treated (control) plants. Plants were grown in a greenhouse for 68 days and watered with 10 mg L⁻¹ of nitrate salt of Cadmium, Copper, Lead and Zinc. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are \pm 1 S.E.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Figure A-2 Stem Count for metal treated and non-treated (control) plants. Plants were grown in a greenhouse for 68 days and watered with 10 mg L⁻¹ of nitrate salt of Cadmium, Copper, Lead and Zinc. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are \pm 1 S.E.



Figure A-3 Nitrogen given to cadmium-treated plants. Comparison of nitrogen given in fertilizer and nitrate salt between treated plants and control and how this relates to dry weight biomass. * Above species/treatment histogram bar indicates a significant difference of p < 0.05 for that particular species/treatment. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are ±1 S.E.


Figure A-4 Nitrogen given to copper-treated plants. Comparison of nitrogen given in fertilizer and nitrate salt between treated plants and control and how this relates to dry weight biomass. * Above species/treatment histogram bar indicates a significant difference of p < 0.05 for that particular species/treatment. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are ± 1 S.E.



Figure A-5 Nitrogen given to lead-treated plants. Comparison of nitrogen given in fertilizer and nitrate salt between treated plants and control and how this relates to dry weight biomass. * Above species/treatment histogram bar indicates a significant difference of p < 0.05 for that particular species/treatment. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are ± 1 S.E.



Figure A-6 Nitrogen given to zinc-treated plants. Comparison of nitrogen given in fertilizer and nitrate salt between treated plants and control and how this relates to dry weight biomass. * Above species/treatment histogram bar indicates a significant difference of p < 0.05 for that particular species/treatment. Ar = <u>Arctophila fulva</u>, Cx = <u>Carex rhynchophysa</u>, My = <u>Menyanthes trifoliata</u>, Sc = <u>Scirpus validus</u> and Ty = <u>Typha latifolia</u>. n = 4 for all histogram bars. Error bars are ± 1 S.E.

			Samplir	ig Dates			
							Season
	6/2/98	6/24/98	7/21/98	8/11/98	9/16/98	10/6/98	Average
Inlet	34.5	19.2	16.4	1.53	7.18	4.77	9.82
Cell 1 - A		5.26	8.05	6.67	4.91	4.75	5.9 3
В	0.32	4.79	6.49	4.23	3.31	4.22	4.61
С		3.97	4.42	3.43	2.13	4.39	3.67
Cell 2 - A		8.03	9.72	9.11	6.22	6.77	7.97
В	0.33	4.78	9.69	6.48	5.59	6.25	6.56
С		3.16	6.35	4.89	5.13	6.1	5.13
Cell 3 - A		6.56	5.96	6.48	5.51	5.32	5.97
В	0.54	5.49	6.52	6.24	5.65	5.3	5.84
С		7.06	6.17	6.06	5.85	4.77	5.98
Cell 4 - A		5.39	8.83	6.31	5.13	5.04	6.14
В	0.16	5.05	7.21	4.02	2.68	4.36	4.66
С		3.65	5.06	3.12	0.91	3.5	3.25
Cell 5 - A		6.41	9.28	8.04	4.42	5.75	6.78
В	0.17	5.57	6.95	4.34	2.39	3.86	4.62
С		4.97	5.61	3.92	1.13	2.18	3.56
Avg.sta.A		6.27	8.97	7.53	5.17	5.58	6.70
Avg.sta.B	0.38	5.05	7.59	4.77	3.49	4.67	5.11
Avg.sta.C		3.94	5.36	3.84	2.33	4.04	3.90

. 1: \sim

Figure A-7 Example data sheet for Table 3-2: 1998 Total Phosphorus.

Data was collected by cell and station on the dates indicated, then averaged over the season. The season averages were used to compile Table 3-2. Data is in mg/L. Cell 3 is the unvegetated cell and is not replicated.

	C _{in} (kg/m ³)	C _{out} (kg/m ³)	C _{in} /C _{out}	In(C ₁ /C _{out})	Q (m³/yr)	A (m ²)	Q/A	k (m/yr)	
1998									120 days flow a t 5.678 m ³ /day
Sta. A	0.0098	0.0067	1.462687	0.38027486	136.27	15.36	8.87	3.37	
Sta. B	0.0098	0.0051	1.921569	0.65314185	136.27	30.72	4.44	2.90	
Sta. C	0.0098	0.0039	2.512821	0.92140583	136.27	46.08	2.96	2.72	
1999									125 days flow at 5.678 m ³ /day
Sta. A	0.00708	0.0075	0.944	-0.0576291	141.95	15.36	9.24	-0.53	
Sta. B	0.00708	0.0069	1.026087	0.0257525	141.95	30.72	4.62	0.12	
Sta. C	0.00708	0.0056	1.264286	0.23450731	141.95	46.08	3.08	0.72	
2000									113 days flow at 5.678 m ³ /day
Sta. A	0.0296	0.0257	1.151751	0.14128337	128.32	15.36	8.35	1.18	
Sta. B	0.0296	0.0236	1.254237	0.22652765	128.32	30.72	4.18	0.95	
Sta. C	0.0296	0.0221	1.339367	0.29219675	128.32	46.08	2.78	0.81	Average for 3 yrs at Sta. C = 1.42

To calculate the uptake rate constant k, rearrange the equation

$$A = \frac{Q}{k} \bullet \ln\left[\frac{C_m}{C_{back}}\right] \quad \text{to give} \quad k = \frac{Q}{A} \bullet \ln\left[\frac{C_m}{C_{out}}\right]$$

where

A = area required

Q = loading rate (m³/yr)

C_{in} = concentration coming in (mg/L)

C_{out} = concentration going out (mg/L)

C_{back} = background concentration (mg/L)

k = uptake rate constant (m/yr)

The C_{back} value is used when there is a certain background concentration that needs to be met. This is changed to the actual output concentration, C_{out} , when calculating the existing k value for the uptake rate constant.

Figure A-8 Example for calculating the uptake rate constant k for Total Phosphorus in Table 3-1.



Figure A-9 Side-by-side comparison of vegetation coverage to pollutant reduction in 1998. Ranks: 1 = low, 2 = medium-low, 3 = medium, 4 = medium-high, 5 = high Vegetation coverage data was taken from Figures 4-4 through 4-8. Pollutant reduction percentages were taken from Table 3-2.























Figure A-13 Average redox potentials for vegetated and unvegetated cells for 1998. The platinum probes were buried at five depths, from the surface of the sediment to the bottom of the sediment (on top of liner). 0 mm, 25 mm, 76 mm, 152 mm and 305 mm. x-axis dates are the same for all depths. n = 4 for the vegetated cells and n = 1 for the unvegetated cell.





Figure A-14 Average redox potentials for vegetated and unvegetated cells for 1999. The platinum probes were buried at five depths, from the surface of the sediment to the bottom of the sediment (on top of liner). 0 mm, 25 mm, 76 mm, 105 mm and 305 mm. x-axis dates are the same for all depths n = 4 for the vegetated cells and $n \approx 1$ for the unvegetated cell

Plant	Dry wgt.	Cd	Cd detected in	micrograms	mg of Cd
Part	Grams	added/mg	micrograms/g	in plant part	in plant
AG 1		15.21			
Stem	7.50		34.26	256.95	0.26
Leaf	21.4		21.75	465.45	0.47
Flower					
Root	9.18		128.7	1181.47	1.18
Plant total	38.08		184.71	1903.87	1.90
Soil	25.9		102.889	2664.83	2.66
AG 2		26.27			
Stem	31.3		25.41	795.33	0.80
Leaf	28.2		19.72	556.10	0.56
Flower	5.7		4.8	27.36	0.03
Root	29.6		36.65	1084.84	1.08
				· · · · · · · · · · · · · · · · · · ·	
Plant total	94.80		86.58	2463.64	2.46
Soil	14.4		198.15	2853.36	2.85
AG 3		35.18			
Stem	41.3		22.8	941.64	0.94
Leaf	30.4		29.09	884.34	0.88
Flower	6.3		2.53	15.94	0.02
Root	40.1		119.04	4773.50	4.77
				··· · · · · · · · · · ·	
Plant total	118.10		173.46	6615.42	6.62
Soil	13.3		209.867	2791.23	2.79
		77 57			
AG 4	22.5	27.57	40.22	047.52	0.05
Joof	23.5		40.32	<u>947.52</u> 570.01	0.95
Flower	<u> </u>		23.13	09.63	0.30
Root	24.0		15.04	30.03	2 79
	24.3		101.07	3110.30	3.70
Plant total	77.90		235.06	5401.75	5.40
Soil	15.3		120.958	1850.66	1.85
	•				····

Average cadmium removed by <u>A. fulva</u> 4.10

Figure A-15 Data set for Arctophila fulva treated with Cadmium. This is an example of t data used for calculations in Chapter 2. Each plant and metal was replicated four times. AG 1 = A. fulva replicate 1

142

Plant	Dry wgt.	Cd	Cd detected in	micrograms	mg of Cd
part	grams	added/mg	micrograms/g	in plant part	in plant
CX 1		23.77			
Stem	17.2		87.125	1498.55	1.50
Leaf	16.4		7.569	124.13	0.12
Flower	3.5		35.659	124.81	0.12
Root	23.5		91.612	2152.88	2.15
Plant total	60.60		221.965	13451.08	3.90
Soil	27.2		134	3644.80	· · · · _ · · _ · · ·
CX 2		16.88		<u> </u>	
Stem	9		38.928	350.35	0.35
Leaf	10.1		4.103	41.44	0.04
Flower	2.6				
Root	10.6		170.095	1803.01	1.80
Plant total	32.30		213.126	6883.97	2.19
Soil	34.9		98.943	3453.11	
CX 3		27 10			
Stem	18.8		59 339	1115 57	1 12
Leaf	22.7	· ····	1 971	AA 7A	0.04
Flower	4.5		1.571		0.04
Root	21.5		113.824	2447.22	2.45
Plant total	67.50		175.134	11821.55	3.61
Soil	24.8		131.539	3262.17	3.26
oX 4					
	40	24.72	A 4 979	4005 47	
Stem	16		64.073	1025.17	1.03
Lear	20.4		8.246	168.22	0.17
Prower			450 304		
R001	15		150./91	2261.87	2.26
Plant total	51.40		223.11	11467.85	3.46
Soil	32.3		90.722	2930.32	2.93

Average cadmium removed by <u>C. rhyncophysa</u> 3.29

Figure A-16. Data set for Carex rhynchophysa treated with cadmium. This is an example of the data used for calculations in Chapter 2. Each plant and metal was replicated four times. CX 1 = \underline{C} . rhynchophysa replicate 1.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Plant	Dry wgt.	Cd	Cd detected in	micrograms	mg of Cd in
part	grams	added/mg	micrograms/g	in plant part	plant part
			·····		
MY 1		22.58			
Stem	10.7		99.60	1065.68	1.07
Leaf	8.4		31.38	263.57	0.26
Flower					
Root	7.1		85.15	604.57	0.60
Plant total	26.20		216 124	5662.45	1 03
Soil	7 1		115 77	821 93	1.55
00	<u> </u>		115.77	021.95	
MY 2		24.36			
Stem	10.3		161.53	1663.79	1.66
Leaf	8.2		50.55	414.51	0.41
Flower					
Root	7.7		128.55	989.84	0.99
	00.00				
Plant total	26.20		340.634	8924.61	3.07
501	37.8		105.81	3999.50	
MY 3		20.32			
Stem	93		46.99	437.01	0 44
Leaf	83		19.40	161.01	0.16
Flower	0.0				0.10
Root	7.5		161.63	1212.22	1.21
Plant total	25.10		228.018	5723.25	1.81
Soil	44.2		114.74	5071.64	
MY 4		23.06			
Stem	12.5		48.78	609.74	0.61
Leaf	7.6		36.45	277.04	0.28
Flower	4.6		0.12	0.56	0.00
Root	8		144.54	1156.28	1.16
Plant total	32.70		229,888	7517.34	2.04
Soil	59.8		67.70	4048.46	

Average cadmium removed by M. trifoliata 2.21

Figure A-17 Data set for Menyanthes trifoliata treated with cadmium. This is an example of the data used for calculations in Chapter 2. Each plant and metal was replicated four times. MY 1 = M. trifoliata replicate 1.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Plant	Dry wgt.	Cd	Cd detected in	micrograms	mg of Cd
part	Grams	added/mg	microrgrams/g	in plant part	in plant
SC 1		28.05			
Stem	27.4		15.21	416.70	0.42
Flower	3.1		40.22	124.69	0.12
Root	16		291.67	4666.69	4.67
Rhizome	26.8		38.73	1037.88	1.04
Plant total	73.30		385.827	28281.12	6.25
Soil	27.8		122.21	3397.33	3.40
SC 2		27.93			
Stem	24.5		17.74	434.68	0.43
Flower	4.4				
Root	12		424.77	5097.28	5.10
Rhizome	26		52.40	1362.50	1.36
Plant total	66.90		494.919	33110.08	6.89
Soll	27.3		76.56	2090.03	2.09
SC 3		29.95			
Stem	21.8		34.74	757.40	0.76
Flower	12.7		2.20	27.98	0.03
Root	12.5		386.49	4831.09	4.83
Rhizome	22.5		65.79	1480.23	1.48
Plant total	69.50		489.221	34000.86	7.10
Soil	22.9		109.20	2500.73	2.50
SC 4		32.56			
Stem	26.8		33.90	908.60	0.91
Flower	4.6		1.86	8.53	0.01
Root	9.4		321.40	3021.13	3.02
Rhizome	27		51.95	1402.65	1.40
Plant total	67.80		409.105	27737 32	5 34
Soil	36.6		190.67	6978.56	6.98

Average cadmium removed by <u>S. validus</u> 6.40

Figure A-18 Data set for Scirpus validus treated with cadmium. This is an example of the data used for calculations in Chapter 2. Each plant and metal was replicated four times. SC 1 = S. validus replicate 1.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Plant	Dry wgt.	Cd	Cd detected	micrograms	mg of Cd
part	Grams	added/mg	micrograms/g	in plant part	in plant
TY 1		34.82			
Stem	21		24.62	517.02	0.52
Leaf	26		19.448	505.65	0.51
Flower					
Root	19		666.689	12667.09	12.67
Rhizome	53.9		22.987	1239.00	1.24
Plant total	119.90		733.744	14928.76	14.93
Soil	17.8		217.578	3872.89	3.87
TV 2		43.62			
Stem	15.4	43.02	7 777	119 77	0.12
Leaf	26.5		10.655	282.36	0.12
Flower	20.0		10.000	202.00	0.20
Root	17.5		504 737	8832 90	8.83
Rhizome	53.8		12 202	656 47	0.00
I VIII ZOINE	33.0		12.202	030.47	0.00
Plant total	113.20		535.371	9891.49	9.89
Soil	15.2		253.954	3860.10	3.86
TY 3		33.16		· · · · · · · · · · · · · · · · · · ·	
Stem	25		2.651	66.28	0.07
Leaf	25.5		6.144	156.67	0.16
Flower			······································		
Root	8.8		620.643	5461.66	5.46
Rhizome	26.6		20.116	535.09	0.54
Plant total	85.90		649.554	6219.69	6.22
Soil	43.4		135.555	5883.09	5.88
TY 4		20.56			
Stem	17.2		5.686	97.80	0.10
Leaf	19.9		7.647	152.18	0.15
Flower	· · · · ·				
Root	6.7		597.463	4003.00	4.00
Rhizome	13.6	· · · ·	40.836	555.37	0.56
	57.40		CE1 000	4800.05	
Fiant total	57.40		001.032	4808.35	4.81
001	33.8		98.679	3335.35	3.34

Average cadmium removed by T. latifolia

8.96

Figure A-19 Data set for Typha latifolia treated with cadmium. This is an example of the data used for calculations in Chapter 2. Each plant and metal was replicated four times. TY 1 = T. latifolia replicate 1.