Algal growth in Faro Mine pit lake water

A Project Report submitted to:

SRK Consulting

Prepared by:

Microbial Technologies, Inc.

Contact:

Dr. André Sobolewski
Microbial Technologies, Inc.
1057 Roberts Creek Road, Unit 21
P.O. Box 193
Roberts Creek, B.C.
Canada VON 2W0

Phone: (604) 885-0568
Email: andre@microbialtech.com

November 2004

Executive Summary

Microbial Technologies, Inc. investigated the effects of fertilizers on algal growth in waters from the Faro, Grum, and Vangorda pit lakes. This laboratory study complemented a field trial in the Grum pit to evaluate the removal of zinc by induced algal blooms.

Algae were grown in water from all the pit lakes, even in Vangorda water which has high zinc concentrations. When fertilized, waters from each of these pit lakes was found to sustain a unique algal population. In addition, algae were found to grow on sediments from these pit lakes. However, they always grew attached to these sediments.

Dilution tests showed that elevated zinc concentrations slowed down growth and limited overall yield, but did not prevent algal growth.

Algae grown from an ice sample retrieved from the Grum pit were grown to a high density and were used to inoculate water from each of the pit lakes. Tests were conducted without fertilizer, a low fertilizer dose (1 mg/L ammonia-nitrogen and 0.1 mg/L phosphate-phosphorus), or a high fertilizer dose (5 mg/L ammonia-nitrogen and 0.5 mg/L phosphate-phosphorus). A duplicate test of Grum water was fertilized with fish fertilizer instead of chemical fertilizer.

The high dose fish fertilizer treatment supported the most rapid algal growth in Grum water. Algal growth appeared within nine days. The high fertilizer treatment in Faro water also produced rapid, but not as luxuriant algal growth. Most of the other fertilizer treatments also supported algal growth, but growth was more limited. While adding ammonia-nitrogen to 5.0 mg/L may accelerate production of an algal bloom, its concentration should be maintained at 2.0 mg/L to sustain good algal growth.

A phenomenon of “cell clumping” was observed in Grum and Faro high fertilizer treatments. Planktonic (free-floating) algae that grew to high densities formed clumps that settled from the water column. However, the remaining planktonic algae eventually grew back in the water column.

Algal blooms did not alter water pH significantly. They appeared to increase nitrate concentrations in water, but this was not a very significant effect. Although zinc concentrations decreased in pit lake waters during the study, this effect could not be conclusively attributed to algal blooms.

Table of Contents

List of Tables

List of Figures

1 Introduction

An updated mine reclamation plan is being developed for the Faro Mine complex , with a view to have a workable closure plan by 2006. This reclamation plan includes detoxification and reclamation of the open pits and development of pit lakes in the Faro, Grum, and Vangorda pits.

Previous studies have shown that algae may remove toxic metals from surface waters (Hrycenko and Sobolewski, 1999; Pelletier *et al.*, 2002; Crosius *et al.*, 2002). Another study at the Vangorda Mine suggested that algae may be grown in pit lakes at the mine, despite elevated zinc concentrations (Sobolewski, 2003). Thus, the concept of using algae to remove toxic metals has been proposed, and this was recently identified as a possible water management option for the Grum pit (Gartner-Lee, 2003). However, the results to date can only be considered tentative, and a more thorough evaluation of this concept is necessary.

The laboratory study was conducted as a precursor to a more comprehensive evaluation of this treatment alternative. It assessed the fertilizer requirements to stimulate and support algal growth in waters from the Faro, Grum, and Vangorda pit lakes and examines the potential toxicity of these waters to algae. Finally, the study intended to determine if induced algal blooms remove zinc from these waters.

The study was divided in several tasks. In Task 1, an algal inoculum was developed for use in subsequent tasks. In Task 2, the “toxicity threshold” for pit lake waters was determined. This test determined what dilution of pit lake water may be necessary to obtain observable algal growth. In Task 3, the effects of different doses and types of fertilizer on algal growth and zinc concentrations were determined.

.

2 Materials and Methods

2.1 Sample collection

Water samples collected by Laberge Environmental Services from the Faro, Grum, and Vangorda pit lakes were shipped to Microbial Technologies on May 14, 2004. Nine collapsible 22 L (5 gal) containers were retrieved on May 15, and stored in the cold until May 17, when the study was started. Separately, sediment samples were collected from the near-shore of each pit lake and ice with green algae was collected from the Grum pit. These were also received on May 15. The sediment samples were stored in the cold until used in the study. The thawed ice samples was exposed to sunlight until subsequent use in the study.

2.2 Experimental Set-up

To grow algae for an inoculum used in subsequent tasks, 100 mL of the Grum ice, Grum 1m, Faro 5m and Vangorda 5 m water samples were fertilized to 10 mg/L NH_3-N using $(NH_4)_2SO_4$ and 1 mg/L PO_4-P using Na_2HPO_4 and placed in baffled shake flasks. These were shaken at 120 rpm on an orbital shaker at room temperature under strong full-spectrum illumination (Figure 1). In addition, 100 mL of Grum 1m, Faro 5m and Vangorda 5 m water samples and 20 g of their corresponding sediment samples were fertilized and incubated as above.

Figure 1. Algal cultures in shake flasks on orbital shaker.

Once algae grew in the Grum ice sample, they were used to inoculate test tubes for the toxicity threshold and plastic containers for the fertilizer tests.

For the toxicity threshold test, water from the Faro 5m, Grum 5m, and Vangorda 5m samples were serially diluted To this end, a volume of pit lake water was mixed with an equal volume of dilution

water, resulting in a two-fold diluted subsample. Part of this subsample was used for the toxicity threshold test, the remainder was diluted again. Part of the resulting diluted subsample was used for the toxicity threshold test, the remainder was diluted again, until a series of four two-fold dilution is obtained.

For the above dilutions, purified water was supplemented with 2.25 g/L $CaSO_4.2H_2O$, producing an effective Ca^{+2} concentration of 130 mg/L. The pH of this dilution water was adjusted to 6.9 before use.

After all the dilution series were prepared for all the pit lake waters, fertilizer was added to every sample, bringing ammonia-N concentrations to 10 mg/L using $(NH_4)_2SO_4$ and phosphate-P to 1 mg/L using Na_2HPO_4. Each fertilized 10 mL sample was dispensed in a sterile screw-cap test tube, inoculated with 0.1 mL of the grown Grum ice sample and incubated flat on an orbital shaker under full-spectrum illumination (See Figure 5 for photograph of all the test tubes).

Mine water from the 22-liter containers was distributed evenly among eleven 20-liter plastic pails. Samples collected at 5 and 40 m were mixed before being distributed to each tank. The tanks were kept under high illumination at room temperature, aerated, and covered with clear plastic film to minimize evaporation. During the study, the temperature ranged from 20-26 ºC, the light source consisted of several wide spectrum fluorescent tubes for plants and aquariums on a 16/8 hour photoperiod, and aeration in each tank was provided through one 4 inch air rock attached to a Maxima 2.5 psi aquarium air pump. The air flow was restricted with a valve so that every container received approximately the same, gentle bubbling from the air stone.

Water from the Vangorda pit lake contained orange suspended particulates, likely iron oxyhydroxides formed after collection. Since they can adsorb zinc, it was important to remove them from the water before starting the test. Most of these particulates were removed by decanting Vangorda water after they had settled to the bottom of the plastic containers. The decant was returned to the containers and topped up with fresh Vangorda water. A single decant was sufficient to remove virtually all the orange particulates, with only faint traces left. Subsequent chemical analysis showed that zinc concentrations in these samples remained very high.

Each of the filled plastic containers received 4.0 mL of the Grum ice algal inoculum. In addition, some water samples received fertilizer according to the addition rates shown in Table 1. Ammonium sulphate $[(NH_4)_2SO_4]$ was used to supply nitrogen and phosphoric acid (H_3PO_4) was used to supply phosphorus to the chemically-fertilized samples. Alaska Fish Fertilizer™ was used for the Grum fish fertilizer treatment.

Table 1. Treatments during the algal growth study.

Treatment	Name	Algal inoculum	Amount N (mg/L)	Amount P (mg/L)
Controls	Ctrl	4.0 mL	-	-
Faro	low NP	4.0 mL	1	0.1
	high NP	4.0 mL	5	0.5
Vangorda	low NP	4.0 mL	1	0.1
	high NP	4.0 mL	5	0.5
Grum chemical	low NP	4.0 mL	1	0.1
	high NP	4.0 mL	5	0.5
Grum fish fertilizer	low FF	4.0 mL	1	0.1
	high FF	4.0 mL	5	0.5

2.3 Measurements

The instruments and techniques used during the study are listed in Table 2. Other chemical analyses (metals, Chlorophyll "a") were performed by a contract laboratory (ALS Environmental, Vancouver, BC). These analyses were done on cooled, unpreserved 50 mL samples that were filtered immediately upon receipt at ALS. The filter cake was analyzed for Chlorophyll "a", whereas the filtrate was preserved with nitric acid and analyzed for metals by ICP.

Table 2. Types of measurements taken and instruments used in study.

Measurement	Instruments Used
pH	VWR Model SP21 portable pH/ISE meter with pH electrode.
Conductivity	HANNA 8733 Conductivity meter.
Dissolved Oxygen	ORION model 810 with DO probe.
Ammonium	ORION model 290A with 95-12 ammonia probe.
Nitrate	GENESYS 6 Spectrophotometer with NitraVer 5 reagent kit.
Phosphate	GENESYS 6 Spectrophotometer with PhosVer 3 reagent kit.

2.3.1 pH

Solution pH was measured using a calibrated pH electrode. The pH meter was calibrated every week before taking readings using standard solutions of pH = 7.01 and pH = 4.00.

2.3.2 Conductivity

Solution conductivity was measured using a calibrated conductivity electrode. The conductivity meter was calibrated at the beginning of the study using a 10.00 mS conductivity standard.

2.3.3 Dissolved Oxygen

Dissolved oxygen concentrations were measured using an oxygen-specific electrode. The dissolved oxygen meter was calibrated immediately before taking readings. In addition, every week new filling solution was added to the probe.

2.3.4 Ammonia

Ammonia-nitrogen concentrations were measured using an ammonia-specific electrode. The ammonia electrode was calibrated throughout the study using 0.0, 1.0, 3.0, and 10 mg/L NH_3-N using an ammonium chloride standard solution. Measurements in mV were taken after adding 1 ml of ammonia pH adjusting solution to 50 mL of each standard. The mV readings for each of the standards were plotted to generate a calibration curve. The curve was subsequently used to determine mg/L ammonia from mV readings taken in this study. The electrode was recalibrated five times during the study.

Ammonia concentrations were determined by taking a 50 mL sample from each tank and adding 1 ml of ammonium pH adjusting solution. The solution was mixed with a magnetic stir bar and a measurement, in mV, was taken using the ammonium probe. The mV readings were converted into mg/L of ammonia nitrogen by using the calibration curve.

2.3.5 Nitrate

Nitrates were measured by the cadmium reduction method (APHA, 1995), using a reagent kit supplied by Hach Inc.. The Spectrophotometer was calibrated at the beginning of this study using standard concentrations of sodium nitrate. A NitraVer 5 reagent pillow was added to 10 mL of each standard or sample, mixed with a vortex mixer and allowed to stand for 10 minutes before absorbance

was measured at 500 nm. Absorbance was converted to concentrations using the above calibration curve.

2.3.6 Phosphate

Phosphate was measured by the ascorbic acid method (APHA, 1995), using a reagent kit supplied by Hach Inc.. The Spectrophotometer was calibrated at the beginning of this study using standard concentrations of potassium phosphate. A PhosVer 3 reagent pillow was added to 10 mL of each standard or sample, mixed with a vortex mixer and allowed to stand for 10 minutes before absorbance was measured at 890 nm. Absorbance was converted to concentrations using the above calibration curve.

2.3.7 QA/QC

On Week 2 of the study, samples from each container were collected and shipped to ALS for nitrate and phosphate analysis. Duplicate samples were analyzed at Microbial using the Hach reagent kits. The two sets of results are compared in Table 3.

Table 3. Comparison of analytical results from ALS with those from Microbial.

Sample ID	1	2	3	4	5	6	7	8	9	10	11
NO_3-N ALS	0.19	0.080	0.71	0.18	0.19	0.086	0.098	-	1.0	1.0	0.93
Microbial	1.4	0.29	0.29	0.43	2.3	0.43	0.57	0.43	0.86	1.1	8.7
PO_4-P ALS	0.007	0.0051	0.011	0.0056	0.014	0.0038	0.0034	-	0.0081	0.0074	0.061
Microbial	0.0093	0.0046	0.0046	0.13	0.30	0.44	4.5	0.097	0.43	0.18	0.60

The Microbial results were generally higher than the ALS data and their correlation was relatively poor.

2.3.8 Cell Counts

Algal cells were counted under transmission microscopy at 400X magnification on a Zeiss Standard microscope using a Petroff-Haueser counting chamber. At the beginning of the study, cells with distinct morphologies were examined at 400X and at 1,000X magnification for unambiguous identification as an alga (presence of coloured pigments, chloroplasts, etc). Subsequently, cells with known morphologies were counted as algae.

2.3.9 Secchi Depth

A conventional method for assessing algal density in a lake is to measure the "Secchi Depth". In this application, a Secchi disk[1] is lowered into the water of a lake until its alternating black and white quadrants are no longer distinct. This depth of disappearance, called the Secchi depth, is a measure of the ***transparency*** of the water. Transparency decreases as water color, suspended sediments, or algal abundance increases. In this study, this technique was adapted to measure algal density (Figure 2).

[1] An 8-inch (20 cm) disk with alternating black and white quadrants

Figure 2. Secchi depth measurement for algal cultures.

A sectored disk was placed at the end of a ruler, and this ruler was lowered until the sectors could no longer be distinguished. This was designated as the Secchi depth.

3 Results

3.1 Initial Sample Characterisation

Water samples collected by Laberge Environmental were analyzed for a complete suite of parameters (See Appendix I). Key parameters that were measured throughout the study are presented in Table 4.

Table 4. Key parameters measured at the outset of the study.

Sample	pH	Cond	NH_3-N	P[1]	Zn[1]	Chl "a"
		µS	mg/L	mg/L	mg/L	µg/L
Faro 5m	7.17	1220	1.70	<0.01	12.3	2
Faro 40 m	6.64	1395	1.94	0.01	2.90	n/a
Grum 5m	7.39	1070	<0.05	0.01	12.9	<1
Grum 40m	7.46	1035	<0.05	0.05	13.1	<1
Vangorda 5m	5.97	1930	0.90	<0.01	116	<1
Vangorda 40m	5.96	2000	0.94	0.02	119	<1

[1] Expressed as Total Metals. Differences between dissolved and total were negligible.

The water analysis shows there are a few differences between water from each pit lake. Vangorda water is slightly acidic, whereas Faro and Grum waters are circumneutral. Vangorda water has much higher conductivity and zinc concentrations. The zinc concentration is 116 mg/L in the 5m Vangorda sample compared with 12.3 and 12.9 mg/L for Faro and Grum, respectively.

Nutrient concentrations are low in all the water samples, except for ammonia, with concentrations around 1 mg/L in both Faro and Vangorda water. Chlorophyll "a" concentrations are also very low in all the pit lakes.

3.2 Task 1: Inoculum Development

A sample of ice apparently containing green algae was collected at the surface of the Grum pit lake. This sample was placed in a shake flask, fertilized with 10 mg/L NH_3-N and 1 mg/L P, and incubated at room temperature under illumination. Water samples from Faro, Grum, and Vangorda pit lakes were similarly fertilized and incubated. A duplicate set of pit lake sample received sediments collected in shallow near-shore areas.

Each of the above samples grew algae, however, the onset of visible algal growth, as well as the type of algae that grew differed markedly (Figure 3).

Figure 3. Algal growth in various water samples. Top row: Grum ice sample (left), Vangorda water (middle), and Grum water with sediments. Bottom row: Faro and Grum water (left), and Faro water with sediments.

Growth in the Grum ice sample was first seen 2-3 weeks after fertilization, as algae attached to the bottom of the shake flask. However, there was distinct planktonic (free-floating) growth a week later (June 14). By their appearance in the microscope, these comprised a mix of Chlorella or Euglena. In addition, there was visible growth on the surface of sediments from the Grum and Faro samples. The latter algae were filamentous and remained attached to the sediments for the entire three months of the lab study, never producing visible planktonic growth.

Planktonic growth appeared later in the fertilized Vangorda (Week 5), Faro (Week 6-7), and Grum (> 2 months) pit lake water samples. The algae in each of these samples were distinctly different. Vangorda algae were green-brown (possibly diatoms), Faro algae were bright green, whereas the Grum algae were blue-greens (See Figure 3). No attempt was made at taxonomic identification, as this was beyond the scope of the project.

3.3 Task 2: Toxicity Threshold

This test was conducted to determine if the pit lake waters are toxic to algal growth. It is not a toxicity test *per se*, but rather a test to determine the effect of dilution on algal growth. Water from each pit lake was fertilized, inoculated with algae from the Grum ice sample, and incubated at full-strength, half-strength, quarter-strength, and so on.

Algae were found to grow in water from every pit lake, even in undiluted water. However, observable growth occurred sooner in the more diluted waters. The rate of growth was somewhat difficult to quantify because much of the early growth was of algae attached onto the inner surface of the glass test tubes (Figure 4, taken one week after inoculation).

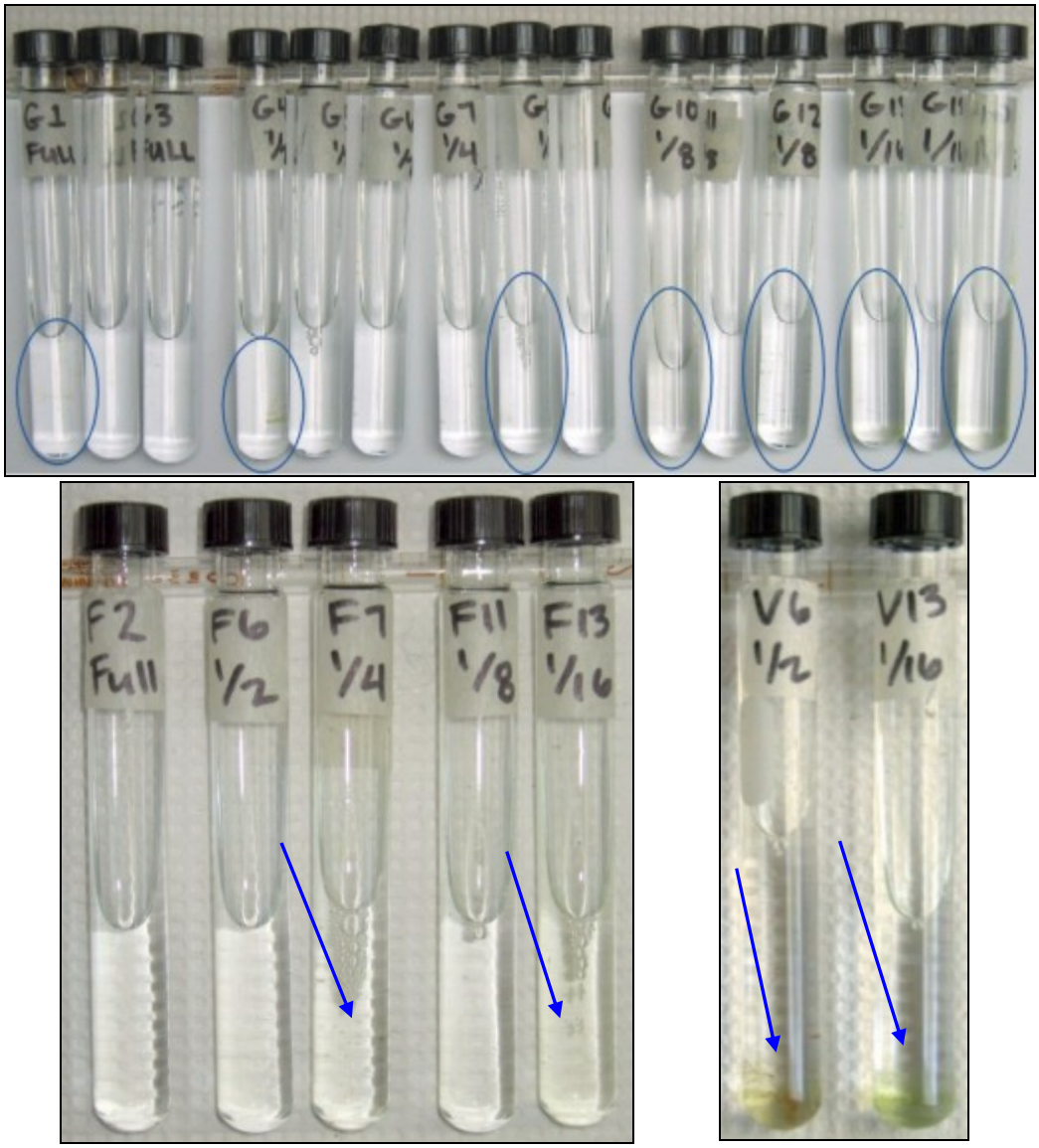


Figure 4. First algal growth in toxicity threshold test. Top: Grum water (tubes with growth are circled). Bottom left: Faro water. Bottom right: Vangorda water. Dilutions are indicated on labels.

Algal numbers were counted at the end of the test (Table 5). On average, all the full-strength water samples had lower cell numbers than diluted water. For the Grum sample, there were five times fewer cells in full-strength water compared with diluted water. Moreover, diluted Grum water averaged nearly twice more cells (8.8×10^6) compared with diluted Faro (4.9×10^6) and diluted Vangorda (4.6×10^6) waters.

However, these results do not reflect all growth because attached algae could not be counted. While, planktonic algae predominated in Faro water, attached algae predominated in Grum and Vangorda waters (Figure 5). Thus, the cell counts results underestimate the differences between Faro, Grum and Vangorda waters. Still, it is clear that algae can grow in full-strength pit lake water, albeit slower than in diluted water.

Figure 5. Algal growth at the end of toxicity threshold test. Note mix of attached and planktonic growth.

Table 5. Counts of planktonic algae at the end of the toxicity threshold test.

	Cell Count (Cells/mL)		
Dilution	Faro	Grum	Vangorda
Full-strength	3.0×10^6	1.2×10^6	2.0×10^6
Half	6.5×10^6	1.1×10^7	3.5×10^6
Quarter	5.3×10^6	8.5×10^6	4.0×10^6
Eighth	2.7×10^6	7.3×10^6	4.5×10^6
Sixteenth	5.1×10^6	8.5×10^6	6.5×10^6
Average of diluted samples	4.9×10^6	8.8×10^6	4.6×10^6

3.4 Task 3: Algal Growth in Fertilized Pit Lake Water

Water from each pit lake was fertilized with high and low fertilizer doses according to the regime shown in Table 1. Algal growth and water chemistry were measured in waters from the pit lake throughout the six-week incubation. Parameters measured routinely during the study included:

- pH
- Temperature
- Conductivity
- Dissolved oxygen
- Ammonia nitrogen
- Nitrate
- Phosphate
- Dissolved zinc concentrations

3.4.1 Water Chemistry

3.4.1.1 Water pH

Except for Faro high fertilizer, the changes in pH in all the fertilizer treatments were comparable with those in the non-fertilized controls (Figure 6). Water pH in the Vangorda water remained between 7.1 and 7.4 throughout the study, that in the Grum treatments remained at approximately 8.5, whereas water pH in the Faro treatments remained between 8.0 and 8.5.

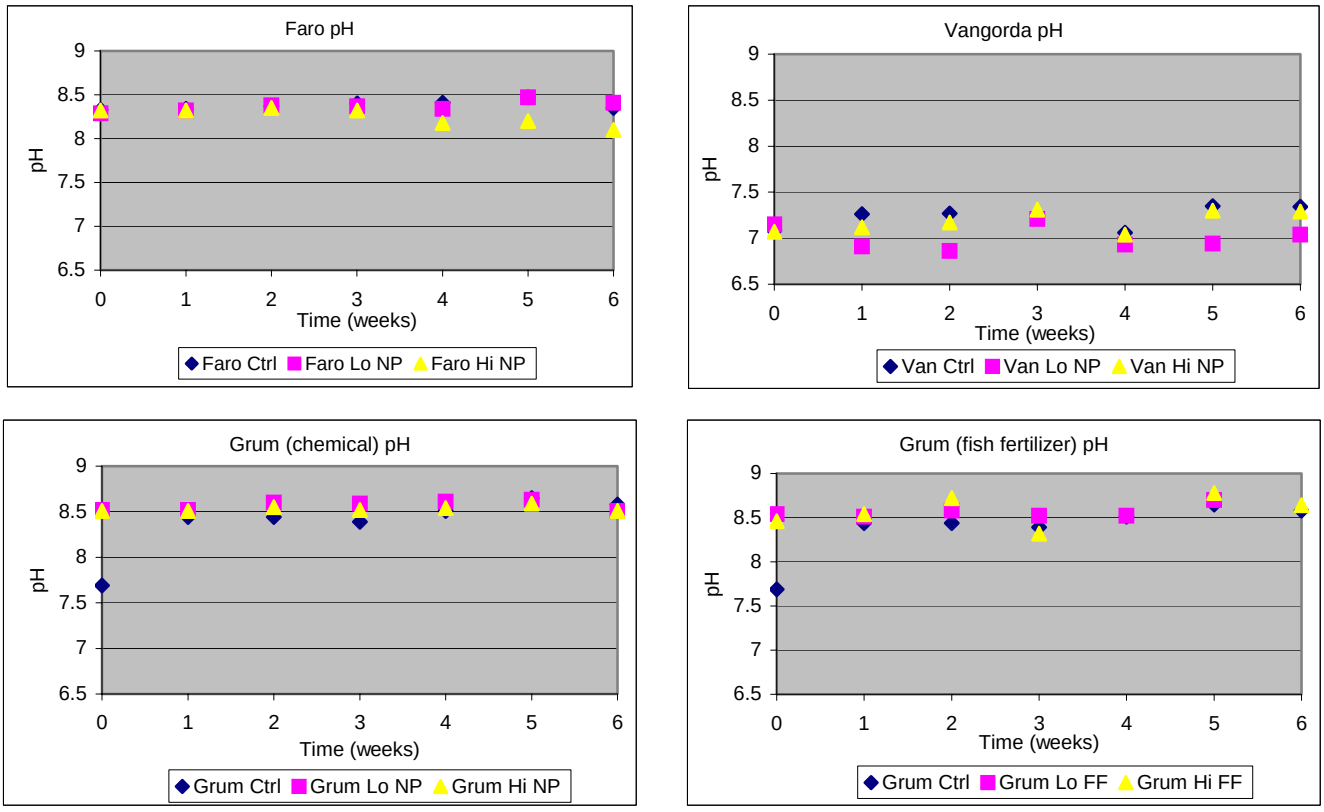


Figure 6. Water pH in Faro, Grum, and Vangorda fertilized water.

3.4.1.2 Temperature

Water temperature ranged from 20.4 to 26.9 ºC and averaged 24.3 ºC during the entire study. No single treatment was warmer of cooler than the others, indicating there was no bias introduced by the position of the containers during the six week incubation.

3.4.1.3 Conductivity

Conductivity varied somewhat during the study due to evaporative water losses and water replenishment (Figure 7).

Grum water had the lowest conductivity, ranging from 1017 to 1393 µS. Conductivity in the chemical fertilizer treatment averaged 1251 µS, whereas the fish fertilizer treatment averaged 1147 µS, a relatively insignificant difference.

Faro water had a conductivity averaging 1344 µS, ranging from 1119 to 1503 µS. Water was replenished in the high fertilizer treatment on Weeks 4 and 6, and in the Control incubation on Week 6, resulting in marked decreases in conductivity.

Vangorda water had the highest conductivity during the study, averaging 2122 µS and ranging from 1907 to 2500 µS. Conductivity increased steadily in all the treatments during the study, apparently from high evaporative losses. Water was replenished in the low fertilizer treatment on Weeks 4 and 6, and in the high fertilizer incubation on Week 6, resulting in marked decreases in conductivity.

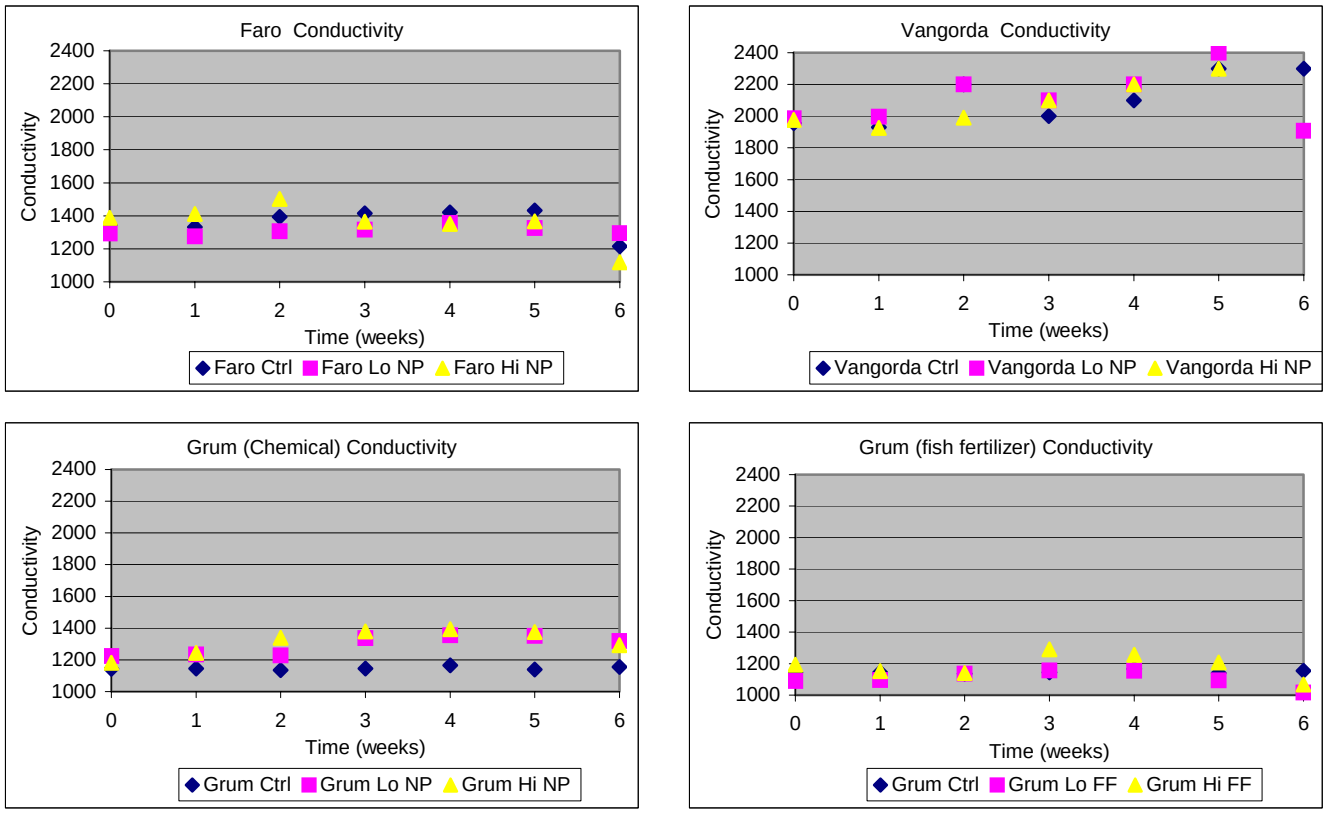


Figure 7. Conductivity in Faro, Grum, and Vangorda fertilized water.

3.4.1.4 Dissolved Oxygen

Dissolved oxygen remained fairly constant between 6.0 and 8.0 mg/L during the entire study, averaging 6.76 ± 0.080 mg/L. This reflects the good aeration provided during the study as well as the production of oxygen by photosynthetic algae. Given its constancy, dissolved oxygen measurement were discontinued after Week 4.

3.4.1.5 Ammonia

Ammonia is the preferred source of nitrogen for algae, hence its use in this study. Ammonia concentrations typically decreased in treatments where algal growth was significant (Figure 8). Thus, ammonia concentrations decreased gradually in the Faro high and low NP, Vangorda low NP, and Grum high and low NP and high and low FF treatments, where algae grew well (See Table 6). In contrast, ammonia concentrations remained low and constant in all the Control incubations.

The Faro high NP treatment had a gradual decrease in ammonia concentrations, starting from 6.8 mg/L to approximately 0.5 mg/L by Week 4. The ammonia decrease was more muted in the Faro low NP treatment, but the trend was similar.

Ammonia concentrations remained elevated in the Vangorda high NP treatment. However, it followed the same pattern of gradual decrease in the low NP treatment, starting from approximately 2 mg/L to less than 0.1 mg/L by the end of the study.

Ammonia concentrations decreased rapidly in all the Grum treatments (Figure 8). This was most rapid in the Grum high FF, decreasing from initial concentrations of approximately 5 mg/L to approximately 1mg/L by Week 2. More fertilizer was added by Week 3, but its concentrations

continued to decrease rapidly thereafter. In the low NP and low FF treatments, ammonia also decreased rapidly, down to approximately 0.1 mg/L by Week 2.

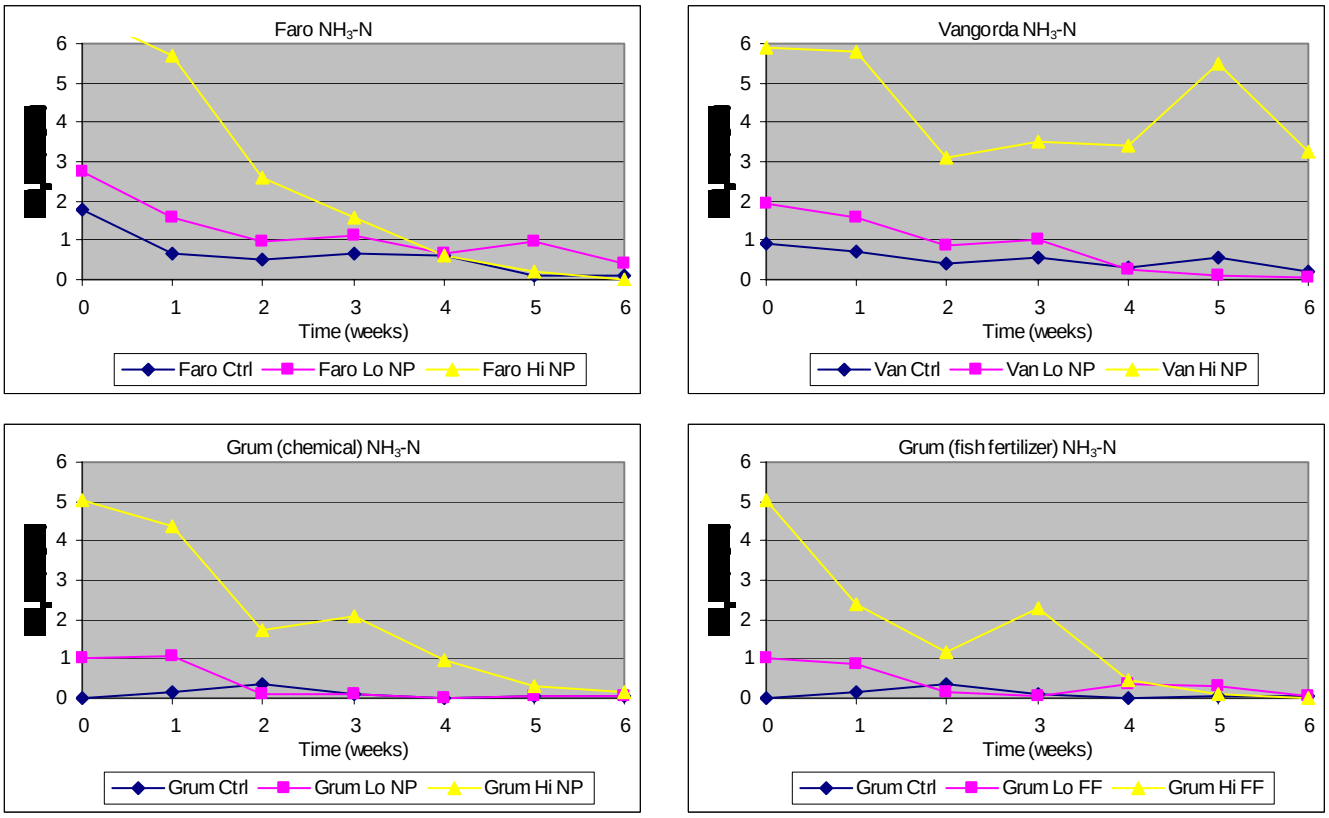


Figure 8. Ammonia-nitrogen in Faro, Grum, and Vangorda fertilized water.

3.4.1.6 Nitrate

Nitrate-nitrogen can be used as a nitrogen source in the absence of ammonia. It is also produced when algae die and accumulate at the bottom (of lakes or our containers). This only occurs to a measurable extent when algal biomass is high, as we observed in previous laboratory studies. Finally, it can be produced by bacterial oxidation of ammonia (nitrification).

Nitrate exhibited an interesting pattern during this study (Figure 9). Nitrate-nitrogen was very low in all the treatments except for Faro high NP and Grum high fish fertilizer. The increase in nitrate concentrations in the Faro and Grum treatments coincide with periods of high algal biomass (Figure 18). Coincidentally, algae in these treatments also formed clumps at those times (See below). However, these results must be taken tentatively in light of the QA/QC results.

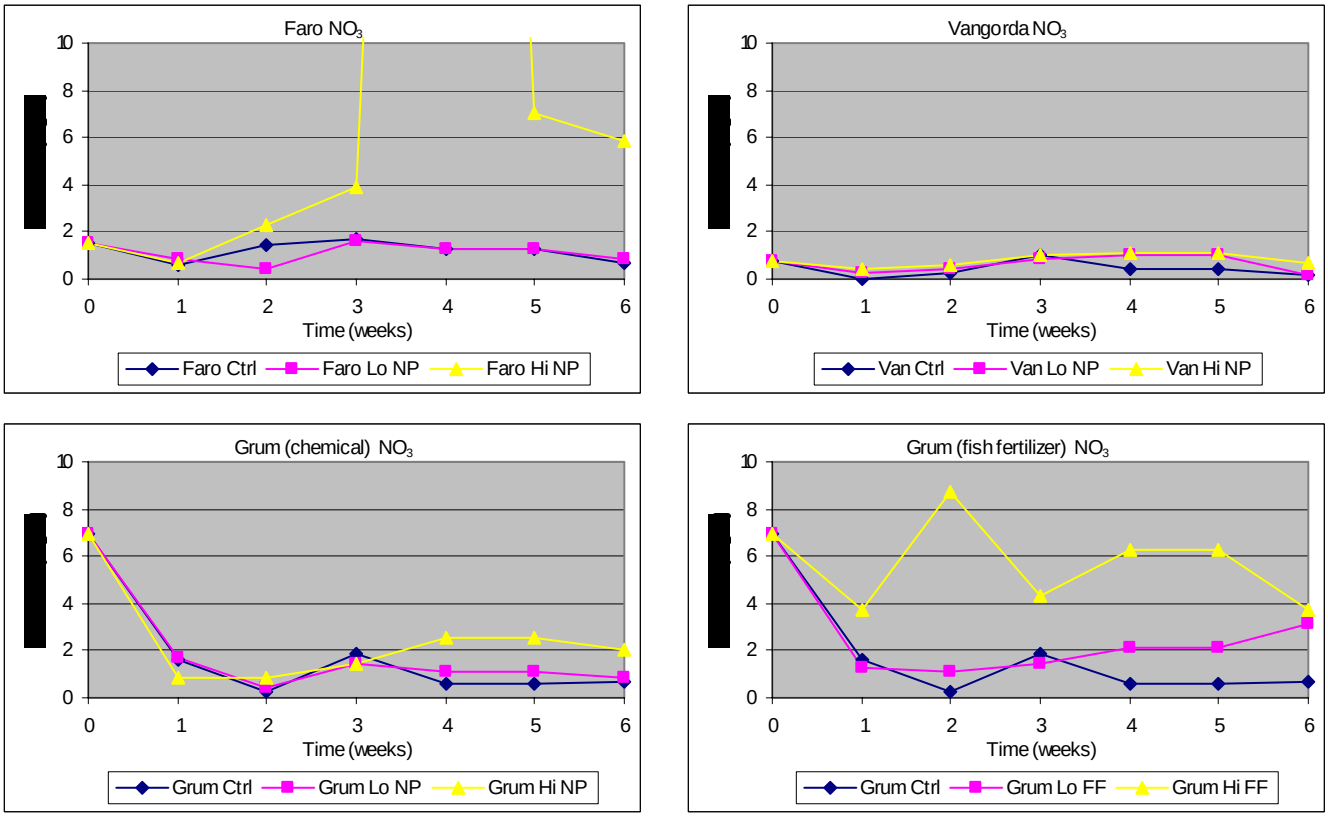


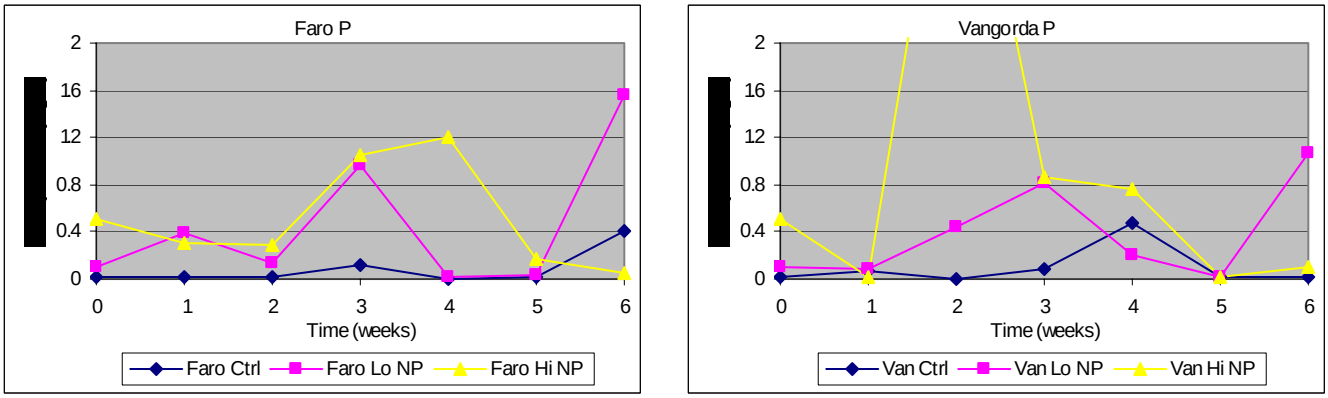
Figure 9. Nitrate-nitrogen in Faro, Grum, and Vangorda fertilized water.

3.4.1.7 Phosphate

Phosphate concentrations did not follow a consistent pattern during this study (Figure 10). Even in the Control incubations, phosphate concentrations were low for the Faro and Vangorda waters, but they were more erratic for the Grum Controls. It is possible that some substance in the Grum water interfered with the phosphate assay, given the discrepancies noted in QA/QC analysis.

Ignoring a few outliers, phosphate concentrations appeared to follow a decreasing trend in the Faro treatments. However, the Vangorda treatments did not show such a pattern.

Taken altogether, the results of these analyses are inconclusive.



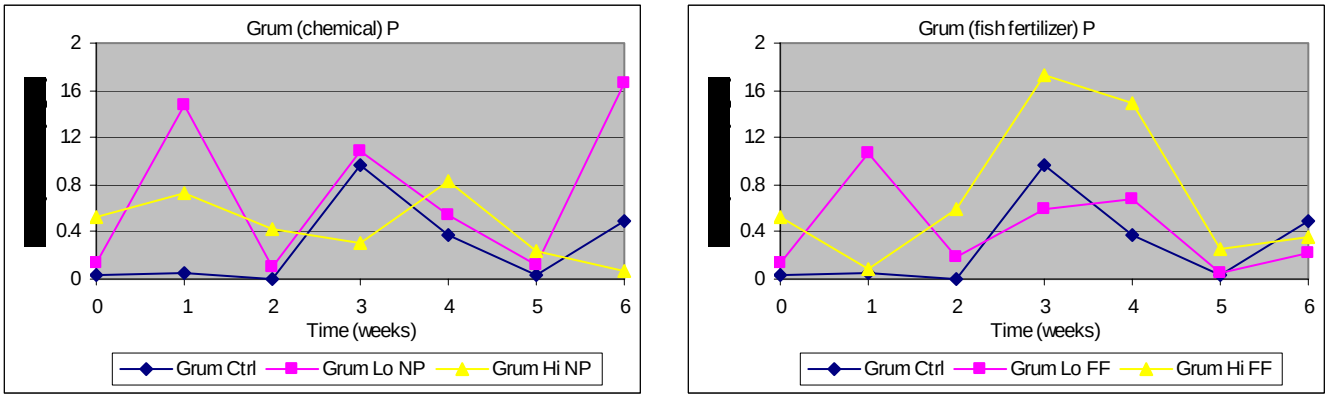


Figure 10. Soluble P in Faro, Grum, and Vangorda fertilized water.

3.4.1.8 Zinc

Zinc concentrations did not follow the expected pattern during the study. In every treatment, zinc concentrations decreased at the same rate as in the Control incubation (Figure 11). Moreover, the initial zinc concentrations in Grum and Vangorda waters were much higher than in any subsequent sampling. The Vangorda 5 and 40m samples averaged 117 mg/L zinc, whereas it concentrations two weeks later averaged 79 mg/L in the Control and fertilized treatments. Similarly, zinc concentrations in Grum water was measured at 12.9 mg/L initially, but by Week 2, it averaged 0.57 in the Control and fertilized treatments.

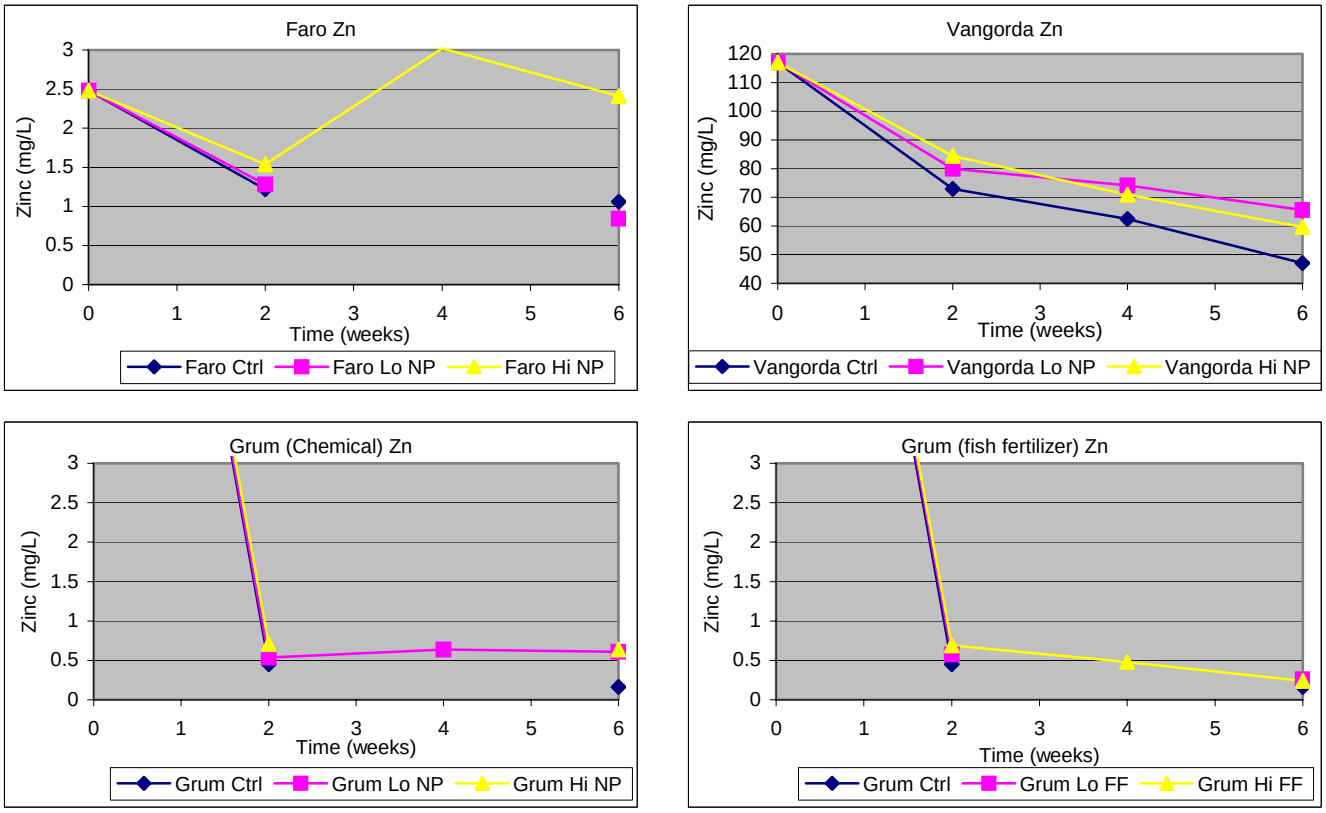


Figure 11. Zinc concentrations in Faro, Grum, and Vangorda fertilized water. Zinc concentrations in Grum water were 12.9 mg/L initially (off-scale on y-axis).

The fact that zinc concentrations decreased in all the treatments and in Control incubations suggests that its removal was independent of algal biomass. Nor was it dependent on water pH, since it decreased as well in alkaline water (Faro, Grum) as in neutral water (Vangorda). It is possible that it adsorbed to the walls of the containers used for the test[2], but there was no evidence for this. At present, there is no explanation for this observation.

3.4.2 Algal Growth

Algal growth was evaluated in four different ways:

- Visual observations
- Cell count
- Chlorophyll "a" density
- Secchi disk depth

The results of these different tests are presented below.

3.4.2.1 Visual Observations

Visually, there were obvious differences between the treatments, though the same algal inoculum was added to every fertilized water sample. None of the Control incubation exhibited any visible sign of algal growth during the test, whereas there was obvious green growth in the other treatments (Figure 12).

Figure 12. Photograph[3] of containers at end of test. White arrows point to Control incubations.

Within nine days, an algal bloom started to develop in the Grum high fish fertilizer treatment (Figure 13). By Week 2, water in this treatment was bright green (Figure 14). However, most of the algae in that treatment were clumped (See Figure 15), so that cell counts did not reveal the true extent of algal growth. Towards the end of the study, a second algal bloom developed, but much of the algal growth was also on the walls of the container, again preventing an accurate cell count (Figure 16, both for Grum high FF [left] and Vangorda low NP [right]).

[2] These are plastic containers, which should be unreactive towards metals.

[3] All the photographs were taken with the same camera settings, but lighting varies, affecting picture colour. As little as possible software compensation was applied to restore colour fidelity.

Figure 13. Algal growth in Grum high fish fertilizer treatment, far right. Taken on Day 9.

Figure 14. Algal growth in Grum high fish fertilizer treatment, far left, as well as in Faro high NP, far right. Taken on Day 15.

Figure 15. Algae growing as clumps in the Grum high fish fertilizer treatment.

Figure 16. Algae growing on wall of container in Grum high fish fertilizer treatment (White arrows). Taken on Week 6.

Algal growth was also visible in the Faro high fertilizer treatment by Day 15, and in most other treatments later on. Growth was mostly planktonic in these treatments (Figure 17).

Figure 17. Algal growth in Grum low fish fertilizer treatment. Notice the absence of growth on the walls of the container. Taken on Week 6.

Algal growth was first observed during the study at the times indicated in Table 6. These times do not correspond completely with cell counts (See below). These observations show that fertilization was required to produce observable algal growth. In addition, high fertilizer applications usually resulted in faster growth, except for the Grum chemical fertilization (Grum low NP faster than Grum high NP).

Table 6. Time of first observable algal growth.

Treatment	First observable algal growth
Faro Control	None
Grum Control	None
Vangorda Control	None
Faro low NP	None
Faro high NP	Day 9
Vangorda low NP	Day 30
Vangorda high NP	Day 15
Grum low NP	Day 15-18
Grum high NP	Day 21
Grum low FF	Day 25
Grum high FF	Day 9

3.4.2.2 Cell counts

Cell counts indicated the number of planktonic algae in a water sample. This number does not account for algae growing in clumps or on the container surface. However, these data reveal some of the population dynamics that occurred in response to the various fertilizer addition.

Unfertilized Faro water supported little planktonic growth (Figure 18). Similarly, Faro water receiving the low fertilizer dose only supported modest algal growth near the end of the study. In contrast, Faro water receiving the high fertilizer dose supported good algal growth throughout the study, with maximum cell counts of 1.6×10^6 cells/mL. A bloom develop by Week 1 and was sustained until Week 4. This bloom crashed by Week 5, but algal growth had resumed by the following week.

Algal growth was negligible in the Control and the high fertilizer treatment in Vangorda water. Cell counts never reached higher than 1.0 x 10^5 cells/mL. Algal growth was somewhat better in the low fertilizer treatment, but its onset was delayed until the end of the study, when cell numbers reached 1.8 x 10^6 cells/mL.

There was modest algal growth in the Grum chemically-fertilized water. Cell numbers started to increase after Week 3, peaking at around 10^6 cells/mL on Week 4 before declining by Week 5. However, this decline in planktonic cell numbers coincides with the appearance of algal growth on the container walls, suggesting that algae merely switched to an attached mode of growth.

Grum water receiving fish fertilizer produced the highest cell numbers in this study, with planktonic algae reaching 2.4 x 10^6 cells/mL by Week 1. Cell counts would suggest that this population crashed within a week, but visual observations show that they actually switched to an attached/clumped mode of growth (See Figure 15). However, the population of planktonic algae gradually increased again, exceeding its original high numbers by Week 6, when they reached 2.8 x 10^6 cells/mL.

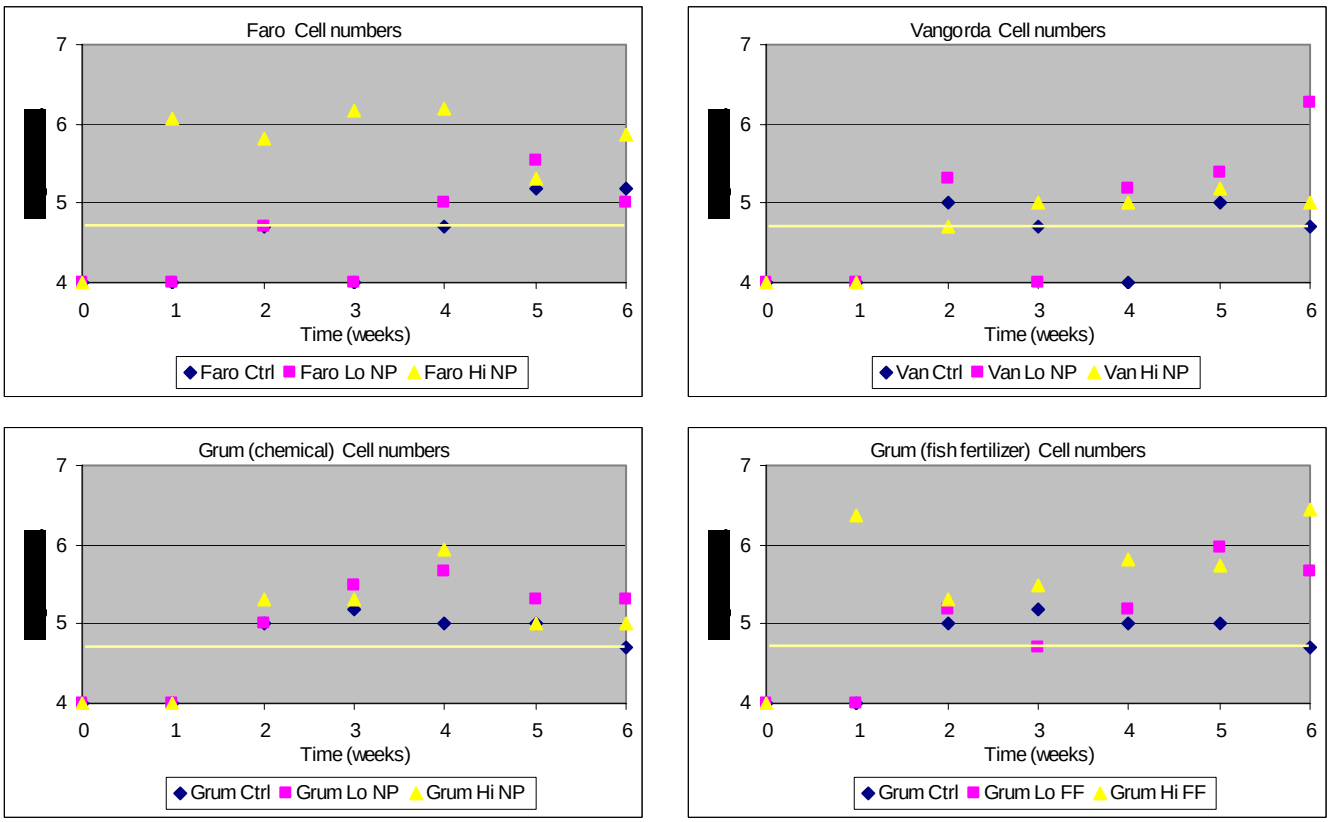


Figure 18. Cell numbers in Faro, Grum, and Vangorda fertilized water. Detection limits indicated by light yellow bar.

The above results indicate that algae growth began shortly after inoculation and fertilization in Faro and Grum waters, whereas it was delayed considerably or negligible in Vangorda water. This pattern coincides with that of zinc concentrations measured in these water. In addition, it appears that algal growth changed from a planktonic mode to an attached or clumped mode when their numbers exceed 1-2 x 10^6 cells/mL, as seen in the Faro high NP and Grum high FF treatments, and possibly in the Grum high NP treatment. In both the former cases, planktonic algae grew back shortly after their population decreased below 1 x 10^6 cells/mL.

3.4.2.3 Secchi depth

Secchi depth was only partly useful in measuring algal density. The Secchi depth for all the Controls, Grum (chemical) and Vangorda treatments, and in the Faro low NP, was greater than 10 inches, the depth of water in the containers used in this study. For the other treatments, the Secchi depth is shown in Figure 19.

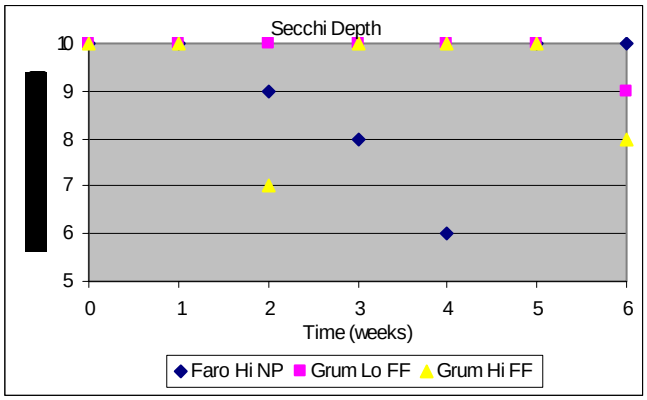


Figure 19. Secchi depth of treatments during algal growth study.

3.4.2.4 Chlorophyll "a"

Chlorophyll "a" is the pigment used by green algae and plants for photosynthesis[4]. Therefore, its concentration in water provides a direct quantitative measure of algal density. Chlorophyll "a" concentrations were measured on three occasions during the study to provide additional data from which to calculate algal biomass[5].

Its concentrations were high in the Faro and Grum (fish) high fertilizer treatments, but otherwise low in the other treatments (Figure 20). Interestingly, chlorophyll "a" concentrations increased steadily with time in the Faro high NP treatment, in parallel with the steady increase in Secchi depth (Figure 19), but despite an apparently *constant* cell number (Figure 18). Evidently, there *must* have been more algae in this treatment, but these were not being counted, since they were in clumps.

Similarly, chlorophyll "a" concentrations peaked on Week 2 in the Grum high FF treatment, but cell numbers were actually lower on Week 2 than on Week 1 (Figure 18)[6]. Again, this suggests that the cell counts underestimated the actual cell number, likely due to cell clumping.

Chlorophyll "a" concentrations in all the other treatments remained low during the study. They increased slightly in the Grum chemical fertilizer treatments, consistent with earlier observations (e.g., Table 6). Somehow, chlorophyll "a" concentrations never increased in the Vangorda high NP treatment, despite showing signs of algal growth early during the study. In contrast, chlorophyll "a" concentrations increased slightly at the end of the study in the Vangorda low NP treatment, consistent with other results (e.g., Table 6).

[4] There are other chlorophylls in different algae, but chlorophyll "a" is common to all.

[5] The algal biomass (ash-free dry weight) is estimated by multiplying the chlorophyll "a" content by a factor of 67.

[6] Cell counts on Week 4 are only slightly lower than on Week 1, but the chlorophyll "a" concentration for Week 4 is much lower, indicating that algal biomass had peaked on Week 2 – according to the chlorophyll "a" data, rather than on Week 1 – according to the cell count data.

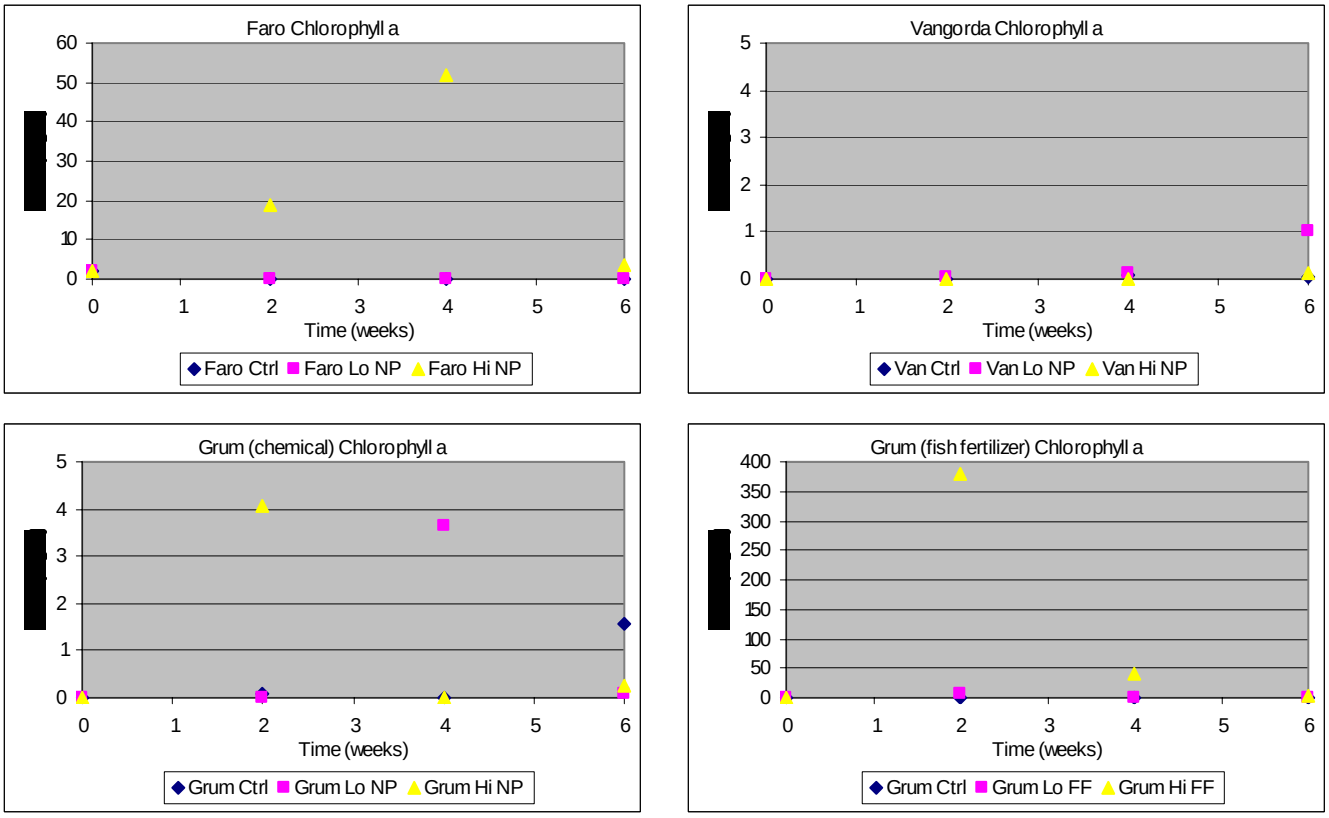


Figure 20. Chlorophyll "a" concentrations in Faro, Grum, and Vangorda waters during the study. Note the different scales in the above graphs.

4 Discussion and Recommendations

The initial analysis of water samples showed that Faro, Grum, and Vangorda pit lakes had limiting nutrient concentrations (Table 4). Faro water had some ammonia, but little phosphorus. Grum water had some phosphorus, but little ammonia, and Vangorda had little of either nutrient. Thus, fertilization would be expected to be necessary for growing algae in these waters

Algae from the Grum ice sample proved to be the best source of inoculum for this study, as algae grew very rapidly from this sample. However, every fertilized water sample grew distinct algae, indicating that algae were present in every pit lake water sample (Figure 3). Algae also grew from every sediment sample, but their growth was epiphytic, not planktonic. These facts suggest that each pit lake will develop its own algal population.

Algae grown from the Grum ice inoculum were used to determine potential toxic threshold concentrations in each pit lake water sample. Algae grew in every full-strength pit lake water sample. This was somewhat surprising at the time, since the Vangorda water had high zinc concentrations. It was clear that growth in dilute pit lake water (for all the pit lakes) was faster than in full-strength water. Thus, zinc was impairing growth, but did not prevent it. Of course, only zinc-resistant algae grew while zinc-sensitive species did not.

The above finding suggests that, in a pit lake, algal growth will be best immediately after snow melt, when water at the lake surface will be diluted. A remedial strategy may be to apply fertilizer directly over the ice before breakup.

Much of the algal growth in the toxicity threshold test was on the test tube walls (Figure 4). This caused us to underestimate the true extent of algal growth in this test and in the subsequent fertilizer test.

The fertilizer tests produced a number of interesting findings. First, the study demonstrated that 5 mg/L NH_3-N and 0.5 mg/L PO_4-P is adequate to produce an algal bloom in any of the pit lakes. The lower fertilizer dose did not produce the same level of growth.

While the fish fertilizer and chemical fertilizer both supported algal growth, there appeared to be a qualitative difference between them. The algal bloom resulting from the fish fertilizer seemed more luxuriant than that from the chemical fertilizer (See Figure 13 and Figure 14). This may be due to the presence of growth factors in fish fertilizer that enhance cell growth. If so, fish fertilizer may be preferable to chemical fertilizer to establish an initial algal bloom.

Both the Faro high NP and Grum high FF treatments produced good algal blooms early in the test. In both cases, there is evidence that cells formed clumps when they reached high densities. For the Grum high FF treatment, this was obvious visually (Figure 15) and was supported by an analysis of Secchi depth, cell counts, and chlorophyll “a”. These test results showed that, while cell counts peaked at Week 1, Secchi depth and chlorophyll “a” peaked at Week 2, when cell counts were low. Similar results were obtained with the Faro high NP treatment.

An interesting observation for the Faro high NP, and particularly the Grum high FF treatment, is that planktonic cell growth resumed towards the end of the six-week test (Figure 18). Thus, it may be that algal blooms go through cycles of planktonic growth to a high cell density, clumping and settling to reduce cell density, followed by another round of planktonic cell growth. This cycling appears to have been accelerated by the second addition of fish fertilizer in the Grum high FF treatment on Week 3.

Conceivably, an algal bloom induced in a pit lake may undergo several cycles of planktonic growth, clumping and settling during a growing season, if nutrients are not depleted from surface water. This

suggest a process for enhancing metal removal in surface waters. The cycling of planktonic-clumped-planktonic growth could be promoted by repeated fertilization. Assuming that metals are removed from surface waters when algae clump and settle away from the water column, this cycling process may accelerate metal removal. However, fertilizer dosage would have to be monitored to avoid excessive nitrogen and phosphorus concentrations.

The algal blooms that developed in all the treatments never reach such high levels as to affect general water chemistry significantly. Thus, water pH in all the treatments remained the same as in Controls. Somewhat surprisingly, there were also no obvious decreases in zinc concentration caused by these algal blooms, when compared with Controls. This is contrary to expectations and remains unexplained.

Algae appeared to use up ammonia-nitrogen quickly (Figure 8). This was most obvious in the Grum High FF treatment, where a second fertilizer application was made after NH_3-N concentrations had decreased from 5 mg/L to approximately 1 mg/L. Despite this second application on Week 3, the continued consumption of NH_3-N maintained its concentration to less than 1 mg/L.

Unfortunately, the corresponding critical concentration for phosphate could not be determined in this study because the test results were inconclusive.

Given the above findings, a number of recommendations follow for the treatment of a pit lake:

Fertilizer dose

The application of 5.0 mg/L NH_3-N and 0.5 mg/L PO4-P-P is adequate to initiate the development of algal blooms. After a bloom is established, NH_3-N concentrations should be maintained at 2 mg/L.

Fertilizer type

The study results suggest that fish fertilizer may be preferred to initiate an algal bloom, as it appears to promotes more luxuriant algal growth at the same dose as the chemical fertilizer. However, subsequent fertilization can be done using chemical fertilizers.

Fertilizer application

It would be preferable to apply fertilizer before ice breakup, since algae grew best when pit lake water was slightly diluted and melt water dilutes surface water. Once an algal bloom is established, fertilization should be continued to maintain ammonia-nitrogen concentrations around 2 mg/L.

5 References

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Eaton, A.D., L.S. Clesceri, and A.E. Greenberg (Eds). American Public Health Association. Washington, DC.

Crosius, J., P. Whittle, D. Kramer, R. Pieters, T. Pedersen, G. Lawrence, J. McNee, D. Dunbar, R. Goldblatt, and A. Leung. Testing metal removal strategies in the Equity Silver pit lakes using limnocorrals. Presented at the 9th Annual British Columbia Ministry of Energy and Mines – MEND Metal Leaching and Acid Rock Drainage Workshop. Vancouver, BC. December 4 and 5, 2002.

Gartner-Lee, Ltd. 2003. Anvil Range Mine 2003 Study of Care and Maintenance Management Alternatives for Grum Pit. Report prepared for Anvil Range Mining Corporation (Interim Receivership) by Gartner-Lee. 40 p. + Appendices

Hrycenko, M. and A. Sobolewski. 1999. A process-oriented approach to the biological removal of selenium. Proceedings of the 23rd Annual British Columbia Mine Reclamation Symposium, September 20-23, Kamloops, BC. p. 22-31

Pelletier, C., D. Muggli, M. Wren, and G. Poling. 2002. Biogeochemical treatment of ARD at the Island Copper Mine pit lake. Presented at the 9th Annual British Columbia Ministry of Energy and Mines – MEND Metal Leaching and Acid Rock Drainage Workshop. Vancouver, BC. December 4 and 5, 2002.

Sobolewski, A. 2003. Detoxifying Pit Lakes by Controlled Algal Blooms (Aquatic Phytoremediation): Laboratory Trials and Pilot Field Tests at the Little Creek Pond at Vangorda Pit near Faro, Yukon Territory, Canada. Report submitted to Mining Environment Research Group (MERG) and Environmental Biotechnology Application Division, Environmental Technology Advancement Directorate, Environment Canada. 22 p.