THE EFFECTS OF NUTRIENT ADDITIONS ON THE SEDIMENTATION OF SURFACE WATER CONTAMINANTS IN A URANIUM MINED PIT-LAKE

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ABSTRACT

I investigated the usefulness of phytoplankton for the removal of surface water contaminants. Three experiments, consisting of nine large mesocosms (92.2 m^3) were suspended in the flooded DJX uranium pit at Cluff Lake (Saskatchewan, Canada), and filled with contaminated mine water. During the summer of 2003, each mesocosm was fertilized with a different amount of phosphorus throughout the 35 day experiment to stimulate phytoplankton growth, and to create a range in phosphorus load (g) to examine how contaminants may be affected by different nutrient regimes. Algal growth was rapid in fertilized mesocosms as demonstrated by chlorophyll *a* profiles. As phosphorus loads increased there were significant declines in the surface water concentrations of As, Co, Cu, Mn, Ni, and Zn. This decline was near significant for uranium. The surface water concentrations of Ra^{226}, Mo, and Se showed no relationship to phosphorus load. Contaminant concentrations in sediment traps suspended at the bottom of each mesocosm generally showed the opposite trend to that observed in the surface water, with most contaminants (As, Co, Cu, Mn, Ni, Ra^{226}, U, and Zn) exhibiting a significant positive relationship ($P < 0.05$) with phosphorus load. Sediment trap concentration of Se and Mo did not respond to nutrient treatments.

Similar experiments were repeated during the mid- and late-summer of 2004, with 5 mesocosms being fertilized with phosphorus, and another 4 with both phosphorus and ammonium to create different nutrient gradients. Results from these experiments were much more variable than those seen in the experiment conducted in 2003, and

small samples (n = 5 for phosphorus treatments and n = 4 for both phosphorus and ammonium treatments) yielded insufficient statistical power to effectively determine statistically significant trends. However, contaminant sedimentation tended to respond to phosphorus treatments in a similar manner as results from 2003; phosphorus-with-ammonium treatments had little positive effect on contaminant sedimentation rates.

My results suggest that phytoremediation has the potential to lower many surface water contaminants through the sedimentation of phytoplankton. Based on our results from 2003, we estimate that the Saskatchewan Surface Water Quality Objectives (SSWQO) for the DJX pit would be met in approximately 45 weeks for Co, 65 weeks for Ni, 15 weeks for U, and 5 weeks for Zn if treated using phytoremediation.

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1. INTRODUCTION

1.1 Introduction

Pit-lakes result from the cessation of open-pit mining operations. Open pit mining intercepts groundwater which must be pumped from surrounding wells to ensure the pit remains dry (Miller *et al.* 1996). Once mining operations are terminated, the pit may passively fill by surface water run-off or an inflow of groundwater, or a combination of both (Castro and Moore 2000; Miller *et al.* 1996; Shevenell *et al.* 1999), or the pit may be actively filled with water from a nearby source. However, pit-lakes commonly become contaminated with metals (Castro and Moore 2000) and sulfates (Miller *et al.* 1996) and other inorganic ions.

Open-pits are a common method of ore extraction around the world. For example, Castro and Moore (2000) reported 86 open-pit mining operations extracting precious and/or base metals other than iron or aluminum in the U. S., 19 in Canada, 74 in Australia, and 37 in Chile. As a result, it is expected that contaminated pit-lakes will continue to be a major water issue in the near future (Castro and Moore 2000; Shevenell *et al.* 1999).

Canada is the world's leading producer of newly-mined uranium. All of the uranium produced in Canada is mined in the Athabasca Basin in Northern Saskatchewan, which contains very high grade uranium ore deposits. In 1996 the average uranium ore grades in the Athabasca basin were approximately ten times higher than ore grades elsewhere (Akin *et al.* 1996). Currently, there are seven uranium mines

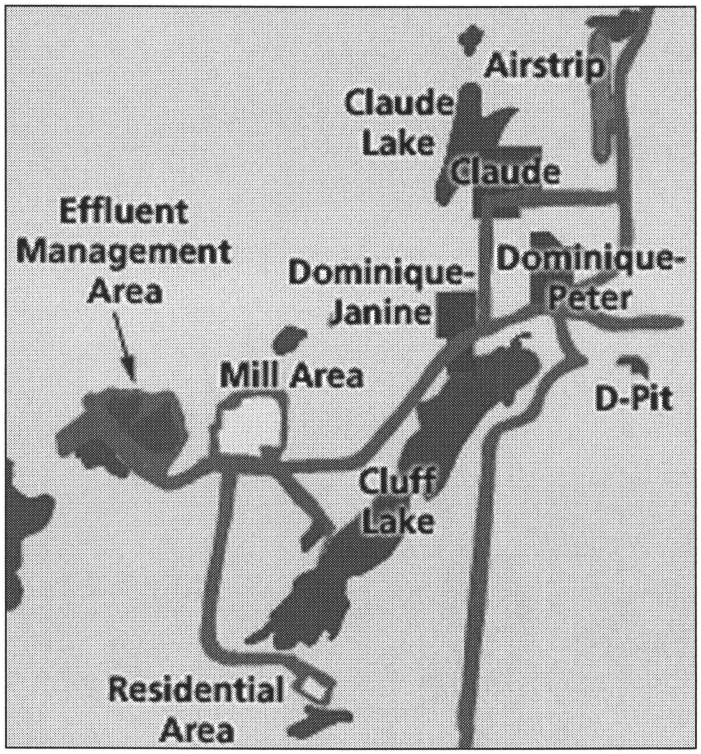
which have been proposed, are in operation, or are in the process of being decommissioned in Saskatchewan and are located at: Cluff Lake, Key Lake, McArthur River, McClean Lake, Cigar Lake, Rabbit Lake, and Midwest. Of the seven uranium mines, five have or plan to have open pits.

In August 1998, COGEMA announced that it would indefinitely suspend mining and milling operations at Cluff Lake due to depleted economically viable ore reserves. Furthermore, the Tailings Management Area (TMA), which is where contaminants are removed from solution, was approaching maximum authorized capacity (Canadian Nuclear Safety Commission 2003), and thus, Cluff Lake mine site soon would lack storage space for contaminants removed from the milling and mining process. In 2002, milling officially ceased, and Cluff Lake mine began preparations for decommissioning. Cluff Lake would become the first high grade mine to be decommissioned in Canada (MineWatch Canada 2004).

Cluff Lake mine site is wholly owned by COGEMA, a Canadian company with headquarters in Saskatoon and part of the Areva conglomerate based in France. COGEMA is one of the largest producers of uranium in the world (Canadian Nuclear Safety Commission 2003). Cluff Lake mine site is located in northern Saskatchewan in the Athabasca Basin (58° 22' N, 109° 31' W), about 700 km north of Saskatoon, 150 km south of Uranium City (Hynes 1990), approximately 15 km east of the Alberta border, and 75 km south of Lake Athabasca (Canadian Nuclear Safety Commission 2003).

Development of the Cluff Lake area began with exploration activities in the 1960's. However, initial site development at the Cluff Lake mine (Figure 1-1) did not begin until 1979 with the mining of D pit, a high grade uranium ore deposit with significant gold reserves, and the construction of a mill to process the uranium ore

Figure 1-1 Map of Cluff Lake uranium mine site. Dominique-Janine area includes DJN and DJX pit. Map from http://www.cri.ca/publications/natural.html.



(Canadian Nuclear Safety Commission 2003). Mining of the Dominique Janine (DJ) area did not begin until 1989 with the mining of the DJ north or DJN pit. The DJN pit mining ceased in 1991; in total 203 800 tonnes of uranium ore was removed from the DJN at 0.317% U, equalling 646 tonnes total U. Mining of the DJ extension pit, or DJX pit, occurred from 1994 to 1997. At this point, the DJN pit was back-filled with waste rock from the DJX pit. The DJX pit ore body was 394 928 tonnes at 0.299% U, equalling 1182 tonnes U (Neal 2004, pers. comm.). Upon the termination of mining activities, the DJX pit was 90 m deep, 240 m wide, 340 m long, with a volume of 2 704 864 m^3 to the top of the bedrock, and a volume of 3 243 324 m^3 to the top of the overburden (Hallborg 2004, pers. comm.).

Water was allowed to collect in the DJX pit, and in 1999, mine operators began pumping water from a nearby pit, Claude pit (Figure 1-1), into the DJX pit. Claude pit water was more contaminated than water contained in the DJX pit. Around the same time, 257 m^3 day^{-1} of water was being pumped from the DJX pit on a seasonal basis to the water treatment plant which represented catchment area runoff and groundwater inputs, as well as water seepage from the DJN pit into the DJX pit (Canadian Nuclear Safety Commission 2003). As a result, water levels in the DJX pit have been variable.

1. 2 General Water Chemistry and Water Quality Objectives for DJX Pit

The DJX pit contains elevated concentrations of a variety of contaminants. These contaminant concentrations have varied since the onset of the study. Saskatchewan surface water quality objectives (SSWQO) for the DJX pit are: 3 $\mu g\ L^{-1}$ for As; 20 $\mu g\ L^{-1}$ for Co; 10 $\mu g\ L^{-1}$ for Cu; 500 $\mu g\ L^{-1}$ for Mo; 25 $\mu g\ L^{-1}$ for Ni; 10 $\mu g\ L^{-1}$ for Se; 2 mg L^{-1} for U; 50 $\mu g\ L^{-1}$ for Zn; and 0.11 Bq L^{-1} for Ra^{226} (Acott 2004, pers. comm.). Surface

water contaminant concentration for the DJX pit must meet SSWQO before the pit can be fully decommissioned.

Initial contaminant concentrations in the summer of 2003 exceeded SSWQO for: Co (240 $\mu g\ L^{-1}$); Mo (950 $\mu g\ L^{-1}$); Ni (1300 $\mu g\ L^{-1}$); U (3.4 $mg\ L^{-1}$); Zn (200 $\mu g\ L^{-1}$); and Ra^{226} (0.68 $Bq\ L^{-1}$). Initial nutrient concentrations in the summer of 2003 were 11 $mg\ L^{-1}$ of total nitrogen and 6 $\mu g\ L^{-1}$ of total phosphorus. The high nitrogen concentration was likely due to ammonium nitrate explosive residue which has dissolved in the pit water. Most of the nitrogen was in the form of nitrate. The total phosphorus concentration suggested that the DJX pit was oligotrophic, even though the total nitrogen concentration suggested that the DJX was hypereutrophic, which indicated that phosphorus was likely the nutrient limiting phytoplankton growth (Schindler 1974).

Initial contaminant concentrations of the DJX pit were lower during the summer of 2004 compared to initial concentrations observed in 2003. However, Co, Ni, Zn, and Ra^{226} concentrations still greatly exceeded SSWQO. Total nitrogen concentration decreased to 4.93 $mg\ L^{-1}$ while total phosphorus concentration increased to 14 $\mu g\ L^{-1}$ which was likely due to the addition of phosphorus to DJX pit by COGEMA during the fall of 2003. Even though phosphorus-limitation may have been relaxed or weakened by phosphorus additions by Cogema, the atomic total N to total P ratio was still approximately 779:1 which greatly exceeds the Redfield ratio of 16 N: 1 P (Redfield 1958; Redfield *et al.* 1963). The Redfield ratio characterizes the proportion C, N, and P, required by phytoplankton, and deviations from this ratio may suggest specific nutrient-limitation. Wetzel (2001) suggests that severe P-limitation occurs when the N:P ratio exceeds 23:1. Thus, the N:P ratio found in the DJX pit strongly suggests that phosphorus remained the limiting nutrient in this system.

1. 3 Mine Water Treatment

Cluff Lake's Tailings Management Area (TMA) or Effluent Management Area is located upstream of Snake and Island Lakes (Canadian Nuclear Safety Commission 2003), and it receives all water contaminated by milling and mining operations (Hynes 1990). The TMA was first created in 1980, but there have been many modifications since. The TMA contains a solids containment area, a tailings water decantation area, freshwater diversion ditches, and the primary and secondary water treatment systems (Hynes 1990).

To treat contaminated water from the DJX, water must be pumped from the DJX pit located about 4 km from the mill to the primary water treatment system. Here, Ra^{226} is precipitated through the addition of barium chloride and ferric sulphate, and is co-precipitated with barium sulphate (Hynes 1990). The treated water is retained in two settling ponds prior to being transferred to the liquid ponds. Water retained in the liquid ponds allows for further settling of precipitate before the water is decanted to the secondary water treatment system for final polishing. Periodically, pH may be adjusted chemically using soda ash when necessary (Canadian Nuclear Safety Commission 2003).

The secondary water treatment system was added in 1981 which used the same process as the primary water treatment system. The main objective of the secondary water treatment system was the further precipitation of Ra^{226} (Hynes 1990). Again, Ra^{226} would be co-precipitated with barium sulphate through the addition of barium chloride and ferric sulphate. Alum was used to flocculate slow-settling barium-radium-sulphate particles (Hynes 1990). In 1982, a computer-controlled sand filtration system was added

to the secondary treatment system. The treated water is then discharged into Snake Creek and subsequently, Island Lake.

Nonetheless, the treated water may still have been detrimental to the local aquatic ecosystem. Hynes (1990) found that contaminants, such as U and Ra^{226}, were not completely removed from the treated water, and as a result, these contaminants increased in concentration in the tissues of fish and aquatic macrophytes in Island Lake. Furthermore, Pyle *et al.* (2002) found that hatching success of fathead minnows (*Pimephales promelas*) was significantly reduced when exposed to waters receiving contaminants associated with uranium ore milling from Key and Rabbit Lake uranium operations. However, Hynes (1990) found that the greatest impact to Island Lake was probably not caused by metals and radionuclides, but rather the increase in salinity from the sulfates used in the water treatment process. Hynes believed that further increases in salinity to Island Lake would likely lead to a further loss of species.

The cost of conventional water treatment processes is large. The cost to treat contaminated water is about 75 cents m^{-3} (Acott, personal communication). Thus, if the DJX pit were filled to the top of the bedrock with contaminated water, the cost to treat the water is estimated at over 2 million Canadian dollars.

1. 4 Background: Phytoplankton Contaminant Uptake

Due to the great financial and environmental costs of conventional mine water treatment processes, more benign approaches to mine water treatment have been tested. For example, phytoplankton have recently been used to bioremediate the mildly contaminated D-Zone pit at Rabbit Lake uranium mine (Paton 2001; Paton 2002). Crusius et al. (2003) also inverstigated the usefulness of phytoplankton to bioremediate

flooded pits at Equity Silver Mine in B. C., Canada. To do this, Crusius et al. added nutrients to mesocosms to stimulate phytoplankton growth.

Bioremediation uses biological agents to restore contaminated lands. These biological agents often take-up or chemically reduce contaminants in either the soil or water. Organisms such as bacteria are often used to bioremediation contaminated sites. Usually, the growth of the organism of interest is promoted to treat a site more rapidly. Using photosynthetic organisms such as phytoplankton to bioremediate contaminated sites known as *phytoremediation*.

Recently, support has grown for the use of bioremedial techniques to reclaim contaminated sites. Kalin (2004) suggests using ecological engineering principles for mine waste management, specifically by incorporating nature's repair mechanisms using bioremediation. Kalin et al. (2005) further promotes bioremedial techniques by using algae to removal of U from dilute waste water discharge, since algae are able to grow in a variety of extreme environments.

Many experiments have demonstrated the ability of phytoplankton and algae to adsorb and take up contaminants such as: Ni, Zn, Cd, Cu, Pb, U, As, Cr, Mn, and Fe (Awasthi and Rai 2004; Costa and Leite 1990; Fehrmann and Pohl 1993; Gonzalezdavila *et al.* 1995; Hashim and Chu 2004; Knauer *et al.* 1997a; Knauer *et al.* 1997b; Koelmans *et al.* 1996; Mann and Fyfe 1985; Pribil and Marvan 1976; Roy *et al.* 1993; Tien 2002; Vasconcelos *et al.* 2002; Xue *et al.* 1988; Yang and Volesky 1999; Yu *et al.* 1999). In fact, many problem contaminants such as Cu and Mo are also micronutrients and necessary for a variety of metabolic processes (Graham and Wilcox 2000; Llamas *et al.* 2000; Zahalak *et al.* 2004). Even dead algal material has shown a great ability to remove dissolved contaminants (Barkley 1991; Campbell 1999; Zeroual

et al. 2003). Phytoplankton, therefore, are good candidates for bioremediation in pit-lakes.

Generally in aquatic systems, phosphorus is the limiting nutrient for phytoplankton growth (Schindler 1974; Schindler 1977). Moreover, the addition of nutrients may enhance phytoplankton metal uptake (Wang and Dei 2001a; Wang and Dei 2001b). With this in mind, the addition of nutrients to a pit-lake may stimulate phytoplankton growth and subsequent uptake and adsorption of contaminants. As phytoplankton senesce and sediment out of the water column to the pit-bottom, so do the bound contaminants. Unlike most natural lakes, pit-lakes, such as the DJX pit, are narrow, steep-sided and relatively deep (Miller *et al.* 1996), and as a result, have a great relative-depth to area ratio (depth relative to surface area) and often become meromictic. This meromixis prevents complete water column mixing (Castro and Moore 2000; Kalff 2002). Once the lake becomes meromictic, there is little chance of resuspension of contaminants. Theoretically, by stimulating phytoplankton growth, contaminants may be sedimented and trapped in the monolimnetic zone (non-mixing portion of the water column) of a pit-lake, effectively ensuring that surface water contaminant levels remain below surface water quality guidelines.

1. 5 Study Objectives and General Study Design

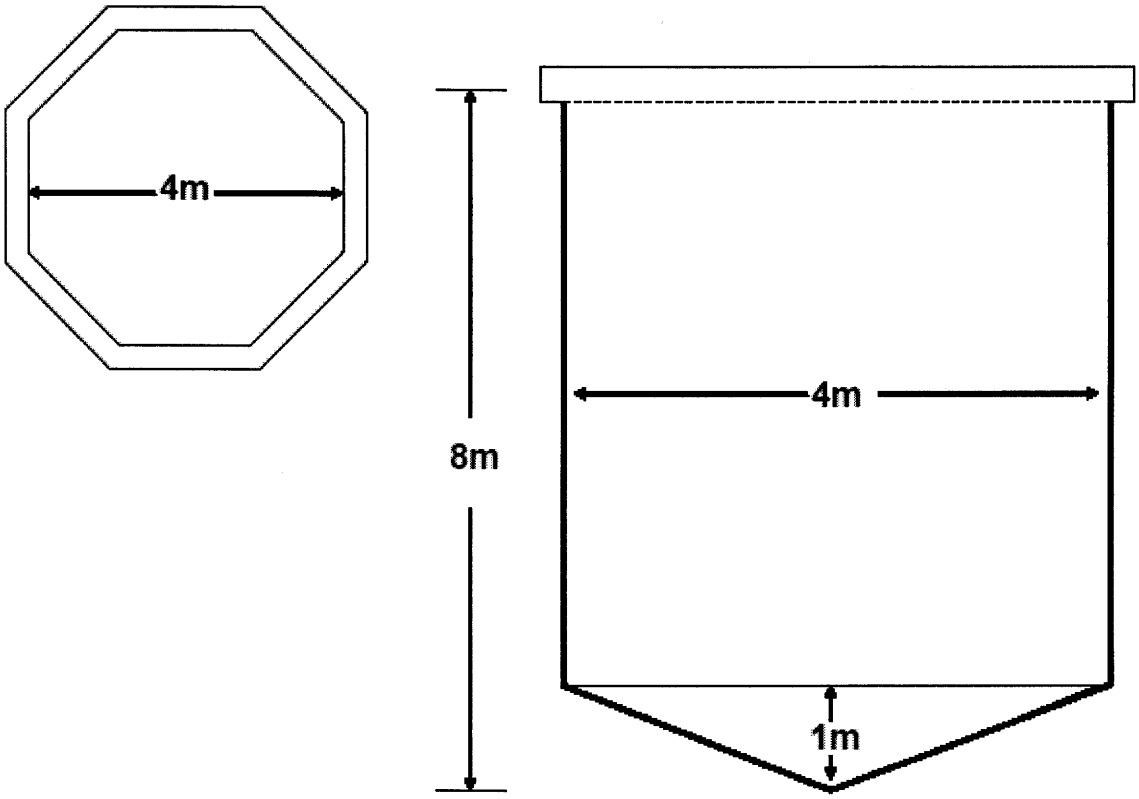
The overall objective of the study was to determine the effectiveness of phytoremediation in a contaminated pit-lake. This would be done through nutrient manipulation, and changes in water chemistry would be compared with changing nutrient concentrations. The main objective of this study was evaluated with the following sub-objectives:

1. To determine if the addition of nutrients would increase phytoplankton growth and biomass in a contaminated pit-lake;
2. To determine if the subsequent growth in phytoplankton would result in a reduction in surface water contaminant concentrations; and
3. To determine if the reduction in surface water contaminant concentration was due to the sedimentation of contaminants.

To test the effects of nutrients on phytoplankton growth and contaminant sedimentation rates, nine 92 m^3 mesocosms were employed. The mesocosms were 4 m in diameter, and 8 m deep with the last meter being tapered (Figure 1-2). The mesocosms were completely enclosed by a translucent, reinforced polyethylene wall, and placed in the DJX pit. Each individual mesocosm represented a nutrient treatment. The mesocosms were filled with contaminated DJX water, attached to one another with a rope, and positioned in the center of DJX pit in an east – west orientation to reduce shading from one mesocosm to the next. Treatments were randomly assigned to each mesocosm. A nutrient gradient was established across the mesocosms to determine the effects of increasing nutrient load on surface water contaminants. Furthermore, different combinations of nutrients were added to the mesocosms to determine their effect on contaminant sedimentation rates.

Nutrients were dissolved in mesocosm water and then added to the mesocosms in a concentration based on the specific treatment designation. Surface water samples were taken from the mesocosms to monitor nutrient, contaminant, and chlorophyll *a* concentrations. Since the Secchi disc causes a lot of turbulence, Secchi depth measurements were only taken on the first day of each experiment before the nutrients were added to the mesocosms, and on the final day of each experiment. This was done to

Figure 1-2 Mesocosm design and dimensions.



prevent the resuspension of sedimented material. Sediment traps were positioned in each mesocosm at a depth of 7 m. The sediment traps were used to collect sedimented phytoplankton and contaminants. Sediment traps were emptied periodically and subsampled, and the subsamples were added to a cumulative sample for metal and radionuclide analyses to get a total contaminant concentration over the course of the experiment.

CHAPTER 2

The Effects of Phosphorus Additions on the Sedimentation of Contaminants in a Uranium Mined Pit-Lake (Based on Dessouki *et al.* 2005)

2. 1 Introduction

Pit-lakes are often created from the mining of ores close to the earth's surface (Castro and Moore 2000; Shevenell *et al.* 1999). Many Uranium deposits in Northern Saskatchewan are mined using open pit methods (Pyle *et al.* 2002). Water from these pit-lakes is often treated and released into local watersheds. However, treated water may still contain elevated levels of contaminants relative to background levels, such as Ra^{226}, Mo, and U in the case of uranium mines (Hynes 1990), which may have detrimental effects on biota in the receiving water (Pyle *et al.* 2002) and negatively effect local watersheds (Bayda *et al.* 1978). In addition, standard water treatment processes are very expensive and may increase the salinity of the receiving water as a result of the chemicals used in the waste water treatment process. Such increases in salinity may have a greater impact on local watersheds than that of trace metals (Hynes 1990).

Bioremediation offers a potentially benign and inexpensive approach for the removal of contaminants from the surface water in pit-lakes. Phytoplankton have been used to remove surface contaminants at Rabbit Lake Uranium Mine, Saskatchewan, Canada (Paton 2001; Paton 2002). Phytoplankton play a key role in the fate and impact of metals (Maeda 1990; Shehata *et al.* 1999), and have been shown to accumulate metals such as Ba, Co, Cu, Ni, U and Zn (Knauer *et al.* 1997; Mann and Fyfe 1985; Pribil 1976) and, therefore, appear to be good candidates for bioremediation.

Many pit-lakes are steep sided and deep and stratify quickly after flooding to become meromictic (remain stratified all year), enhancing their potential for remediation (Castro and Moore 2000). Specifically, if phytoplankton are used to take up contaminants from the surface water and sediment them to the bottom, the contaminants are unlikely to be recirculated to the surface water under permanently stratified conditions. Moreover, this process may be enhanced by adding nutrients to increase algal abundance and biomass, which may result in increased sedimentation of contaminants (Wang and Dei 2001a). The objectives of this study were to determine if the addition of phosphorus would increase the growth and biomass of phytoplankton in a heavily contaminated pit-lake, and whether this would lead to an increased sedimentation of contaminants to deeper water.

This study was conducted in the DJX pit-lake, located at Cluff Lake Uranium Mine (58° 22' N, 109° 31' W) in Northern Saskatchewan, Canada, during July and August, 2003. Uranium ore (1182 tonnes) was mined from the DJX pit from 1994 to 1997. The pit is 240 m meters wide, 340 m long, 90 m deep, and has a total volume of 2,704,864 m^3. Contaminated water was being pumped from Claude Pit into the DJX Pit during the experiment. The pit was filled to a depth of 55 m at the start of the experiment and the water was 65 m deep by the end of the experiment. Initial contaminant and nutrient concentrations in the pit were measured (Table 2-1).

2. 2 Materials and Methods

The study involved a mesocosm experiment where a gradient of nutrient concentrations was created, and the response of the phytoplankton and various contaminants were measured in relation to the addition of nutrients. Nine 92.2 m^3 mesocosms were positioned in the centre of the DJX pit from east to west and filled with

Table 2-1 Initial contaminant and nutrient concentrations (mg L^{-1}) in the DJX Pit. Ra^{226} is reported in Bq L^{-1}.

Contaminant	Concentration (mg L^{-1})	Saskatchewan Surface Water Quality Objectives (SSWQO)
Arsenic	0.003	0.05
Cobalt	0.24	0.02
Copper	0.0095	0.01
Manganese	2.5	N. A.
Molybdenum	0.905	0.5
Nickel	1.3	0.025
Total Nitrogen	11	N. A.
Total Phosphorus	0.006	N. A.
Selenium	0.0066	0.01
Uranium	3.4	2.0
Zinc	0.2	0.05
Radium-226	0.68	0.11

N. A. - Not Applicable

contaminated pit water. The mesocosms were cylindrically shaped, 4 m in diameter and 8 m deep, with a closed, tapered bottom to prevent resuspension of sedimented contaminants (Figure 1-2).

Ambient total phosphorus concentrations in the DJX pit were low relative to that of total nitrogen (Table 2-1), and therefore, as previously mentioned (Chapter 1-2), the pit water was considered phosphorus deficient for phytoplankton growth (Schindler 1977). Consequently, analytical grade potassium phosphate was added to eight treatment mesocosms on days 0, 18 and 25 of the experiment to create a nutrient gradient. One mesocosm was left untreated to act as a control (Table 2-2). Treatment and control mesocosms were arranged in a random order. The experiment was run for 37 days, July 6th to August 12th, 2003. Total phosphorus was analysed following the method described in Wetzel and Likens (2000).

Phytoplankton growth was monitored using an algal fluorescence probe (YSI 600 OMS profiler). Water column fluorescence profiles were measured twice weekly in the morning, and once weekly at dusk. Surface water samples were collected concurrently with algal fluorescence profiles in order to measure chl *a* by spectrophotometry. The surface water was filtered onto glass fibre filters (GF/F, nominal pore size 0.7 μm) and then stored in the dark at -20°C until chl *a* was analysed. Chl *a* pigment was extracted and analysed using the methods described in Bergmann and Peters (1980) with the following changes based on Arvola (1981): absorbance was read at 665nm rather than 655nm, and chl *a* samples were left in 95% ethanol for 24 hours at room temperature rather than refrigerated. The relationship between the chl *a* estimates and their corresponding YSI fluorescence measurements were analysed with Model I linear regression forced through the origin (see below). Fluorescence values were

Table 2-2 Total phosphorus load (g) and corresponding Secchi depths (m) in each mesocosm at the end of the experiment (Day 37).

Mesocosm position	Phosphorus Load (g)	Secchi Depth (m)
5 (control)	0.58	> 8
2	2.54	2.4
6	4.08	2.45
1	5.00	1.95
8	7.59	2.0
3	9.75	1.95
4	11.91	1.75
9	12.00	1.75
7	13.34	1.8

converted to chl *a* estimates using the following linear model: (Y = 5.583X). Chl *a* profiles were then compared between mesocosms. Secchi depths were taken on the final day of the experiment (day 37) to prevent sediment resuspension in the mesocosms. Secchi depths are measured using a Secchi disc, which is lowered into the water until it is no longer visible, at this point the depth is recorded. Secchi depths are often used in limnological studies as an easy and rapid measure of water clarity or transparency (Kalff 2002).

Surface water samples for water chemistry were taken at the beginning of the experiment (day 0), day 21, and on day 35. Cylindrical sediment traps (7.6 cm diameter by 45.7 cm long), which were suspended 7 m below the surface in the centre of each mesocosm, were emptied on days 12, 24 and 36. These samples were combined to obtain an estimate of the total sediment for each mesocosm. Samples for metal and Ra^{226} analyses were preserved by adding nitric acid to a final concentration of 0.2%. Water contaminant analysis was performed by Saskatchewan Research Council (SRC, Saskatoon, Saskatchewan). Most metals were analysed using inductively coupled plasma atomic emission spectrometry (ICP-AES). As, Se, and U were analysed using inductively coupled plasma-mass spectroscopy (ICP-MS), and Ra-226 was analysed using alpha-spectroscopy (Chiu and Dean 1986; Clesceri *et al.* 1998).

Phytoplankton samples were taken from the surface water of the mesocosms on day 35. Sediment traps were sub-sampled for phytoplankton identification whenever the sediment traps were emptied. Phytoplankton sediment sub-samples were added to a cumulative sample for final identification, enumeration, and biomass analyses. Phytoplankton samples were taken to examine the species that have grown and may have been responsible for binding to contaminants. Phytoplankton samples taken from

the surface water and sediment traps of the mesocosms were analyzed and identified by AlgaTax Consulting and sample preparation and preservation followed the protocol outline by AlgaTax Consulting (Appendix A).

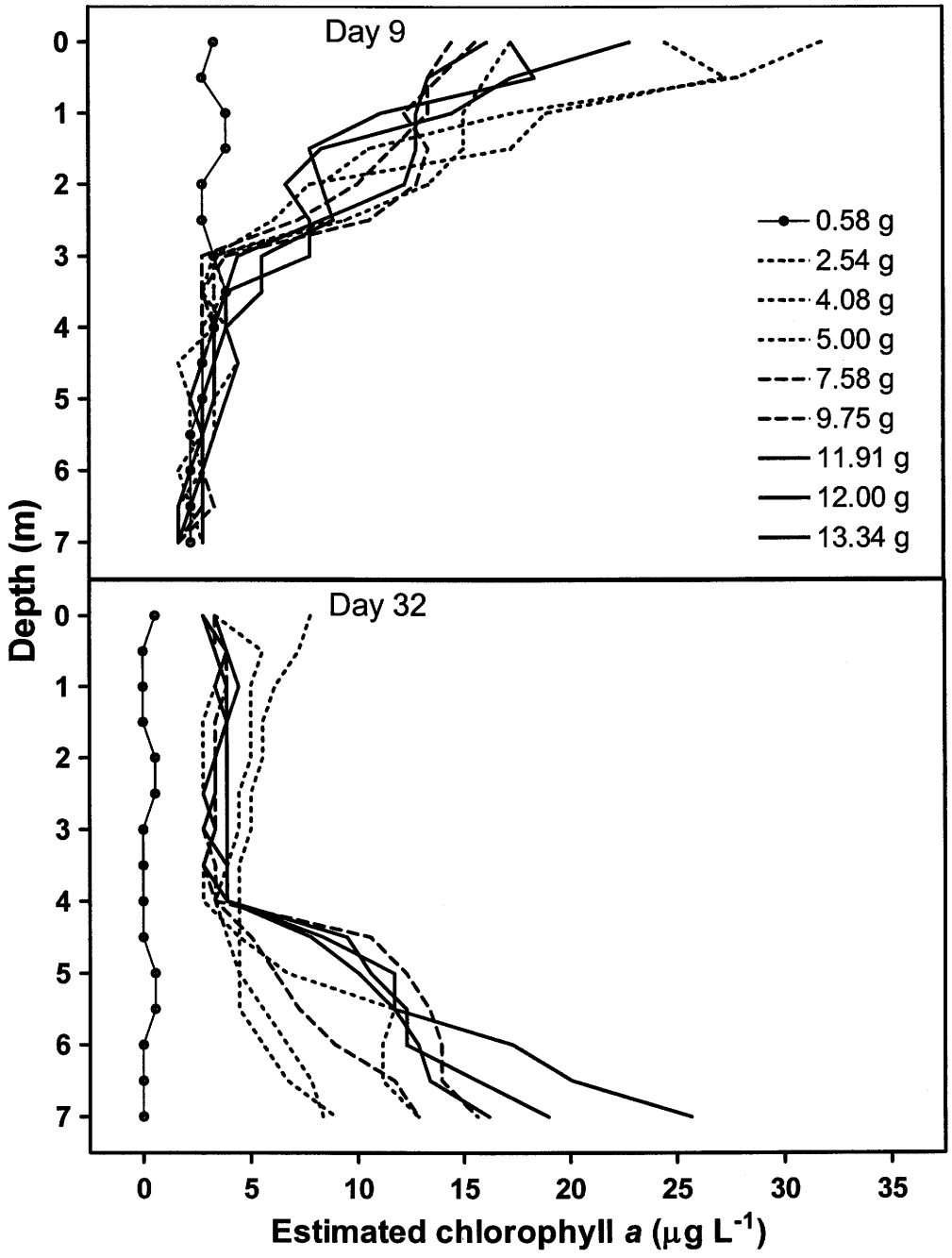
I expected that contaminants would decrease in concentration in the surface water with increasing phosphorus concentration, and that contaminants would increase in the sediment trap with increasing phosphorus concentration. Surface water and sediment trap contaminant concentrations (dependent variable) were plotted against P-load (g) (independent variable). The relationship between contaminant concentration and P-load was analysed with Model I regression analyses (Microsoft excel and SigmaStat) with an alpha level set at 0.05.

2. 3 Results

Phytoplankton chl *a* concentrations increased in all fertilized mesocosms compared to the control mesocosm, and this increase was maintained over the course of the experiment (Fig. 2-1). Chl *a* profiles from day 9 indicated that most algal biomass was concentrated near the surface, whereas profiles from day 32 indicated that algal biomass was concentrated in the bottom half of the mesocosms. Secchi depths taken at the end of the experiment (day 37) were less in fertilized mesocosms than the control mesocosm (Table 2-2).

The results show increased phytoplankton density and biomass in treated mesocosms when compared to the control (see Appendix B). Furthermore, the results from the phytoplankton samples indicate dominance by phytoplankton from the phylum Chlorophyta, specifically in the surface waters by the genus *Chlamydomonas*, a well studied unicellular, flagellated genus. Chlorophytes from the genera *Planktosphaeria* and *Sphaerellopsis* were also high in density and biomass in the surface water.

Figure 2-1 Depth profiles of chlorophyll *a* estimated using an algal fluorescence probe (YSI 600 OMS profiler) on day 9 and day 32 in each mesocosm. YSI fluorescence values were converted to chlorophyll *a* using the conversion value of y = 5.583X (linear regression forced through the origin, $n = 34$, $r^2 = 0.21$, $P > 0.01$). Legend refers to total phosphorus load in each mesocosm. The control mesocosm had a P-load of 0.58 g (ambient total P concentration in the pit).


Day 9
Day 32
0.58 g
2.54 g
4.08 g
5.00 g
7.58 g
9.75 g
11.91 g
12.00 g
13.34 g
Depth (m)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
Estimated chlorophyll a (μg L-1)

Phytoplankton results from sediment traps corroborate the results from the surface water, with the phylum Chlorophyta comprising the majority of the biomass (Appendix B-1). Here too, the genera *Chlamydomonas*, *Planktosphaeria* and *Sphaerellopsis* had the greater biomass, however, the majority of the biomass in the sediment trap seems to have been dominated by an unidentified Chlorophyte species distinguished by a thick wall (Appendix B). Although the unknown, thick-walled species does appear in the surface waters of all mesocosms where it was as abundant in terms of biomass as either *Planktosphaeria* or *Sphaerellopsis*, it composed less of the total phytoplankton biomass in the surface water than *Chlamydomonas*.

Surface metal concentrations (As, Co, Cu, Mn, Ni, and Zn) decreased significantly ($P < 0.05$, $r^2 > 0.5$) with increasing phosphorus load (Table 2-3; Fig. 2-2A). The decline in U was not significant ($P = 0.065$, $r^2 = 0.41$). The relationship between total phosphorus load and Ra^{226}, Se and Mo was not significant (Table 2-3; Fig. 2-2A). Sediment trap metal concentrations (As, Co, Cu, Mn, Ni, U, Ra^{226} and Zn) increased significantly ($P <0.05$, $r^2 > 0.46$) with increasing phosphorus load (Table 2-4; Fig. 2-2B), but Se and Mo showed no significant trend. An outlier was excluded from the As analysis (Table 2-4; Fig. 2-2B) (Dixon's Test, $n = 8$, $P < 0.01$) (Sokal and Rohlf 1995). One sediment trap was lost during the course of the experiment in mesocosm 4 (11.91 g P-load) lowering our sample size from 9 to 8.

2. 4 Discussion

The relationship between phosphorus limitation, algal growth, and chl *a* has long been established in many temperate lakes (Litchman *et al.* 2003; Schindler 1977) and streams (Van Nieuwenhuyse and Jones 1996). Phytoplankton fluorescence increased rapidly in the surface water of mesocosms with nutrient amendments (Fig. 2-1), and are

Table 2-3 Surface water contaminant concentrations (arranged by p – value) as a function of total phosphorus loads (X) in grams (model I linear regression; N = 9) in the 9 mesocosms. Samples were taken on day 35 of the 37 day experiment. All contaminants reported in mg L^{-1} except Radium-226 which is reported in Bq L^{-1}. Values in the last column represent the percentage decrease in contaminants in the mesocosm receiving the highest nutrient load (mesocosm 7) relative to those in the control mesocosm (mesocosm 5).

Contaminant	Model	*p*	r^2	Observed % Decrease
Arsenic	0.0024 – 0.00003X	0.0008	0.82	21
Zinc	0.2078 – 0.0024X	0.0024	0.75	76
Cobalt	0.2331 – 0.0049X	0.0063	0.68	28
Nickel	1.2692 – 0.0271X	0.0078	0.66	27
Copper	0.0081 – 0.0003X	0.023	0.55	40
Manganese	2.5303 – 0.0336X	0.0257	0.53	16
Uranium	3.0371 – 0.023X	0.0652	0.41	13
Selenium	No trend	0.4874	0.07	-8
Radium 226	No trend	0.6237	0.04	7
Molybdenum	No trend	0.769	0.01	-2

Figure 2-2 Contaminant concentration versus phosphorus load (g) in the surface water (A) and corresponding sediment traps (B). Solid lines represent statistically significant relationships ($P < 0.05$) and dashed lines non-significant relationships. Surface water samples were taken on day 35 and final sediment trap samples were taken on day 36. Ra^{226} reported in Bq L^{-1} in surface water and Bq m^{-2} d^{-1} in sediment traps.

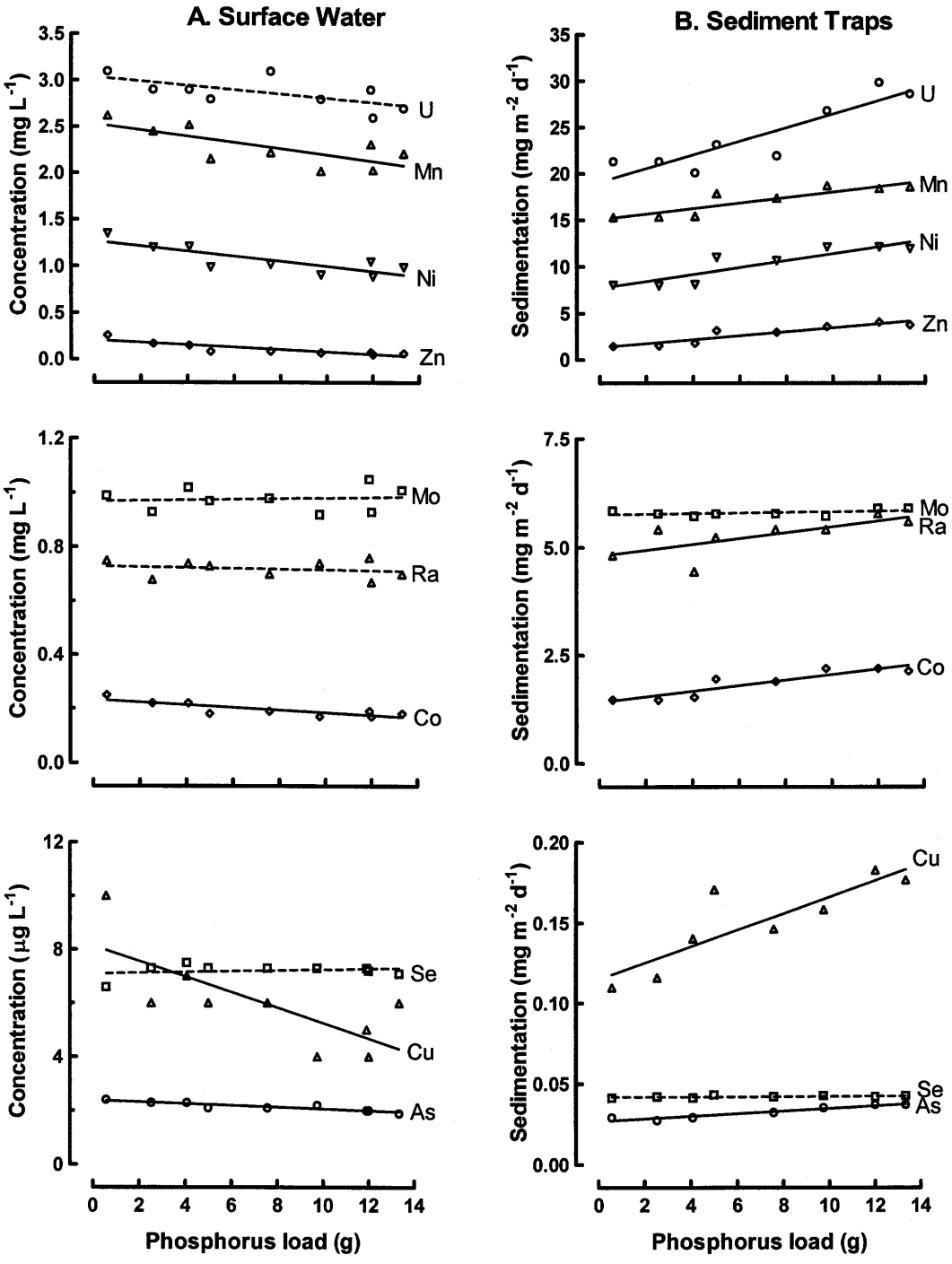

A. Surface Water
B. Sediment Traps
Concentration (mg L-1)
Sedimentation (mg m-2 d-1)
U
Mn
Ni
Zn
Mo
Ra
Co
Concentration (µg L-1)
Sedimentation (mg m-2 d-1)
Se
Cu
As
Phosphorus load (g)
Phosphorus load (g)

Table 2-4 Total contaminant load (mg m^{-2}) in sediment traps (arranged by *P* – value) as a function of total phosphorus load (X) in grams (model I linear regression). All contaminants reported in mg m^{-2} day^{-1} except Radium-226 which is reported as Bq m^{-2} day^{-1}. For all contaminants n = 8 except for As (n = 7). Values in the last column represent the percentage increase of contaminants in the mesocosm receiving the highest nutrient load (mesocosm 7) relative to those in the control mesocosm (mesocosm 5).

Contaminant	Model	*p*	r^2	Observed % Increase
Arsenic	0.027 + 0.0008X	0.0007	0.92	27
Zinc	1.34 + 0.2136X	0.0009	0.86	158
Cobalt	1.41 + 0.0645X	0.0017	0.83	46
Uranium	19.11 + 0.7339X	0.0025	0.81	34
Nickel	7.7 + 0.372X	0.0027	0.80	48
Manganese	15.1 + 0.2955X	0.0033	0.79	22
Copper	0.115 + 0.0051X	0.0057	0.75	61
Radium 226	4.81 + 0.0678X	0.0484	0.50	16
Selenium	No trend	0.2708	0.20	3
Molybdenum	No trend	0.2714	0.20	1

similar to results observed in other large-scale nutrient amendment experiments (Strauss *et al.* 1994). As the experiment progressed, fluorescence profiles became greater near the bottom of the mesocosms (Fig. 2-1), which may indicate either the movement of living phytoplankton or the sedimentation of dead or senescing phytoplankton biomass or a combination of the two.

At the end of the experiment (day 37), the Secchi depth for the control mesocosm (P-load 0.58g) exceeded the depth of the mesocosm (8 metres); however, Secchi depths were much shallower in phosphorus amended mesocosms (Table 2-2). Secchi depth tended to decline with increasing P-load, but the weakness of this trend probably reflects the ineffectiveness of Secchi depth measurements to differentiate beyond chl *a* concentrations greater than 20 $\mu g\ L^{-1}$ (Canfield and Bachmann 1981; Horne and Goldman 1994). More importantly, the difference in water clarity between control and amended mesocosms indicates that the addition of nutrients stimulated algal growth in the pit lake water.

Phytoplankton biomass increased in the surface water and sediment traps in the treated mesocosm when compared to the control. This increase in phytoplankton biomass in the treated mesocosms indicates that the DJX pit was P-limited, and that phytoplankton growth can be stimulated regardless of the high contaminant concentrations present in the pit-lake. Chlorophytes dominated the phytoplankton community, especially *Chlamydomonas* in the surface water and an unidentified chlorophyte species in the sediment trap. *Chlamydomonas* similarly dominated the phytoplankton community at another uranium mine pit-lake near Wollaston Lake, Saskatchewan (Kalin *et al.* 2001). The reasons for the greater biomass of the unidentified chlorophyte rather than *Chlamydomonas* in the sediment trap are unknown.

Speculatively, a more rapid sedimentation rate, a lack of mobility, or a combination of both may explain the differences. The unidentified species seems to be mainly distinguished by its thick wall. It is possible that this thick wall makes it denser, and thus, it may sink more rapidly through the water column. Furthermore, the unidentified, thick-walled species is slightly larger than the *Chlamydomonas* sp. which may increase its sedimentation rates due to greater cell density (Stolte *et al.* 1994). Moreover, *Chlamydomonas* are flagellated and motile (Graham and Wilcox 2000), and this allows them to move through the water column, potentially avoiding sedimentation. It is unknown if the unidentified, thick-walled species was motile like many of the other Chlorophyte species observed in the sediment traps (Appendix B).

Nonetheless, phytoplankton biomass clearly increased in treated mesocosms, and phytoplankton species which are abundant in the surface water also tended to be abundant in the sediment traps. Most importantly, the increases in phytoplankton density and abundance corroborate the observed results from the chl *a* profiles and Secchi depth measurements.

The surface water concentrations of As, Co, Cu, Mn, Ni, and Zn significantly declined as more phosphorus was added to the mesocosms (Table 2-3; Fig. 2-2A), and this was consistent with the significant increase of these metals in the corresponding sediment traps (Table 2-4; Fig. 2-2B). Although there may be direct chemical precipitation of contaminants by the addition of phosphorus, I suspect that the decline of metals in the surface water was largely a result of these metals binding to algal cell surfaces or being taken up by algae, followed by the sedimentation of algae with the metals. For example, some forms of As are taken up by phytoplankton (Cullen *et al.* 1994; Kobayashi *et al.* 2003; Sanders and Riedel 1993); Cu has been shown to

accumulate intracellularly in freshwater algae (Knauer *et al.* 1997); and phytoplankton take up more Zn as nutrient concentrations increase (Wang and Dei 2001a). Thus, our results are consistent with the observations in the literature.

The decline of surface water U concentrations along the nutrient gradient was non-significant ($P = 0.0652$; Table 2-3; Fig. 2-2A), but the increase in concentration in the sediment traps was statistically significant (Table 2-4; Fig. 2-2B). Reasons for the non-significant trend in the surface water concentration of U may be due to low sample size, and thus, low power, or it may be possible that the experimental length was insufficient to result in a significant trend. However, taking into account the significant trend in the sedimentation of U, it seems fairly clear that U increasingly sedimented from the surface water as P-load increased. This observation is consistent with those of Lovley and Phillips (1992a; 1992b) and Lovley *et al.* (1991) who have shown that microorganisms have the capacity to enzymatically reduce U from soluble U(VI) to insoluble U(IV), and of Mann and Fyfe (1985), and Pribil and Marvan (1976) who have shown that algae can accumulate U.

The results for Ra^{226} were inconsistent. As P-loads increased, there was no change in concentration in the surface water of Ra^{226}, but there was a significant increase in the sediment traps. I have no explanation for this discrepancy.

Se and Mo appeared to be unaffected by increasing P-loads. Both metals appear to be essential to some phytoplankton (Harrison *et al.* 1988; Paulsen *et al.* 1991; Zahalak *et al.* 2004) and are expected to be taken up by phytoplankton, as has been shown for Se (Wang and Dei 2001a). However, the uptake of Se has been negatively correlated with P-concentrations (Wang and Dei 2001b), and the uptake of Mo by tomato plants decreases in the presence of phosphate because of increased competition between

phosphate and molybdate for uptake sites (Heuwinkel *et al.* 1992). Furthermore, molybdate uptake may be inhibited by the presence of sulphate or tungstate (Cole *et al.* 1993). Even though sulphate was not measured in the mesocosms, sulphate is often a problem contaminant in many pit-lakes (Miller *et al.* 1996). Selenate uptake is also inhibited by sulphate concentrations, and selenite uptake is significantly increased when phosphate concentrations are low, indicating competitive inhibition (Riedel and Sanders 1996). Moreover, Baines *et al.* (2001) found that when comparing Se uptake among a variety of marine phytoplankton groups, that the Chlorophyceae (*Dunaliella terrtiolecta*) incorporated the least amount of dissolved Se when compared to the other phytoplankton groups. Thus, it is possible that Se uptake would be minute due the dominance of the Chlorophyceae in the mesocosms (Appendix B), a factor which may have been compounded by the pre-existing low concentrations of Se in the mesocosms (Table 2.1). Such factors may explain the lack of effect of phosphorus additions on Mo and Se concentrations in our experiment.

Saskatchewan Surface Water Quality Objectives (SSWQO) for the DJX pit vary by contaminant (Table 2-1). If the rate of decrease of these specific contaminants between the highest treatment and the control mesocosm were to remain unchanged (Table 2-3), I would expect SSWQO to be met in approximately 45 weeks for Co, within 65 weeks for Ni, in approximately 15 weeks for U, and approximately 5 weeks for Zn. The duration of the growing season for phytoplankton in northern Saskatchewan is approximately about 20 weeks, and therefore, it would take at least two years for our phytoremediation process to bring the DJX pit water to meet SSWQO. It should be noted that there was little change in contaminant concentrations in the control mesocosm

between the beginning and end of the experiment, and so I would expect that it would take several years for the pit to reach SSWQO if left untreated.

If filled to the top of the bedrock with contaminated water, I estimate that it would cost over 2 million $CDN to treat DJX pit using conventional water treatment processes. Taking the approximate time needed to meet SSWQO listed above into account, I conservatively estimate that it would cost 50 000 $CDN to treat DJX pit using phytoremediation. Since DJX pit has such an extreme relative depth ratio (maximum depth relative to surface area) of 27%, DJX pit would become meromictic and incompletely mix (Kalff 2002). Furthermore, the likely development of a chemocline in DJX pit will assist in maintaining long-term stratification. The sedimented contaminants therefore may be trapped in the non-mixing portion of the pit-lake. In support, Castro and Moore (2000) suggest that the non-mixing portion may be utilised to remediate pit-lakes.

The potential for reduction of contaminants in the surface water through nutrient enhancement appears to be a useful tool for pit lake remediation. Through nutrient addition, and subsequent phytoplankton growth, most contaminants were lost by sedimentation from the surface water. However, the results suggest that a maximum sedimentation rate has not been reached over the range of phosphorus amendments used in this experiment. Future experiments will use a broader range of P-load to determine the optimum load for the most rapid rate of removal of surface contaminants in the DJX Pit.

The results also indicate that phytoremediation may be an effective and inexpensive approach that may be used in tandem or as a substitute for traditional mining water treatment processes. Future studies will focus on increasing sedimentation

rates with increased phosphorus additions, and on the removal of contaminants which were not affected by nutrient additions (e.g., selenium and molybdenum).

2. 5 Conclusions

1. The addition of phosphorus to contaminated surface water resulted in an increase in phytoplankton biomass, particularly in the Chlorophytes.
2. The addition of phosphorus to contaminated surface water resulted in a reduction of most metals, probably as a result of increased algal growth and sedimentation.
3. However, the addition of phosphorus did not lead to an increase in sedimentation of Se or Mo from the surface water.
4. The linear increase in sedimentation rate as phosphorus load increased suggests that the sedimentation of contaminants could be further increased by adding more phosphorus.
5. Phytoremediation may be an effective and inexpensive method to improve the water quality of contaminated flooded pits, which may be used in tandem or as a substitute for traditional mining water treatment processes
6. Future research will focus on increasing sedimentation rates with increased phosphorus additions, and on the removal of contaminants which were not affected by nutrient additions (such as Se and Mo).

CHAPTER 3

The Effect of Phosphorus and Ammonium Additions on the Sedimentation of Contaminants in a Uranium Mined Pit-Lake

3. 1 Introduction

In the summer of 2003 (see chapter 2), I performed a phytoremediation experiment in the DJX pit. The purpose of this experiment was to measured the effects of increased phosphorus loads on the sedimentation of contaminants using mesocosms. I found that the sedimentation rates of As, Co, Cu, Mn, Ni, U, Zn, and Ra^{226} significantly increased with increasing phosphorus loads, however, Se and Mo sedimentation rates were not affected by the phosphorus additions. I also found that maximum sedimentation rates were not met with our high phosphorus treatment. The objectives of this study were to increase the phosphorus loads to determine if I could reach a maximum sedimentation rate. I also wanted to determine if different sources of nitrogen would affect sedimentation rate. The water in the DJX pit is high in nitrate, however, ammonium is preferentially taken up by phytoplankton and can suppress nitrogen fixation in Cyanobacteria (Takahashi and Saijo 1981; Takahashi and Saijo 1988; Wheeler and Kokkinakis 1990). Different sources of nitrogen may affect phytoplankton community composition and succession (Burford and Pearson 1998) and ammonium has been shown to increase macroalgae diversity under lower nitrogen concentrations (Bracken and Nielsen 2004). If certain mesocosms received ammonium to supplement both the phosphorus additions as well as the pre-existing high nitrate concentrations mesocosms only received phosphorus additions, I could compare the effects of the different sources

of nitrogen on contaminant sedimentation rates. I believed that since the ammonium-supplemented treatments may contain a more diverse phytoplankton community, these treatments may result in greater contaminant sedimentation rates. In addition, I want to determine if contaminants which did not respond to my previous nutrient enhancements (Mo, Se) would respond to the ammonium-supplemented treatments.

Water was being pumped from the pit to the water treatment plant during the course of the experiment, and as a result, pit water depth fluctuated from 65 m at the start of the experiments in July of 2004 to 55 m deep by the end of the experiments in September of 2004. Initial contaminant and nutrient concentrations in the pit were measured (Tables 3-1 and 3-2).

3. 2 Materials and Methods

Two similar studies were performed during the mid- and late-summer of 2004 in the DJX pit. The mid-summer study lasted for 33 days from July 2nd to August 4th, and the late-summer study lasted for 29 days from August 21st to Sept 19th. Increasing phosphorus and phosphorus-with-ammonium gradients were created in both studies and the response by the phytoplankton communities and various contaminants were measured in relation to the addition of nutrients. Mesocosms were filled and positioned in the DJX pit (described in Chapter 1, Section 5). Nine 92.2 m^3 mesocosms (Figure 1-2) were positioned in the centre of the DJX pit in an east to west orientation and filled with contaminated pit water.

Ambient total phosphorus concentrations in the DJX pit were low relative to that of total nitrogen for both experiments (Table 3-1 and 3-2), and therefore, I considered the pit water to be phosphorus-deficient for phytoplankton growth for both experiments (Schindler 1974; Schindler 1977). Because I wanted to measure the difference in

Table 3-1 Initial contaminant and nutrient concentrations in the DJX Pit on July 2, 2004. *Ra^{226} reported as Bq L^{-1}.

Contaminant	Concentration (mg L^{-1})	Saskatchewan Surface Water Quality Objectives (SSWQO)
Arsenic	0.003	0.05
Cobalt	0.19	0.02
Copper	0.01	0.01
Manganese	1.75	N.A.
Molybdenum	0.50	0.5
Nickel	0.94	0.025
Total Nitrogen	4.93	N. A.
Total Phosphorus	0.014	N. A.
Selenium	0.003	0.01
Uranium	1.8	2.0
Zinc	0.14	0.05
Radium-226*	0.50	0.11

N. A. – Not Applicable

Table 3-2 Initial contaminant and nutrient concentrations in the DJX Pit on Aug. 21, 2004. * Ra^{226} reported as Bq L^{-1}.

Contaminant	Concentration (mg L^{-1})	Saskatchewan Surface Water Quality Objectives (SSWQO)
Arsenic	0.005	0.05
Cobalt	0.200	0.02
Copper	0.011	0.01
Manganese	1.963	N.A.
Molybdenum	0.493	0.5
Nickel	0.970	0.025
Total Nitrogen	6.40	N. A.
Total Phosphorus	0.023	N. A.
Selenium	0.003	0.01
Uranium	2.233	2.0
Zinc	0.143	0.05
Radium-226*	0.60	0.11

N. A. – Not Applicable

contaminant sedimentation rates in both experiments using phosphorus, or phosphorus-with-ammonium treatments, the mesocosms were separated into two different treatments: those consisting of only phosphorus amendments, and those consisting of phosphorus-with-ammonium amendments. Analytical grade potassium phosphate was added to four treatment mesocosms in order to create a nutrient gradient, while a fifth mesocosm was left as a control (Table 3-3 and 3-4). The phosphorus gradient increased in 5 g phosphorus-load increments beginning from the control. The phosphorus-with-ammonium treatments consisted of four mesocosms. Analytical grade potassium phosphate was added to four treatment mesocosms in 5 g phosphorus-load increments in order to create a phosphorus gradient similar to that of the phosphorus only treatment.

Analytical-grade ammonium chloride was added to each mesocosm along with the phosphorus additions in an atomic ratio of 16N:1P, to remain consistent with the Redfield ratio (Redfield 1958; Redfield *et al.* 1963). The Redfield ratio, which is the atomic ratio of 106C:16N:1P, is useful in determining macronutrient-limitation. Nitrate is not as readily useable by phytoplankton. Therefore, in the phosphorus-with-ammonium treatments, I added ammonium at a final concentration to follow the Redfield ratio.

Final phosphorus treatment loads for both experiments are found in Tables 3-3 and 3-4. In both experiments, half of the nutrients were added on day 0, with the remaining nutrients added evenly on days 7, 14, and 21. I wanted to sustain maximum algal growth over the entire experiment, and as a result, I decided that adding the nutrients throughout the experiment may be more effective at maintaining algal growth. Total phosphorus was analysed following the method described in (Wetzel and Likens 2000).

Table 3-3 Treatment, total phosphorus load (g), Secchi depth, phosphorus and ammonium concentration ($\mu g\ L^{-1}$) in each mesocosm at the end of the mid-summer experiment and corresponding Secchi depths (m).

Treatment	Phosphorus Load (g)	Secchi Depth (m)	P Conc. ($\mu g\ L^{-1}$)	NH_4 Conc. ($\mu g\ L^{-1}$)
No Treatment	2.02	> 8	5.2	261.3
+ 5g P	6.14	1.4	63.6	6.0
+ 10g P	11.54	1.3	104.2	6.7
+ 15g P	16.21	2.0	153.0	43.2
+ 20g P	21.10	0.9	147.2	8.3
+ 5g P + NH_4	6.16 + NH_4	1.4	62.6	387.0
+ 10g P + NH_4	11.31 + NH_4	1.4	121.9	744.7
+ 15g P + NH_4	15.96 + NH_4	1.9	177.1	1554.1
+ 20g P + NH_4	21.32 + NH_4	1.4	219.4	1713.0

Table 3-4 Treatment, total phosphorus load (g), Secchi depth, phosphorus and ammonium concentration ($\mu g\ L^{-1}$) in each mesocosm at the end of the late-summer experiment and corresponding Secchi depths (m).

Treatment	Phosphorus Load (g)	Secchi Depth (m)	P Conc. ($\mu g\ L^{-1}$)	NH_4 Conc. ($\mu g\ L^{-1}$)
No Treatment	2.17	6.25	8.9	235.6
+ 5g P	6.74	2.0	38.3	5.9
+ 10g P	12.00	4.5	85.5	41.6
+ 15g P	17.23	3.8	137.5	23.6
+ 20g P	22.04	4.0	210.9	25.0
+ 5g P + NH_4	7.45 + NH_4	4.0	35.8	503.6
+ 10g P + NH_4	11.94 + NH_4	2.5	78.5	918.0
+ 15g P + NH_4	17.07 + NH_4	2.4	146.6	1429.1
+ 20g P + NH_4	22.12 + NH_4	1.5	214.2	1812.7

Phytoplankton growth was monitored using the algal fluorescence probe and chl *a* collection methods described in chapter 2. 2. Water column fluorescence profiles were only measured in the morning. The relationship between the chl *a* estimates and their corresponding YSI fluorescence measurements were analysed with model I linear regression forced through the origin (Chapter 2. 2). Fluorescence values were converted to chl *a* estimates using these conversion factors described in Figures 3-1 and 3-2. Chl *a* profiles were then compared between mesocosms. Secchi depths, a measurement of water clarity, were taken on the final day of the each experiment (day 33 and day 29 respectively) to prevent the resuspension of sedimented materials in the mesocosms.

Surface water samples for water chemistry were taken at the beginning of each experiment (day 0), on day 14 for both experiments, and on day 32 for the first experiment and day 28 for the second experiment. Surface water samples were taken on day 0 to get an idea of initial contaminant concentrations, on day 14 to determine the contaminant concentrations at an intermediate stage, and on day 32 and day 28 for the first and second experiment respectively to determine the final surface water contaminant concentrations. Cylindrical sediment traps (described in chapters 1. 5 and 2. 2) were emptied on days 10, 20 and 29 for both experiments. These samples were combined to obtain an estimate of the total sediment for each mesocosm. Metal and Ra^{226} samples were preserved and analysed using the methods described in chapters 2. 2.

The relationships between phosphorus load and contaminant concentrations, and the relationships between phosphorus-with-ammonium and contaminant concentrations in both the surface water and sediment traps were analysed using Model I linear regression (Chapter 1. 5). All regression analysis was performed with SigmaStat, which also provided *a posteriori* power analyses. *A posteriori* power analysis was used to

Figure 3-1 Depth profiles of chlorophyll *a* during the mid-summer study (July 2nd to August 4th, 2004) estimated using an algal fluorescence probe (YSI 600 OMS profiler) on day 16 and day 23 in each mesocosm. YSI fluorescence values converted to chlorophyll *a* using the conversion value of y = 5.0624X (linear regression forced through origin, $n = 9$, $r^2 = 0.87$, $p > 0.01$) for day 16, and y = 4.1495X (linear regression forced through origin, $n = 9$, $r^2 = 0.91$, $p > 0.01$). Legend refers to total phosphorus load in each mesocosm. The control mesocosm had a P-load of 2.02 g (initial ambient P concentration in the mesocosm).

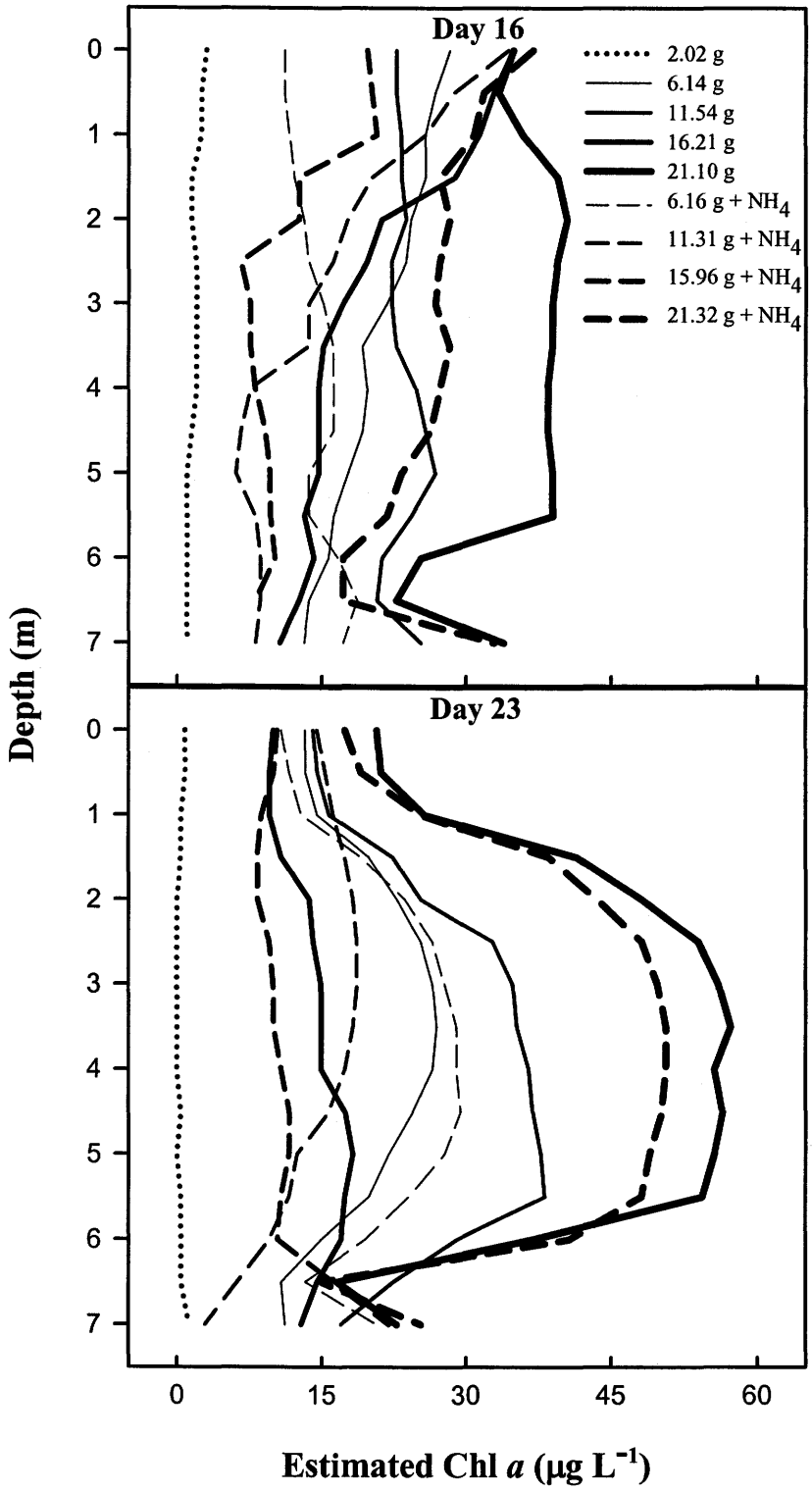
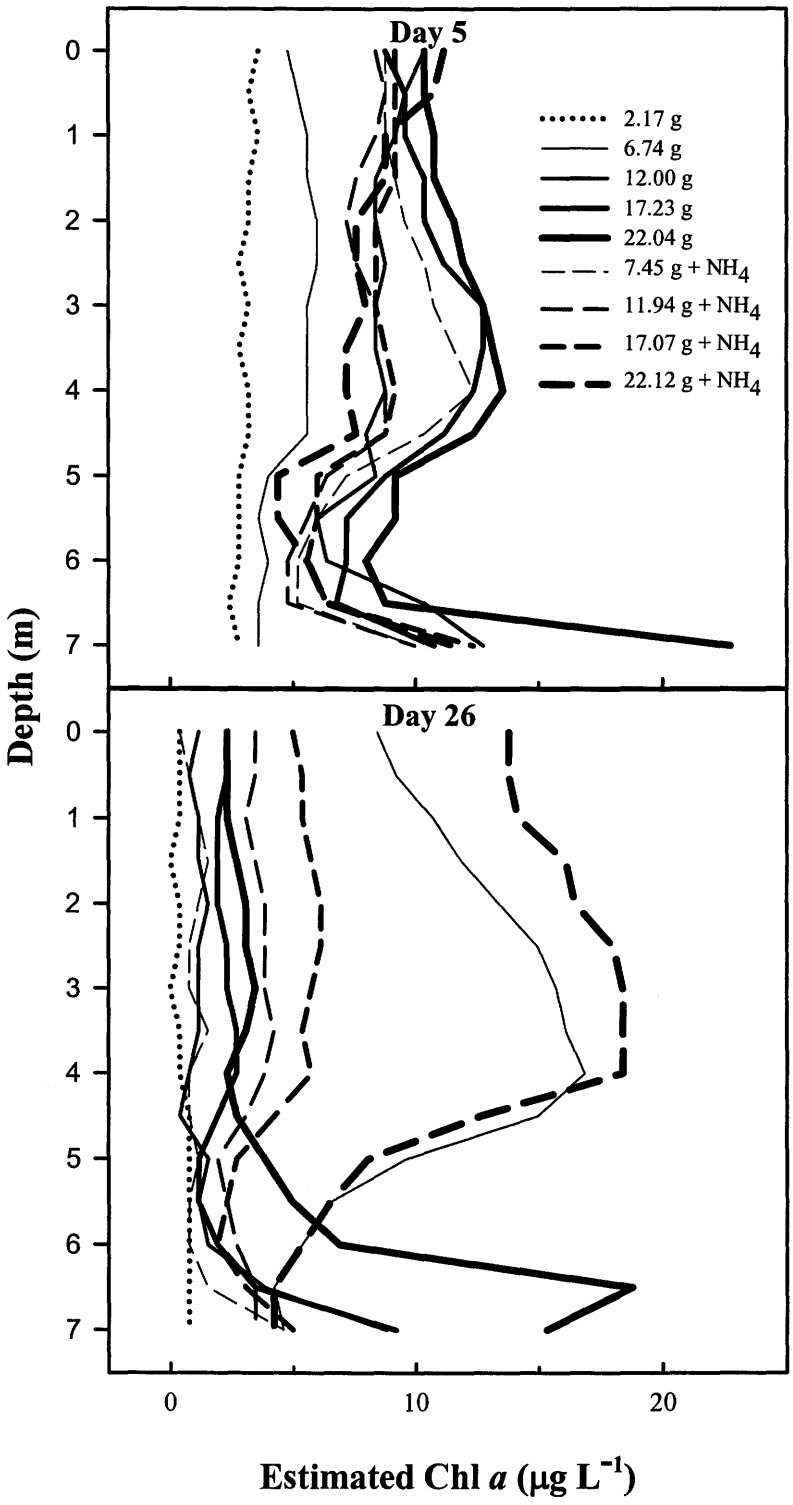

Day 16
Day 23
2.02 g
6.14 g
11.54 g
16.21 g
21.10 g
6.16 g + NH4
11.31 g + NH4
15.96 g + NH4
21.32 g + NH4
Depth (m)
0
1
2
3
4
5
6
7
0
15
30
45
60
Estimated Chl a (µg L-1)

Figure 3-2 Depth profiles of chlorophyll *a* estimated during the late-summer study (August 21st to Sept 19th, 2004) using an algal fluorescence probe (YSI 600 OMS profiler) on day 16 and day 23 in each mesocosm. YSI fluorescence values converted to chlorophyll *a* using the conversion value of y = 3.9873X (linear regression forced through origin, $n = 9$, $r^2 = 0.64$, $p > 0.01$) for day 5, and y = 3.8312X (linear regression forced through origin, $n = 9$, $r^2 = 0.71$, $p > 0.01$). Legend refers to total phosphorus load in each mesocosm. The control mesocosm had a P-load of 2.17 g (initial ambient P concentration in the mesocosm).


Day 5
Day 26
2.17 g
6.74 g
12.00 g
17.23 g
22.04 g
7.45 g + NH4
11.94 g + NH4
17.07 g + NH4
22.12 g + NH4
Depth (m)
0
1
2
3
4
5
6
7
0
10
20
Estimated Chl a (µg L-1)

determine if sample size was sufficient to detect a significant effect ($P \leq 0.05$).

Two-way ANOVAs were performed using SigmaStat to compare contaminant concentrations in the phosphorus and phosphorus-with-ammonium treatments in relation to phosphorus loads (5g, 10g, 15g, 20g). However, the effect of phosphorus load on contaminants was analysed using Model I regression analysis (see above); I was only interested in the comparison of the nutrient treatments. The Two-way ANOVAs were performed independently for the mid-summer and late-summer experiments.

3. 3 Results

3. 3. 1 Chlorophyll *a*

Water column profiles showed an increase in estimated chl *a* concentrations in the treatment mesocosms relative to the control in both experiments (Fig. 3-1 and 3-2). During the mid-summer study, both day 16 and day 23 profiles treated mesocosms show a large increase in chl *a* concentration relative to the control throughout the majority of water column (Fig. 3-1). Chl *a* concentration from day 23 seems to indicate that most of the algal biomass is located in the middle portion of the treated mesocosms rather than being relatively evenly distributed through the water column as seen on day 16. Secchi depths taken on day 33 of the mid-summer study show a decrease in water clarity in the treated mesocosms relative to that of the control mesocosm in the mid-summer study (Table 3-3), which corroborates the results from the algal fluorescence profiles.

Although the late-summer study chl *a* concentrations were greater in the treated mesocosms than in the control, the difference between them was less than that seen in the mid-summer study (Fig. 3-2). Furthermore, this relative lack of difference between treated and the control mesocosms was reflected in the Secchi depths taken on day 29 during the late-summer study (Table 3-4). Day 5 shows a higher concentration of chl *a*

within the treated mesocosms when compared to day 26 (Fig. 3-2). Overall, chl *a* concentrations were lower in the late-summer study compared to the mid-summer study.

3. 3. 2 Phosphorus Treatments

Surface metal concentrations in phosphorus treatment mesocosms from the mid-summer study had non-significant ($0.15 \leq P > 0.05$) declining trends for Cu, U, and Zn with increasing phosphorus load (g); Mn, Ni, Ra^{226}, Co, Mo, Se, and As had no significant trend ($P > 0.15$) with increasing phosphorus load (Table 3-5A; Fig. 3-3A). Results from *a posteriori* power analyses were below the desired statistical power ($\beta < 0.8$). Sediment trap concentrations for Zn and Ra^{226} increased significantly ($P < 0.01$, $r^2 \geq 0.93$) with increasing phosphorus load; Se significantly decreased ($P = 0.0143$, $r^2 = 0.90$) with increasing phosphorus load; U, Ni, Mn, and Cu had non-significant increasing trends ($0.15 \leq P > 0.05$, $r^2 \geq 0.58$) with increasing phosphorus load; As and Mo showed no significant trend (Table 3-5B; Fig. 3-3B). Only Zn and Ra^{226} had sufficient statistical power ($\beta > 0.8$).

Surface concentrations of Se in the phosphorus treatment mesocosms from the late-summer study significantly increased as phosphorus load increased ($P = 0.0072$, $r^2 = 0.93$); Mn had a non-significant increasing trend ($P = 0.1342$, $r^2 = 0.58$) as phosphorus load increased; As, Co, Cu, Mo, Ni, U, Zn, and Ra^{226} showed no significant trend ($P > 0.15$) with increasing phosphorus concentration in the late-summer study (Table 3-6A; Fig. 3-4A). Only the analysis of Se had sufficient statistical power ($\beta < 0.8$). Sediment trap metal concentrations showed a non-significant declining trend for Se ($P = 0.0716$, $r^2 = 0.71$); As, Co, Cu, Mn, Mo, Ni, U, Zn, and Ra^{226} showed no significant trend ($P > 0.15$) (Table 3-6B; Fig. 3-4B). Insufficient statistical power ($\beta < 0.8$) was observed for these analyses.

Table 3-5 Contaminant concentrations as a function of total phosphorus loads (X) in grams (model I linear regression) (n = 5) from the mid-summer experiment in the surface water (A) and sediment traps (B). Surface water samples were taken on day 32 and final sediment trap samples were taken on day 29 of the 33 day experiment. All contaminants reported in mg L^{-1} in the surface water and mg m^{-2} except Ra^{226} which is reported in Bq L^{-1} in the surface water and Bq m^{-2} in the sediment traps.

A. Surface Water

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.8841	0.01
Cobalt	no trend	0.5218	0.15
Copper	0.0108 - 0.0001X	0.0936	0.66
Manganese	no trend	0.2895	0.35
Molybdenum	no trend	0.9381	0.00
Nickel	no trend	0.1896	0.49
Selenium	no trend	0.3461	0.29
Uranium	2.1801 - 0.0333X	0.0537	0.76
Zinc	0.1210 - 0.0021X	0.0710	0.72
Radium-226	no trend	0.3676	0.27

B. Sediment Trap

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.1957	0.48
Cobalt	no trend	0.3052	0.34
Copper	3.2975 + 0.1303X	0.1006	0.65
Manganese	373.5876 + 1.3310X	0.1326	0.58
Molybdenum	no trend	0.2594	0.39
Nickel	194.6496 + 0.5537X	0.1044	0.64
Selenium	0.5994 - 0.0064X	0.0143	0.90
Uranium	272.2844 + 32.3074X	0.0649	0.73
Zinc	27.9849 + 0.3164X	0.0078	0.93
Radium-226	107.8434 + 3.2029X	0.0084	0.93

Figure 3-3 Contaminant concentration versus phosphorus load (g) in the surface water (A) and corresponding sediment traps (B) during the mid-summer study. Solid lines represent statistically significant relationships ($p < 0.05$), dashed lines represent near significant relationships ($0.15 < p \geq 0.05$), and dotted lines represent non-significant relationships ($p \geq 0.15$). Surface water samples were taken on day 32 and final sediment trap samples were taken on day 29. Ra^{226} reported in Bq L^{-1} in surface water and Bq m^{-2} in sediment traps.

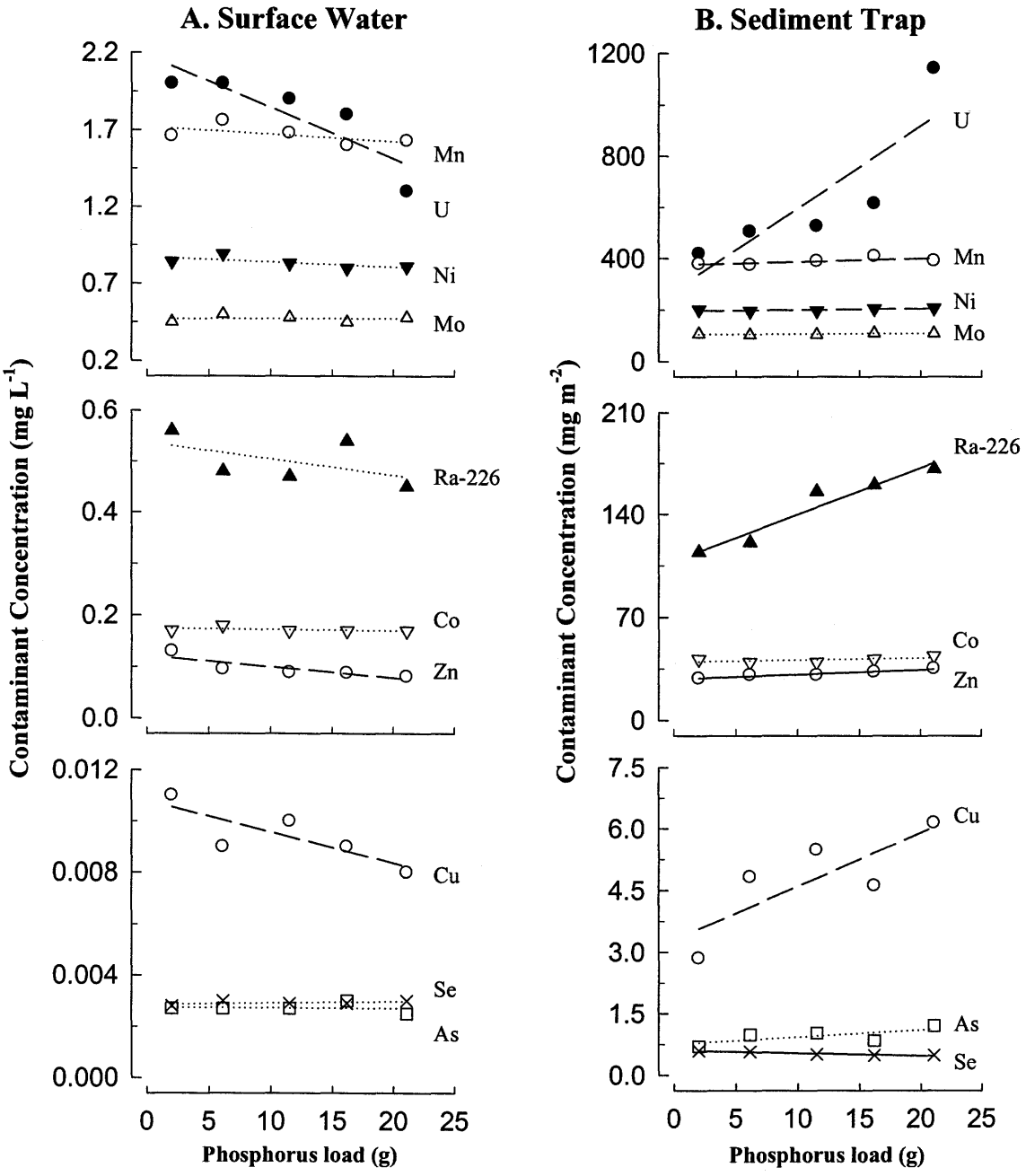

A. Surface Water
B. Sediment Trap
Contaminant Concentration (mg L-1)
Contaminant Concentration (mg m-2)
Mn
U
Ni
Mo
Ra-226
Co
Zn
Cu
Se
As
Phosphorus load (g)
Phosphorus load (g)

Table 3-6 Contaminant concentrations as a function of total phosphorus loads (X) in grams (model I linear regression) ($n = 5$) from the late-summer experiment in the surface water (A) and sediment traps (B). Surface water samples were taken on day 28 and final sediment trap samples were taken on day 29 of the 29 day experiment. All contaminants reported in mg L^{-1} in the surface water and mg m^{-2} except Ra^{226} which is reported in Bq L^{-1} in the surface water and Bq m^{-2} in the sediment traps.

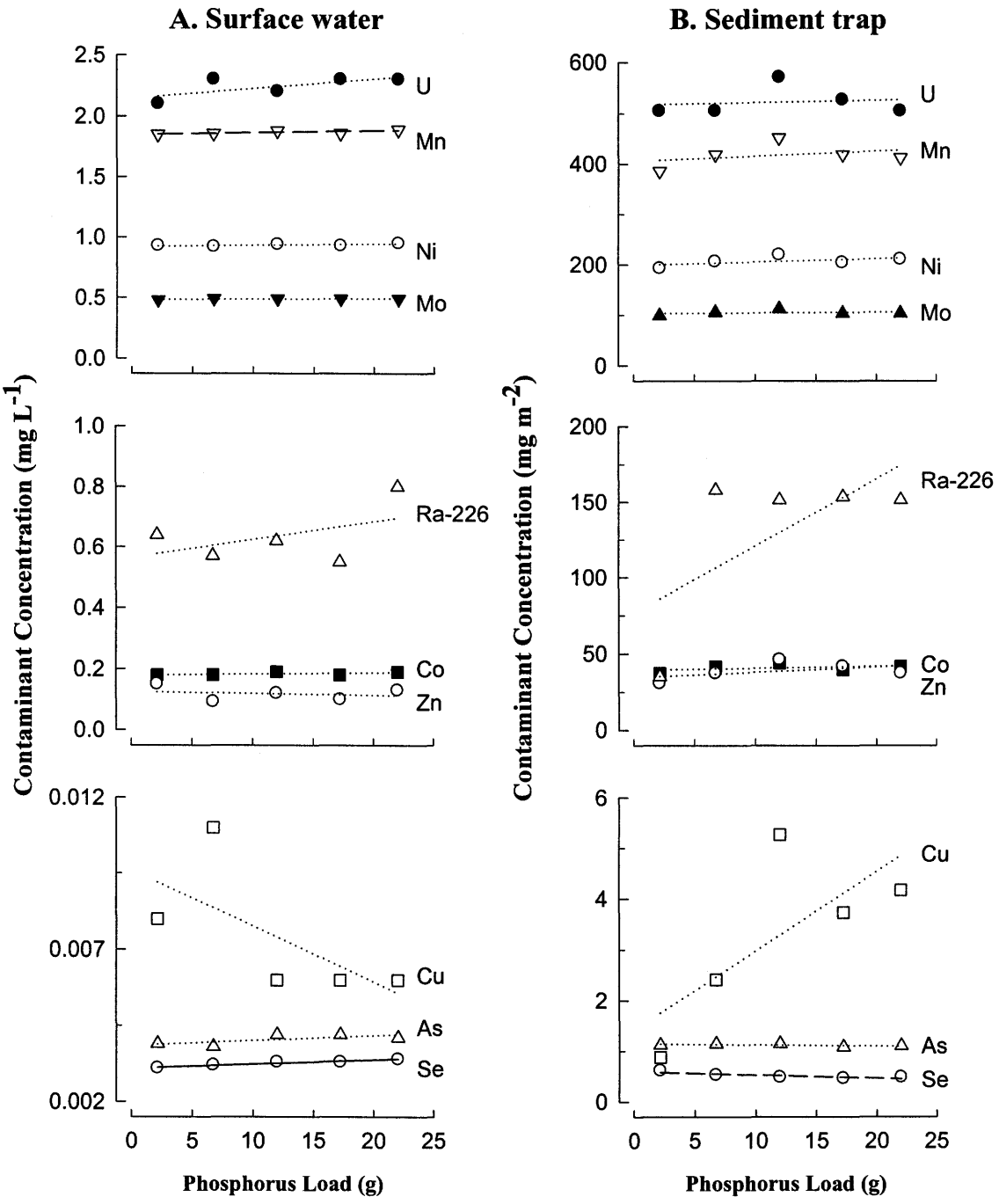
A. Surface Water

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.1823	0.50
Cobalt	no trend	0.3131	0.33
Copper	no trend	0.2218	0.44
Manganese	1.8490 + 0.0016X	0.1342	0.58
Molybdenum	no trend	0.1934	0.48
Nickel	no trend	0.1906	0.49
Selenium	0.0031 + 0.000014X	0.0072	0.93
Uranium	no trend	0.1922	0.48
Zinc	no trend	0.7412	0.04
Radium-226	no trend	0.4133	0.23

B. Sediment Trap

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.2635	0.39
Cobalt	no trend	0.5046	0.16
Copper	no trend	0.1585	0.54
Manganese	no trend	0.5690	0.12
Molybdenum	no trend	0.7407	0.04
Nickel	no trend	0.3585	0.28
Selenium	516.8395 + 0.4415X	0.0716	0.71
Uranium	no trend	0.8442	0.02
Zinc	no trend	0.4124	0.23
Radium-226	no trend	0.2192	0.44

Figure 3-4 Contaminant concentration versus phosphorus load (g) in the surface water (A) and corresponding sediment traps (B) during the late-summer study. Solid lines represent statistically significant relationships ($p < 0.05$), dashed lines represent near significant relationships ($0.15 < p \geq 0.05$), and dotted lines represent non-significant relationships ($p \geq 0.15$). Surface water samples were taken on day 28 and final sediment trap samples were taken on day 29. Ra^{226} reported in Bq L^{-1} in surface water and Bq m^{-2} in sediment traps. Note: first Ra^{226} data point in the sediment trap overlaps with Co and Zn.


A. Surface water
B. Sediment trap
Contaminant Concentration (mg L-1)
Contaminant Concentration (mg m-2)
U
Mn
Ni
Mo
Ra-226
Co
Zn
Cu
As
Se
Phosphorus Load (g)
Phosphorus Load (g)

3. 3. 3 Phosphorus-with-Ammonium Treatments

Surface water contaminants from the mid-summer study had a non-significant declining trend with uranium ($P = 0.0567$, $r^2 = 0.89$) with increasing phosphorus load with ammonium; a non-significant increasing trend with Co ($P = 0.1167$, $r^2 = 0.78$) with increasing phosphorus-with-ammonium load; As, Co, Cu, Mn, Mo, Ni, Se, Zn, and Ra^{226} had no significant trend with increasing phosphorus-with-ammonium load (Table 3-7A, Fig. 3-5A). Insufficient statistical power ($\beta < 0.8$) was observed for these analyses. Sediment trap metal concentrations had a non-significant decreasing trends for Zn ($P = 0.0915$, $r^2 = 0.83$) with increasing phosphorus load with ammonium; As, Co, Cu, Mn, Mo, Ni, Se, U and Ra^{226} showed no trend ($P > 0.15$) with increasing phosphorus load with ammonium (Table 3-7B; Fig. 3-5B). Insufficient statistical power ($\beta < 0.8$) was observed for these analyses.

Surface water contaminant concentrations in the phosphorus-with-ammonium treatment mesocosms from the late-summer study showed a significant increase for Cu ($P = 0.0468$, $r^2 = 0.91$) with increasing phosphorus-with-ammonium load; a non-significant increase for As ($P = 0.1265$, $r^2 = 0.76$) with increasing phosphorus-with-ammonium load; Co, Mn, Mo, Ni, Se, U, Zn, and Ra^{226} had no significant trend ($P > 0.15$) with increasing phosphorus-with-ammonium load (Table 3-8A; Fig. 3-6A). None of these analyses had sufficient statistical power ($\beta < 0.8$). Sediment trap contaminant analyses (As, Co, Cu, Mn, Mo, Ni, Se, U, Zn, and Ra^{226}) showed no significant trend ($P > 0.15$) (Table 3-8B; Fig. 3-6B). None of these analyses had sufficient statistical power ($\beta < 0.8$).

Table 3-7 Contaminant concentrations as a function of total phosphorus loads (X) in grams with ammonia additions (model I linear regression) ($n = 4$) from the mid-summer experiment in the surface water (A) and sediment traps (B). Surface water samples were taken on day 32 and final sediment trap samples were taken on day 29 of the 33 day experiment. All contaminants reported in mg L^{-1} in the surface water and mg m^{-2} except Ra^{226} which is reported in Bq L^{-1} in the surface water and Bq m^{-2} in the sediment traps.

A. Surface Water

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.4741	0.28
Cobalt	0.1742 + 0.0008X	0.1167	0.78
Copper	no trend	0.2137	0.62
Manganese	no trend	0.151	0.72
Molybdenum	no trend	0.2178	0.61
Nickel	no trend	0.202	0.64
Selenium	no trend	0.2143	0.62
Uranium	2.0159 - 0.0140X	0.0567	0.89
Zinc	no trend	0.9678	0.00
Radium-226	no trend	0.6452	0. 12

B. Sediment Trap

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.1920	0.65
Cobalt	no trend	0.6617	0.11
Copper	no trend	0.4184	0.34
Manganese	no trend	0.8272	0.03
Molybdenum	no trend	0.9294	0.01
Nickel	no trend	0.5467	0.21
Selenium	no trend	0.5314	0.22
Uranium	no trend	0.6062	0.16
Zinc	36.6746 - 0.4355X	0.0915	0.83
Radium-226	no trend	0.1724	0.68

Figure 3-5 Contaminant concentration versus phosphorus load (g) with ammonia treatments in the surface water (A) and corresponding sediment traps (B) during the mid-summer study. Solid lines represent statistically significant relationships ($p < 0.05$), dashed lines represent near significant relationships ($0.15 < p \geq 0.05$), and dotted lines represent non-significant relationships ($p \geq 0.15$). Surface water samples were taken on day 32 and final sediment trap samples were taken on day 29. Ra^{226} reported in Bq L^{-1} in surface water and Bq m^{-2} in sediment traps.

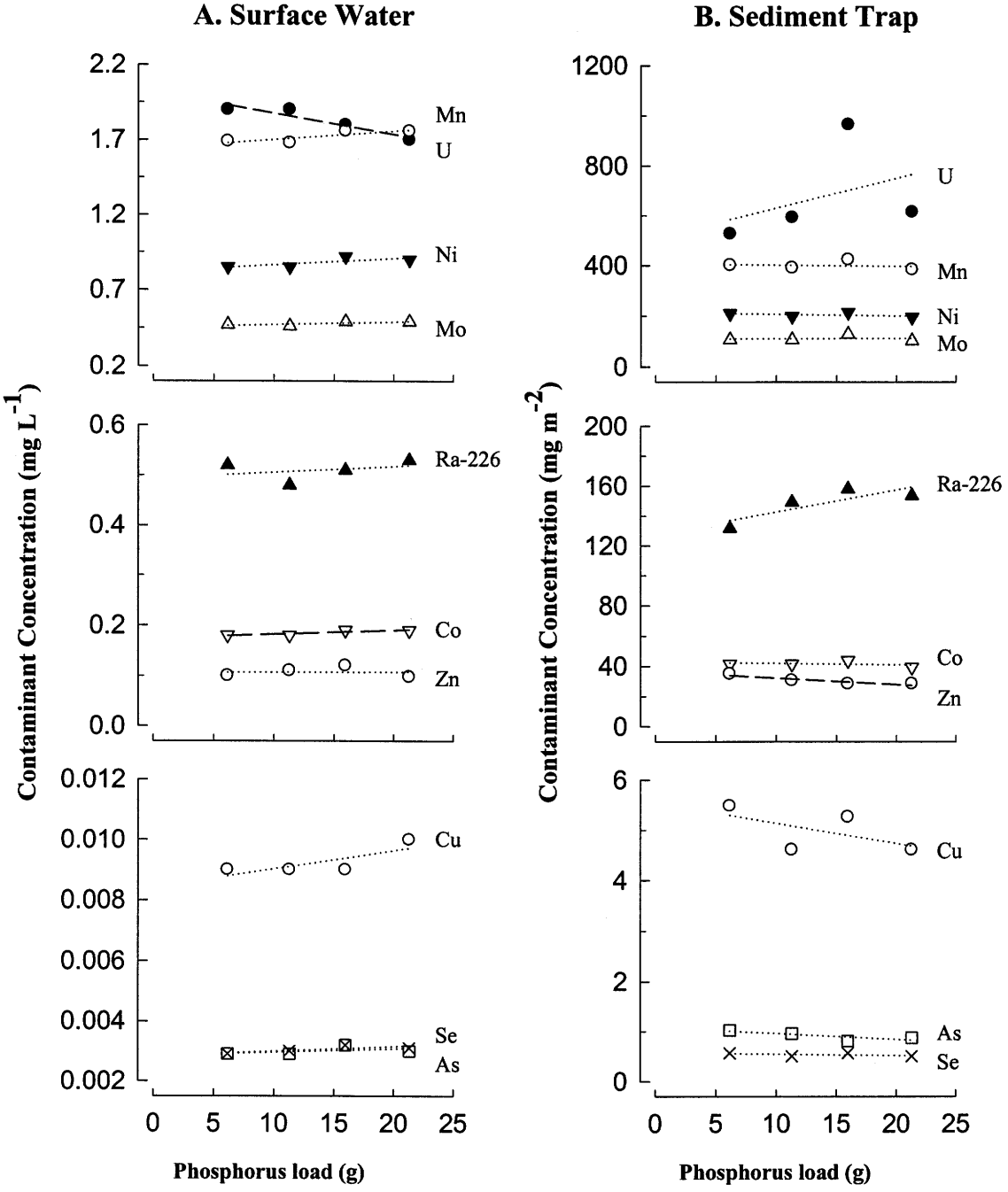

A. Surface Water
B. Sediment Trap
Contaminant Concentration (mg L-1)
Contaminant Concentration (mg m-2)
Mn
U
Ni
Mo
Ra-226
Co
Zn
Cu
Se
As
Phosphorus load (g)
Phosphorus load (g)

Table 3-8 Contaminant concentrations as a function of total phosphorus loads (X) (g) with ammonia additions (model I linear regression) ($n = 4$) from the late-summer experiment in the surface water (A) and sediment traps (B). Surface water samples were taken on day 28 and final sediment trap samples were taken on day 29 of the 29 day experiment. All contaminants reported in mg L^{-1} in the surface water and mg m^{-2} except Ra^{226} which is reported in Bq L^{-1} in the surface water and Bq m^{-2} in the sediment traps.

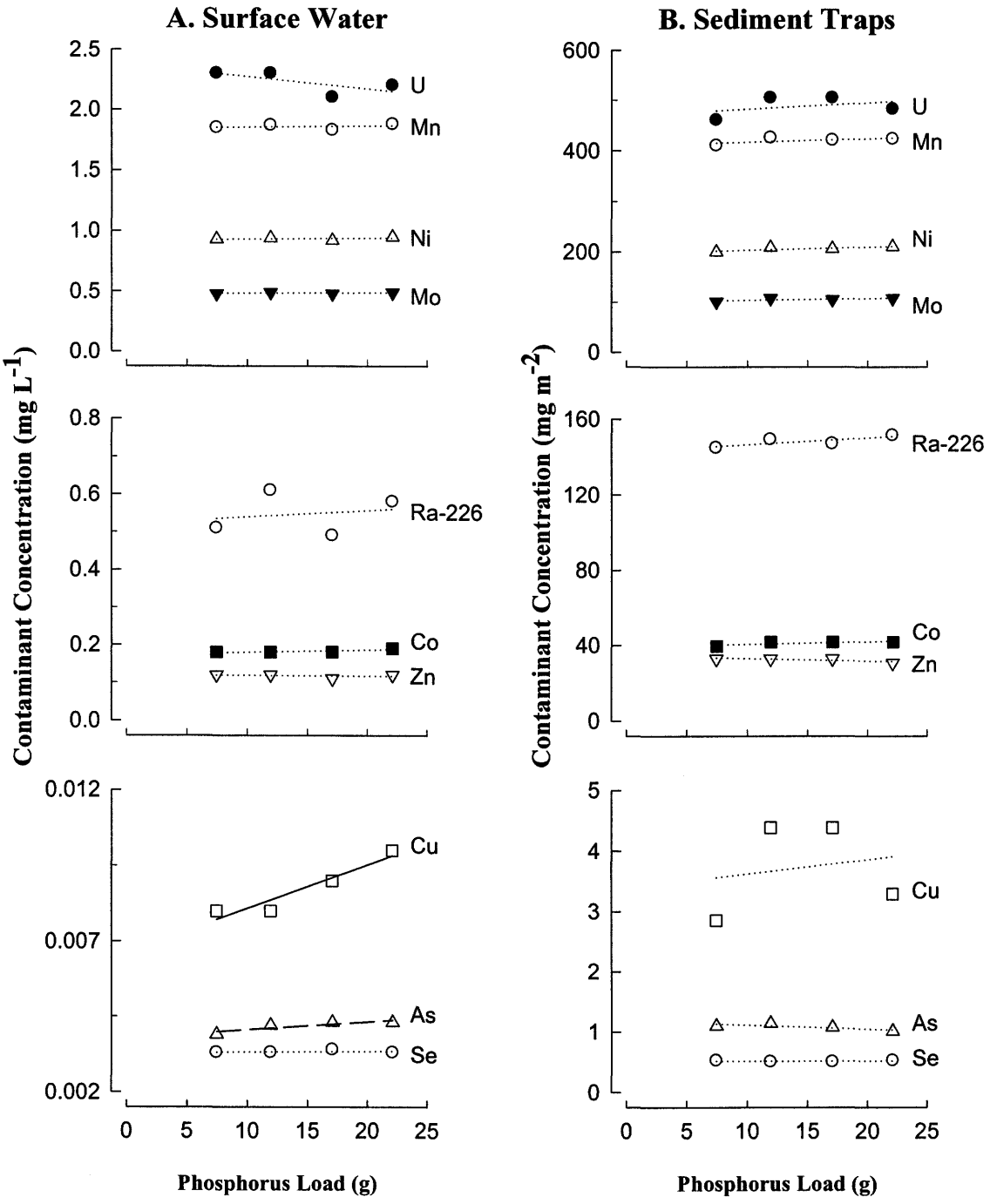
A. Surface Water

Contaminant	Model	*P*	R^2
Arsenic	0.0038 + 0.000026X	0.1265	0.76
Cobalt	no trend	0.2148	0.62
Copper	0.0066 + 0.0001X	0.0468	0.91
Manganese	no trend	0.7119	0.08
Molybdenum	no trend	0.5662	0.19
Nickel	no trend	0.6005	0.16
Selenium	no trend	0.7453	0.06
Uranium	no trend	0.3239	0.46
Zinc	no trend	0.7453	0.06
Radium-226	no trend	0.8112	0.04

B. Sediment Trap

Contaminant	Model	*P*	R^2
Arsenic	no trend	0.2071	0.63
Cobalt	no trend	0.2444	0.57
Copper	no trend	0.8085	0.04
Manganese	no trend	0.3576	0.41
Molybdenum	no trend	0.2886	0.51
Nickel	no trend	0.1776	0.68
Selenium	no trend	0.9744	0.0
Uranium	no trend	0.6208	0.14
Zinc	no trend	0.2148	0.62
Radium-226	no trend	0.2092	0.63

Figure 3-6 Contaminant concentration versus phosphorus load (g) with ammonia treatments in the surface water (A) and corresponding sediment traps (B) during the mid-summer study. Solid lines represent statistically significant relationships ($p < 0.05$), dashed lines represent near significant relationships ($0.15 < p \geq 0.05$), and dotted lines represent non-significant relationships ($p \geq 0.15$). Surface water samples were taken on day 28 and final sediment trap samples were taken on day 29. Ra^{226} reported in Bq L^{-1} in surface water and Bq m^{-2} in sediment traps.


A. Surface Water
B. Sediment Traps
Contaminant Concentration (mg L-1)
Contaminant Concentration (mg m-2)
U
Mn
Ni
Mo
Ra-226
Co
Zn
Cu
As
Se
Phosphorus Load (g)
Phosphorus Load (g)

3. 3. 4 Comparing Phosphorus and Phosphorus-with-ammonium treatments

A comparison of the phosphorus and phosphorus-with-ammonium treatments generally resulted in no significant difference between treatments for most contaminants in both experiments (Table 3-9). However, there was a significant ($p < 0.05$) difference between nutrient treatments with As and Zn in the surface water of the mid-summer experiment, with both contaminants having decreased in concentrations in the phosphorus treatment compared to the phosphorus-with-ammonium treatments. In the sediment traps of the late-summer experiment, there was a significant ($p < 0.05$) difference between nutrient treatments with U and Zn. Both U and Zn had increased concentrations in the phosphorus treatment compared to the phosphorus-with-ammonium treatment.

3. 4 Discussion

3. 4. 1 Chlorophyll *a*

The relationship between phosphorus limitation, algal growth, and chl *a* has been well established in temperate lakes (Litchman *et al.* 2003; Schindler 1977) and streams (Van Nieuwenhuyse and Jones 1996). Phytoplankton fluorescence increased in the water columns of the treatment mesocosms with the addition of nutrients in both the mid- and late-summer studies (Fig. 3-1 and 3-2), and in a similar fashion as the experiment conducted in DJX pit during the summer 2003 (Chapter 2, Fig. 2-1) and similar to other large scale nutrient amendment experiments (Strauss *et al.* 1994). However, late-summer fluorescence profiles of treatment mesocosms show a reduced amount of chl *a* throughout the water column (Fig. 3-2) when compared to mid-summer fluorescence profiles (Fig. 3-1) and profiles from 2003; late-summer fluorescence profiles rarely

Table 3-9 Comparison of phosphorus vs. phosphorus-with-ammonium treatments on contaminants (Two-way ANOVA, *d. f.* = 1) analysis was performed independently for the mid-summer and late-summer experiment.

Contaminant	Mid-Summer Experiment		Late-Summer Experiment	
	Surface water	Sediment Trap	Surface water	Sediment Trap
	No NH_4 vs. NH_4	No NH_4 vs. NH_4	No NH_4 vs. NH_4	No NH_4 vs. NH_4
Arsenic	***P* < 0.05**	$P = 0.34$	$P = 0.09$	$P = 0.22$
Cobalt	$P = 0.08$	$P = 0.76$	$P = 0.39$	$P = 0.64$
Copper	$P = 0.72$	$P = 0.66$	$P = 0.41$	$P = 0.72$
Manganese	$P = 0.39$	$P = 0.37$	$P = 0.06$	$P = 0.54$
Molybdenum	$P = 1.0$	$P = 0.43$	$P = 0.18$	$P = 0.82$
Nickel	$P = 0.28$	$P = 0.59$	$P = 1.00$	$P = 0.29$
Selenium	$P = 0.31$	$P = 0.28$	$P = 0.64$	$P = 0.08$
Uranium	$P = 0.55$	$P = 0.91$	$P = 0.50$	***P* < 0.05**
Zinc	***P* < 0.05**	$P = 0.59$	$P = 0.45$	***P* < 0.05**
$Radium^{226}$	$P = 0.36$	$P = 0.56$	$P = 0.15$	$P = 0.16$

exceeded 20 μg L^{-1} of chl *a* while fluorescence profiles from high P-load treatments from the mid-summer study exceeded 50 μg L^{-1} of chl *a*. The mid- and late-summer runs were subjected to substantially different conditions: July was typified by hot, long, sunny days with very little rain fall; late August and September were typified by cool, substantially shorter, rainy days and longer, cold nights, and sub-zero temperatures near the end of the late summer experiment. Temperature changes may affect the productivity of phytoplankton. Flanagan *et al.* (2003), who suggest that primary productivity may increase with increasing temperature and nutrients. Similarly, models have demonstrated slight changes in productivity in relation to day length (Behrenfeld and Falkowski 1997). Seasonal changes in abiotic factors have resulted in changes in phytoplankton communities (Anneville *et al.* 2002; Ferguson and Harper 1982; Gurbuz *et al.* 2004; Letelier *et al.* 2004) and chl *a* concentrations (Marshall and Peters 1989). Therefore, lower temperatures and light levels may have dampened the treatment effects.

Differences in phytoplankton biomass between experiments were reflected in the Secchi depths on the last day of each experiment (Tables 3-3 and 3-4). Secchi depths taken on the last day (day 33) of the mid-summer experiment showed high water transparency in the control mesocosm (P-load 2.02g), exceeding the 8m depth of the mesocosm; Secchi depths were very shallow in treated mesocosms in the mid-summer (Table 3-3), however, differences between high P-load treatments were indistinguishable which is reflective of the ineffectiveness of Secchi depth measurements to differentiate beyond chl *a* concentrations of 20 μg L^{-1} (Canfield and Bachmann 1981; Horne and Goldman 1994).

Secchi depths taken on the last day (Day 29) of the late-summer experiment show that although treatment mesocosms had reduced water clarity compared to the

control, the difference was much less than that seen in the mid-summer experiment (Table 3-4). Moreover, Secchi measurements from the late-summer experiment reflect patterns seen in chl *a* profiles taken on three days earlier on day 26 (Fig. 3-2); the treatments of 6.74g P-load and 22.12g P-load with NH_4 show the lowest water clarity (Table 3-4) and the greatest chl *a* concentrations on day 26 (Fig. 3-2); the treatments of 11.94g and 17.07g P-load with NH_4 had intermediate water clarity (Table 3-4) and chl *a* profiles (Fig. 3-2); 12.00g, 17.23g, and 22.04g P-load, and 7.45g P-load with NH_4 had greater water clarity (Table 3-4) and lower chl *a* concentration (Fig. 3-2). Nonetheless, the difference in water clarity between control and amended mesocosms indicates that the addition of nutrients was effective at stimulating algal growth in the pit-lake water during both the mid- and late-summer studies.

Overall, chl *a* profiles and Secchi depths indicated lower phytoplankton biomass in the late-summer experiment compared to the mid-summer experiment. The previously mentioned abiotic factors may have largely contributed to differences in phytoplankton growth and biomass between the two experiments. In addition, such changes in the abiotic conditions may have enhanced the toxicity of certain contaminants and at varying nutrient concentrations. For example, As and Cu have been found to inhibit productivity in early spring rather than summer; moreover, both exhibited their toxic effects at varying nutrient levels, with Cu having a greater effect at higher nutrient levels, and As affecting productivity at lower nutrient levels (Riedel *et al.* 2003).

3. 4. 2 Phosphorus Treatments

3. 4. 2. 1 Mid - Summer Study

Surface water concentrations of U, Zn, and Cu had non-significant decreasing trends with increasing phosphorus load (Table 3-5A; Fig. 3-3A); Zn and Ra^{226}

significantly increased and U, Mn, Ni, and Cu had non-significant increasing trends (Table 3-5B; Fig. 3-3B) in the sediment trap with increasing phosphorus loads. This corroborates the decreasing surface water contaminant concentrations and is similar to the results from our previous experiment in the summer of 2003 (see Chapter 2). Although I cannot exclude the effects of chemical precipitation, I suspect that the increase in the sedimentation rates of these contaminants was largely due to sedimentation of algae with bound contaminants (described in chapter 2).

The responses by U, Zn, Cu, and Mn to increasing phosphorus loads are similar to the results from 2003 (see discussion in chapter 2. 4). Evidence for the uptake of these metals by algae has been shown in the literature. For example, U can be enzymatically reduced from soluable to insoluable U (Lovley and Phillips 1992a; Lovley and Phillips 1992b; Lovley *et al.* 1991) and accumulate in algae (Pribil and Marvan 1976); Zn which can accumulate in algae (Meylan *et al.* 2003; Wang and Dei 2001a; Wolterbeek *et al.* 1995; Yu and Wang 2004) and Cu which can accumulate in algae (Knauer *et al.* 1997; Meylan *et al.* 2003); and Mn which is a micronutrient (Graham and Wilcox 2000). These previous experiments suggest that these metals may be accumulating in the algal cells in the mesocosms through cellular uptake processes.

During the mid-summer study, Se concentrations in the sediment traps decreased as P-load increased (Table 3-5B; Fig. 3-3B), but such a trend was not observed in the surface water (Table 3-5A; Fig. 3-3A). Se is required as a micronutrient by a variety of phytoplankton (Harrison *et al.* 1988). However, Se uptake has been found to be minimal by Chlorophytes when compared to other groups of phytoplankton (Baines *et al.* 2001), thus, if Chorophytes dominated the phytoplankton biomass in the mesocosms in a similar manner to the previous experiment in 2003, we would expect little to no Se

uptake. Furthermore, Se uptake has shown to be inhibited by (Yu and Wang 2004) and negatively correlated with P-concentrations (Wang and Dei 2001b;Yu and Wang 2004). Riedel and Sanders (1996) found that selenite uptake was greater when phosphate concentrations were low. These factors may explain the lack of effect of increasing P-load on Se, but they do not explain the decreasing sedimentation rate of Se with increasing P-load. I do not have an explanation for this outcome.

Mo, Co, and As were not affected by increasing P-load in the surface water or sediment trap (Table 3-5; Fig. 3-3). Mo had no trend with increasing P-load in our previous experiment in 2003, however Co and As were both greatly affected by increasing P-load in 2003. Although Mo is a cofactor for enzymes necessary for nitrogen metabolism (Heck and Ninnemann 1995; Paulsen *et al.* 1991; Zahalak *et al.* 2004), molybdate may compete with phosphate for uptake site. For example, Mo uptake by tomato plants was shown to decrease with increasing phosphate concentrations (Heuwinkel *et al.* 1992). This may explain the lack of effect of increasing P-load on Mo concentrations. As previously mentioned (Chapter 2. 4), sulfate was not measured in the mesocosms, even though sulfate concentrations may become a problem in many pit-lakes (Miller *et al.* 1996). Sulfate has shown to inhibit Mo uptake (Cole et al. 1993), which may also explain why Mo is not sedimenting out of the water column. Some species of arsenic are taken up by phytoplankton (Cullen *et al.* 1994; Kobayashi *et al.* 2003; Sanders and Riedel 1993). The green algae, *Chlorella minutissima*, was shown to adsorb large proportions of Co (Roy *et al.* 1993), however, neither Mo, Co, nor As responded to increasing P-load.

3. 4. 2. 2 Late - Summer Study

Selenium concentrations significantly increased in the surface waters. Mn had a non-significant increase in the surface water as P-load increased (Table 3-6A; Fig. 3-4A) in the late-summer study. Selenium had a non-significant decreasing trend in the sediment traps as P-load increased (Table 3-6B; Fig. 3-4B). Surface water and sediment trap concentrations of U, Ni, Mo, Ra^{226}, Co, Zn, Cu, and As concentrations were not affected by P-load (Table 3-6; Fig. 3-4).

I have no explanation for the increase in Se concentrations in the surface water and for the decrease in Se concentration in the sediment traps, nor can I explain the non-significant increasing trend of Mn concentrations in the surface water. However, a lack of effect overall on the various contaminants may be partially explained by the lack of phytoplankton growth. Chl *a* profiles from the late-summer study showed reduced overall growth (Fig. 3-2) when compared to mid-summer chl *a* profiles (Fig. 3-1). In addition, seasonal changes in the phytoplankton community (Harris 1986; Reynolds 1984) may have affected metal sedimentation rates through a potential shift from phytoplankton with high sedimentation rates to a group of phytoplankton with lower sedimentation rates, and by potentially reducing the phytoplankton biomass available to bind and sediment the contaminants from the surface water.

3. 4. 3 Phosphorus-with-ammonium Treatments

Overall, phosphorus-with-ammonium treatments had little effect on the sedimentation rates of contaminants; however, uranium concentrations had a non-significant decreasing trend, while Co concentrations had a non-significant increasing trend in the surface water with increasing P-load-with-ammonium, but Mn, Ni, Mo, Ra^{226}, Zn, Cu, Se, and As had not trend in the surface water with increasing P-load-with-

ammonium in the mid-summer study (Table 3-7A; Fig. 3-5A). Zn concentrations had non-significant decreasing trend in the sediment trap with increasing P-load-with-ammonium, however, U, Mn, Ni, Mo, Ra^{226}, Co, Cu, Se, and As showed no trend in the sediment trap with increasing P-load-with-ammonium in the mid-summer study (Table 3-7B; Fig. 3-5B).

Results from the phosphorus-with-ammonium treatments from the late-summer experiment remained relatively unchanged from mid-summer study results. Copper significantly increased and As had a non-significant increase in surface water concentration with increasing P-load-with-ammonium, however, U, Mn, Ni, Mo, Ra^{226}, Co, Zn, and Se showed no trend in the surface water with increasing P-load-with-ammonium (Table 3-8A; Fig. 3-6A). None of the contaminants showed any trend in the sediment traps from the late-summer study (Table 3-8B; Fig. 3-6B).

I am unsure why certain contaminant concentrations increased in the surface water and decreased in the sediment trap with increasing P-loads-with-ammonium additions. Nitrate is the dominant form of N in DJX pit, and thus, it was also the dominant form of N in the treatments with only phosphorus additions. However, the treatments with ammonium additions may have altered the phytoplankton community structure (Findlay *et al.* 1999) which in turn lowered sedimentation rates. Past studies have shown that ammonium can negatively affect cell size and sedimentation rates. In nitrogen-controlled marine systems, marine phytoplankton were found to have a larger cell size if NO_3, rather than NH_4, was the predominant form for nitrogen available (Stolte and Riegman 1995; Stolte and Riegman 1996). Moreover, when comparing the sedimentation rates of marine phytoplankton grown in NO_3 or NH_4, the phytoplankton grown in NO_3 had more rapid sedimentation rates (Stolte *et al.* 1994). Such factors may

have contributed to the lack of response in the sedimentation rates of contaminants in phosphorus-with-ammonium additions when compared to phosphorus additions alone.

3. 4. 3 Comparison of Nutrient Treatments

Although visually, there seems to be a difference between phosphorus and phosphorus-with-ammonium treatments, statistically the differences between treatments were not different. As and Zn were significantly lower in concentration in the surface water in the phosphorus treatments compared to the phosphorus-with-ammonium treatments in the mid-summer study, and U and Zn were significantly higher in concentration in the sediment traps of the phosphorus treatments compared to the phosphorus-with-ammonium treatments in the late-summer study. Yet the fact few significant differences were apparent between the nutrient treatments was not unexpected due to the low sample size and especially the lack of replication within treatments which led to high variability within the samples.

3. 5 Conclusion

Contaminant sedimentation rates in the phosphorus treated mesocosms in the mid-summer study responded in a similar manner to our experiment in 2003. However, seasonal differences between the mid- and late-summer studies appear to have negatively affected contaminant sedimentation rates in the phosphorus-treated mesocosms in the late-summer experiment. Contaminant sedimentation rates were generally not affected by phosphorus-with-ammonium treatment in either study, suggesting that ammonium additions have a negative effect on contaminant sedimentation rates. Although many contaminants showed no trend with either treatment in both the mid- and late-summer season, I can not assume that the treatments did not affect contaminant sedimentation rates. Almost all of the statistical analyses yielded low

statistical power, and therefore, the negative results must be interpreted cautiously, keeping in mind the danger of committing type II statistical errors (Peterman 1990).

CHAPTER 4

Summary and Conclusions

4. 1 Phytoplankton

As demonstrated by the chl *a* profiles in all the experiments, the treated mesocosms experienced increased phytoplankton growth when compared to the untreated controls. Thus, phosphorus and phosphorus additions were successful in stimulating phytoplankton blooms in contaminated water from the DJX pit.

Results from the phytoplankton identification analyses from 2003 indicated that Chlorophytes dominated the community. Furthermore, there were differences in the sedimentation rates of different phytoplankton genera. For example, *Chlamydomonas* dominated the surface water while an unidentified Chlorophyte tended to have the greatest biomass in the sediment traps. Reasons for the difference in sedimentation rates may be due to differences in cell density, where the unidentified Chlorophyte tended to be slightly larger than *Chlamydomonas*, potentially enhancing its sedimentation rate. Unfortunately, we do not have the phytoplankton taxonomy results to compare phosphorus treatments with phosphorus-with-ammonium treatments in 2004.

4. 2 Phosphorus Additions

Overall, phosphorus had a positive effect on the sedimentation of contaminants. Results from the experiment conducted in 2003 clearly showed that phosphorus significantly affected the sedimentation rates of As, Co, Cu, Mn, Ni, U, Zn, and Ra^{226}. However, phosphorus additions were not successful at removing Mo and Se from the

surface waters in 2003, possibly due to competition between phosphate, selenate and selenite, and molybdate for phytoplankton uptake sites.

Trends from 2004 overall tended to be similar to those seen in 2003, however, fewer contaminants were affected by increasing phosphorus additions, particularly during the fall of 2004. This may indicate a seasonal effect of phosphorus additions on phytoplankton growth and contaminant sedimentation rates, likely caused by abiotic factors (sunlight, temperature), or a change in the phytoplankton community between experiments in 2004. It was apparent that phytoplankton growth was reduced during the late-summer versus the mid-summer experiment in 2004, but it is unclear whether this is the only reason for reduced contaminant sedimentation rates.

Unfortunately, small sample sizes from experiments conducted in 2004 resulted in low statistical power, and as such, very few definitive conclusions can be made about the effects of phosphorus on contaminant sedimentation rates during the summer of 2004. Furthermore, phosphorus added to DJX pit by Cogema during the winter of 2003-2004, based on the results from 2003, may have affected the results by relaxing P-limitation.

4. 3 Phosphorus-with-Ammonium Additions

Phosphorus-with-ammonium treatments proved to have little impact on the sedimentation of contaminants, even though increased phytoplankton growth was observed. However, mid-summer chlorophyll *a* profiles and Secchi depth measurements indicate that high phosphorus treatments experienced slightly greater phytoplankton growth than high phosphorus-with-ammonium treatments (Table 3-3; Fig. 3-1). Different sources of nitrogen may have affected the sedimentation rates of phytoplankton, with phytoplankton in the phosphorus only treatments using nitrate as

their dominant source of nitrogen. As previously mentioned in chapter 3, studies have shown that the source of nitrogen may affect phytoplankton size and sedimentation rates (Stolte *et al.* 1994) which may lend itself to explain the reduced sedimentation rates of phosphorus with ammonium treatments.

4.4 Conclusions

I have reiterated throughout the thesis that, although there is no direct evidence linking the sedimentation of contaminants with that of phytoplankton, I believe that the phytoplankton are greatly influencing the sedimentation of contaminants. There is an abundance of evidence in the literature linking phytoplankton with the fate and transport of contaminants. In addition, the seasonal differences between experiments conducted in 2004 in DJX pit may further attest to the effects of phytoplankton growth on contaminant sedimentation rates. For example, chlorophyll *a* profiles indicate increased phytoplankton growth occurred during the mid-summer experiment (Fig. 3-1) as opposed to the late-summer experiment (Fig. 3-2) during the summer of 2004. Similarly, contaminant sedimentation rates were more highly correlated with phosphorus loads during the mid-summer experiment (Table 3-5) as opposed to the late-summer experiment (Table 3-6) in the phosphorus only treatments.

If contaminant sedimentation rates were solely related to the chemical precipitation of contaminants, I might expect similar contaminant sedimentation rates for all experiments. However, contaminant sedimentation rates varied between experiments conducted during 2003 and 2004, and seasonally between experiments during 2004. Thus, this may indicate that phytoplankton blooms, sedimentation rates, and phytoplankton community structure profoundly impact contaminant sedimentation rates,

albeit, this alone does not provide enough evidence for the exclusion of chemical precipitation of contaminants or a combination of both.

To further tease apart factors influencing contaminant sedimentation rates, phytoplankton and water samples must be examined more closely to determine where and how the bulk of the contaminants are being bound and sequestered. However, this falls outside the scope of this experiment. Here, we wish only to determine the effects of nutrients on the sedimentation rates of contaminants as a whole. With this purpose in mind, it seems clear that phytoremediation does hold much promise for the bioremediation of contaminated pit-lakes. Results from the experiments conducted in 2003 show clear trends between contaminant sedimentation rates and increasing phosphorus load, even if results from the summer of 2004 are less clear. As a result, pit-lake phytoremediation can potentially reduce the environmental impact of mine water treatment and greatly reduce the cost of mine water treatment to Saskatchewan uranium mines.

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APPENDIX A
Phytoplankton Enumeration Protocol Used by AlgaTax Consulting

APPENDIX B

Phytoplankton Taxon, Biomass, Density, and Cell Dimensions Observed in the Surface Water and Sediment Traps of Mesocosms Treated with Varying Phosphorus Loads during the summer of 2003 in DJX Pit

Table B-1 Phytoplankton group biomass ($\mu g\ L^{-1}$) observed in the surface water (A) and sediment trap (B) under different phosphorus treatments throughout the summer of 2003.

A. Surface Water

Phytoplankton Group	Treatment - Phosphorus Load (g)								
	0.58	2.54	4.08	5.00	7.59	9.75	11.91	12.00	13.34
Cyanobacteria Biomass	1.1	13.3	45.5	16.7	8.6	30.9	50.0	7.3	9.3
Chlorophyta Biomass	123.2	12716.6	11150.2	1328.9	2455.8	1795.3	5215.2	2103.3	2542.4
Chrysophyta Biomass	5.5	420.0	93.7	75.0	63.4	56.4	207.9	45.7	84.4
Bacillariophyta Biomass				2.2	0.7				
Pyrrhophyta Biomass	5.5								

B. Sediment trap

Phytoplankton Group	Treatment - Phosphorus Load (g)							
	0.58	2.54	4.08	5.00	7.59	9.75	12.00	13.34
Cyanobacteria Biomass	12.8	78.8	116.1	60.1	313.1	211.5	156.5	159.1
Chlorophyta Biomass	237.9	22522.0	46600.0	54077.5	89710.3	96899.8	82830.3	30517.1
Chrysophyta Biomass	43.1	877.4	2068.5	1754.9	5174.4	3056.6	1937.3	1402.3
Bacillariophyta Biomass	6.1							
Pyrrhophyta Biomass								

Table B-2 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 5 (control) (P-Load 0.58 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	84556	1.1	3.2	2.8
Total	**84556**	**1.1**		
Chlorophyta				
Chlamydomonas sp. (large)	175313	42.7	8.5	7.4
Chlamydomonas sp. (small)	257614	10.7	6.5	3.5
Planktosphaeria spp.	25930	24.0	12.3	12.0
Sphaerellopsis spp.	16911	4.8	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	19729	0.6	4.5	3.5
Unidentified green spp. (thick-wall)	85120	33.8	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	55807	6.5	6.2	6.0
Total	**636424**	**123.2**		
Chrysophyta				
Ochromonas spp.	58062	3.0	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	31567	2.5	5.5	5.2
Total	**89629**	**5.5**		
Pyrrhophyta				
Glenodinium spp. (small)	2818	5.5	17.2	14.7
Total	**2818**	**5.5**		
Overall Total	**813427**	**135.2**		

Table B-3 Phytoplankton Taxa, Density and Biomass in the Surface Water of Mesocosm 2 (P-Load 2. 54 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	1014675	13.3	3.2	2.8
Total	**1014675**	**13.3**		
Chlorophyta				
Chlamydomonas sp. (large)	10823210	2637.8	8.5	7.4
Chlamydomonas sp. (small)	100368360	4377.6	6.8	3.5
Planktosphaeria spp.	3015842	2796.9	12.3	12.0
Sphaerellopsis spp.	7440956	2090.8	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	958305	23.1	4.5	3.2
Unidentified green spp. (thick-wall)	1127417	447.8	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	2931286	342.6	6.2	6.0
Total	**126665376**	**12716.6**		
Chrysophyta				
Ochromonas spp.	2339391	121.5	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	3833220	298.5	5.5	5.2
Total	**6172611**	**420.0**		
Overall Total	**133852662**	**13150.0**		

Table B-4 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 6 (P-Load 4.08 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	3466809	45.5	3.2	2.8
Total	**3466809**	**45.5**		
Chlorophyta				
Chlamydomonas sp. (large)	12063369	2940.0	8.5	7.4
Chlamydomonas sp. (small)	170042768	7416.5	6.8	3.5
Sphaerellopsis spp.	225483	63.4	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	1437457	34.7	4.5	3.2
Unidentified green spp. (thick-wall)	789192	313.5	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	3269511	382.1	6.2	6.0
Total	**187827780**	**11150.2**		
Chrysophyta				
Ochromonas spp.	1465643	76.1	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	225483	17.6	5.5	5.2
Total	**1691126**	**93.7**		
Overall Total	**192985715**	**11289.4**		

Table B-5 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 1 (P-Load 5.00 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	1268344	16.7	3.2	2.8
Total	**1268344**	**16.7**		
Chlorophyta				
Chlamydomonas sp. (large)	1465643	357.2	8.5	7.4
Chlamydomonas sp. (small)	15149675	660.8	6.8	3.5
Sphaerellopsis spp.	155019	43.6	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	352318	8.5	4.5	3.2
Unidentified green spp. (thick-wall)	253668	100.8	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	1352901	158.1	6.2	6.0
Total	**18729224**	**1328.9**		
Chrysophyta				
Ochromonas spp.	916026	47.6	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	352318	27.4	5.5	5.2
Total	**1268344**	**75.0**		
Bacillariophyta				
Navicula spp. (small)	14092	2.2	24.5	4.9
Total	**14092**	**2.2**		
Overall Total	**21280004**	**1422.8**		

Table B-6 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 8 (P-Load 7.59 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	654909	8.6	3.2	2.8
Total	**654909**	**8.6**		
Chlorophyta				
Chlamydomonas sp. (large)	3675145	895.7	8.5	7.4
Chlamydomonas sp. (small)	16347985	681.6	6.5	3.5
Monoraphidium spp.	47261	3.6	24.5	2
Planktosphaeria spp.	396096	367.3	12.3	12.0
Sphaerellopsis spp.	317327	89.2	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	234057	6.8	4.5	3.5
Unidentified green spp. (thick-wall)	474865	188.6	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	1908465	223.0	6.2	6.0
Total	**23401201**	**2455.8**		
Chrysophyta				
Ochromonas spp.	733678	38.1	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	324079	25.2	5.5	5.2
Total	**1057757**	**63.4**		
Bacillariophyta				
Navicula spp. (small)	4501	0.7	24.5	4.9
Total	**4501**	**0.7**		
Overall Total	**25118368**	**2528.5**		

Table B-7 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 3 (P-Load 9.75 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	2353484	30.9	3.2	2.8
Total	**2353484**	**30.9**		
Chlorophyta				
Chlamydomonas sp. (large)	2170279	528.9	8.5	7.4
Chlamydomonas sp. (small)	17235398	751.7	6.8	3.5
Sphaerellopsis spp.	225483	63.4	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	1451550	35.0	4.5	3.2
Unidentified green spp. (thick-wall)	591894	235.1	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	1550199	181.2	6.2	6.0
Total	**23224803**	**1795.3**		
Chrysophyta				
Ochromonas spp.	746914	38.8	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	225483	17.6	5.5	5.2
Total	**972397**	**56.4**		
Overall Total	**26550684**	**1882.6**		

Table B-8 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 4 (P-Load 11.91 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	3805034	50.0	3.2	2.8
Total	**3805034**	**50.0**		
Chlorophyta				
Chlamydomonas sp. (large)	2959471	721.3	8.5	7.4
Chlamydomonas sp. (small)	39234136	1711.2	6.8	3.5
Planktosphaeria spp.	338225	313.7	12.3	12.0
Sphaerellopsis spp.	1155603	324.7	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	2903100	70.0	4.5	3.2
Unidentified green spp. (thick-wall)	1606570	638.2	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	12288853	1436.2	6.2	6.0
Total	**60485958**	**5215.2**		
Chrysophyta				
Ochromonas spp.	3494994	181.6	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	338225	26.3	5.5	5.2
Total	**3833219**	**207.9**		
Overall Total	**68124211**	**5473.1**		

Table B-9 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 9 (P-Load 12.00 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	554689	7.3	3.2	2.8
Total	**554689**	**7.3**		
Chlorophyta				
Chlamydomonas sp. (large)	1791466	436.6	8.5	7.4
Chlamydomonas sp. (small)	10623656	442.9	6.5	3.5
Monoraphidium spp.	162348	12.5	24.5	2.0
Planktosphaeria spp.	378812	351.3	12.3	12.0
Sphaerellopsis spp.	244649	68.7	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	677578	19.6	4.5	3.5
Unidentified green spp. (thick-wall)	356263	141.5	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	5392438	630.2	6.2	6.0
Total	**19627210**	**2103.3**		
Chrysophyta				
Ochromonas spp.	324696	16.9	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	369792	28.8	5.5	5.2
Total	**694488**	**45.7**		
Overall Total	**20876387**	**2156.3**		

Table B-10 Phytoplankton Taxon, Density and Biomass in the Surface Water of Mesocosm 7 (P-Load 13.34 g) on Day 35 of 37 Day experiment, 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	711173	9.3	3.2	2.8
Total	**711173**	**9.3**		
Chlorophyta				
Chlamydomonas sp. (large)	3528860	860.0	8.5	7.4
Chlamydomonas sp. (small)	25278162	1053.9	6.5	3.5
Monoraphidium spp.	159788	12.3	24.5	2.0
Planktosphaeria spp.	306074	283.9	12.3	12.0
Sphaerellopsis spp.	153037	43.0	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	1584386	45.7	4.5	3.5
Unidentified green spp. (thick-wall)	362338	143.9	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	852957	99.7	6.2	6.0
Total	**32225602**	**2542.4**		
Chrysophyta				
Ochromonas spp.	1206294	62.7	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	279068	21.7	5.5	5.2
Total	**1485362**	**84.4**		
Overall Total	**34422137**	**2636.2**		

Table B-11 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 5 (Control) (P-Load 0.58 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Pseudanabaena spp.	19470	3.4	100.0	1.5
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	708715	9.3	3.2	2.8
Total	**728185**	**12.8**		
Chlorophyta				
Chlamydomonas sp. (large)	272582	57.8	8.5	6.9
Chlamydomonas sp. (small)	268688	12.8	7.4	3.5
Planktosphaeria spp.	54516	50.6	12.3	12
Sphaerellopsis spp.	27258	7.7	9.8	7.4
Ulothrix spp. (*Klebsormidium* ?)	15576	1.3	8.5	3.5
Unidentified green flagellate (cf. *Scourfieldia*)	15576	0.3	4.5	3.0
Unidentified green spp. (thick-wall)	151867	60.3	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	809960	47.2	5.5	4.5
Total	**1616023**	**237.9**		
Chrysophyta				
Ochromonas spp.	264794	13.8	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	564635	29.3	4.9	4.5
Total	**829429**	**43.1**		
Bacillariophyta				
Nitzschia spp. (small)	19470	6.1	32.5	3.5
Total	**19470**	**6.1**		
Overall Total	**3193107**	**299.8**		

Table B-12 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 2 (P-Load 2.54 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	5997861	78.8	3.2	2.8
Total	**5997861**	**78.8**		
Chlorophyta				
Chlamydomonas sp. (large)	33619592	7123.7	8.5	6.9
Chlamydomonas sp. (small)	47035864	2232.5	7.4	3.5
Planktosphaeria spp.	5129750	4757.3	12.3	12.0
Sphaerellopsis spp.	16257362	4568.1	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	4261638	90.4	4.5	3.0
Unidentified green spp. (thick-wall)	6392458	2539.2	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	23833608	1210.7	5.5	4.2
Total	**136530272**	**22522.0**		
Chrysophyta				
Ochromonas spp.	3393527	176.3	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	13495189	701.1	4.9	4.5
Total	**16888716**	**877.4**		
Overall Total	**159416849**	**23478.2**		

Table B-13 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 6 (P-Load 4.08 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	8838954	116.1	3.2	2.8
Total	**8838954**	**116.1**		
Chlorophyta				
Chlamydomonas sp. (large)	36776364	7792.6	8.5	6.9
Chlamydomonas sp. (small)	55677520	2642.7	7.4	3.5
Planktosphaeria spp.	9312469	8636.4	12.3	12.0
Sphaerellopsis spp.	24464962	6874.4	9.8	7.4
Ulothrix spp. (*Klebsormidium* ?)	276217	22.6	8.5	3.5
Unidentified green flagellate (cf. *Scourfieldia*)	3314607	70.3	4.5	3.0
Unidentified green spp. (thick-wall)	47706676	18949.8	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	22097386	1611.2	5.8	4.9
Total	**199626201**	**46600.0**		
Chrysophyta				
Ochromonas spp.	8720575	453.1	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	31094178	1615.5	4.9	4.5
Total	**39814753**	**2068.5**		
Overall Total	**248279908**	**48784.6**		

Table B-14 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 1 (P-Load 5.00 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	4577315	60.1	3.2	2.8
Total	**4577315**	**60.1**		
Chlorophyta				
Chlamydomonas sp. (large)	36066088	6170.2	8.5	6.2
Chlamydomonas sp. (small)	72447856	3438.7	7.4	3.5
Planktosphaeria spp.	4814073	4464.6	12.3	12.0
Sphaerellopsis spp.	10180581	2860.6	9.8	7.4
Ulothrix spp. (*Klebsormidium* ?)	78919	11.0	7.4	4.9
Unidentified green spp. (thick-wall)	89020896	35360.4	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	34882300	1772.0	5.5	4.2
Total	**247490713**	**54077.5**		
Chrysophyta				
Ochromonas spp.	8523277	442.8	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	25254154	1312.1	4.9	4.5
Total	**33777431**	**1754.9**		
Overall Total	**285845459**	**55892.5**		

Table B-15 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 8 (P-Load 7.59 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	23833608	313.1	3.2	2.8
Total	**23833608**	**313.1**		
Chlorophyta				
Chlamydomonas sp. (large)	42932064	10463.2	8.5	7.4
Chlamydomonas sp. (small)	99477696	4721.6	7.4	3.5
Planktosphaeria spp.	10101662	9368.3	12.3	12
Sphaerellopsis spp.	21110894	5931.9	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	12942754	312.3	4.5	3.2
Unidentified green spp. (thick-wall)	133057824	52852.6	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	61714840	6060.5	6.2	5.5
Total	**381337734**	**89710.3**		
Chrysophyta				
Ochromonas spp.	13100593	680.6	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	86495480	4493.8	4.9	4.5
Total	**99596073**	**5174.4**		
Overall Total	**504767415**	**95197.8**		

Table B-16 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 3 (P-Load 9.75 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	16099523	211.5	3.2	2.8
Total	**16099523**	**211.5**		
Chlorophyta				
Chlamydomonas sp. (large)	85864128	13757.3	8.5	6.0
Chlamydomonas sp. (small)	175200704	8315.8	7.4	3.5
Planktosphaeria spp.	8838954	8197.2	12.3	12.0
Sphaerellopsis spp.	26832540	7539.6	9.8	7.4
Unidentified green spp. (thick-wall)	136688112	56304.5	11.7	8.2
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	78958696	2785.5	5.5	3.5
Total	**512383134**	**96899.8**		
Chrysophyta				
Ochromonas spp.	21466032	1115.2	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	36618524	1941.3	5.0	4.5
Total	**58084556**	**3056.6**		
Overall Total	**586567213**	**100167.9**		

Table B-17 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 9 (P-Load 12.00 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	11916804	156.5	3.2	2.8
Total	**11916804**	**156.5**		
Chlorophyta				
Chlamydomonas sp. (large)	14521139	3539.0	8.5	7.4
Chlamydomonas sp. (small)	41353676	1962.8	7.4	3.5
Planktosphaeria spp.	25569832	23713.4	12.3	12
Sphaerellopsis spp.	21150354	5943.0	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	8996793	217.1	4.5	3.2
Unidentified green spp. (thick-wall)	108750704	43197.4	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	38986100	4257.5	6.2	5.8
Total	**259328598**	**82830.3**		
Chrysophyta				
Ochromonas spp.	8720575	453.1	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	28568762	1484.3	4.9	4.5
Total	**37289337**	**1937.3**		
Overall Total	**308534739**	**84924.1**		

Table B-18 Phytoplankton Taxon, Density and Biomass observed in the Sediment trap of Mesocosm 7 (P-Load 13.34 g) throughout the summer of 2003.

TAXON	DENSITY (cells L^{-1})	BIOMASS ($\mu g\ L^{-1}$)	CELL DIMENSIONS Length (μm)	CELL DIMENSIONS Width (μm)
Cyanobacteria				
Unidentified cyanobacteria spp. (*cf. Synechocystis, Synechococcus*)	12114102	159.1	3.2	2.8
Total	**12114102**	**159.1**		
Chlorophyta				
Chlamydomonas sp. (large)	7694625	1875.3	8.5	7.4
Chlamydomonas sp. (small)	22255224	1056.3	7.4	3.5
Planktosphaeria spp.	5208669	4830.5	12.3	12
Sphaerellopsis spp.	10535718	2960.4	9.8	7.4
Unidentified green flagellate (cf. *Scourfieldia*)	3590825	86.6	4.5	3.2
Unidentified green spp. (thick-wall)	44668284	17742.9	10.5	8.5
Unidentified green spp. (*cf. Chlorella,* small spp. with lost flagella)	17993586	1965.0	6.2	5.8
Total	**111946931**	**30517.1**		
Chrysophyta				
Ochromonas spp.	10417339	541.2	4.9	4.5
Unidentified chrysophyte spp. (*cf.* monads, broken colonies)	16573039	861.0	4.9	4.5
Total	**26990378**	**1402.3**		
Overall Total	**151051411**	**32078.5**		