Avalon Institute of Applied Science Inc.

Charleswood Technology Centre
3227 Roblin Blvd
Winnipeg MB R3R 0C2
Canada

phone +1 (204) 489-4569 fax +1 (204) 489-5782

Internet http://www.avalon-institute.org

Current Address: 976 Elgin Ave., Winnipeg MB R3E 1B4

RESEARCH REPORT

Biological and Chemical Assessment of the Oxbow Lake Water, St. François Xavier, MB

Submitted to:
Mr. Roger Poitras, Reeve
RM of St. François Xavier
1060 Hwy. 26
St. François Xavier, MB R4L 1A5

April 30, 2006

Table of Contents

Microbiological and chemical analyses undertaken in the preparation of this report were performed by the Avalon Institute of Applied Science, Inc., Winnipeg, Manitoba, in agreement with the RM of St. Francois Xavier MB.

Printed in Canada, 2006.

Introduction

Oxbow Lake is the natural meander of the Assiniboine River that was isolated from the river by a man-made dam. The small island in the centre, and the dam that connects it with the road subdivides this meander into two separate lakes – Eastern and Western Oxbow Lake. In this report, the Eastern Lake will be called **Lake 1** and the Western Lake will be called **Lake 2** (Figure 3). Both lakes have seasonal river water supply when the water level of the Assiniboine River rises, but do not interconnect with each other. Lake 1 connects with the river via a depression in the dam (Figure 1), and Lake 2 connects with the river via underground pipe (Figure 2**b**) and natural depression (Figure 2**a, Figure 3**). Certainly both lakes have seasonal groundwater and surface water supply, which significantly influences their levels, and also their water quality.

The central island and western side of the Lake 2 are almost completely covered with the trees, growing wild as a small unmaintained forest, with no immediate contact to human settlement. While the eastern side of the Lake 1 has houses build along the lake, surrounded by an open area covered by the lawn, that spreads up to the lake waterline. Only the southeast part of Lake 1 is covered with the wild growing trees.

The Avalon Institute has been contacted by the RM Council to give an overview of the water situation and suggest the remedial plan for the algae bloom problem of Oxbow Lake.

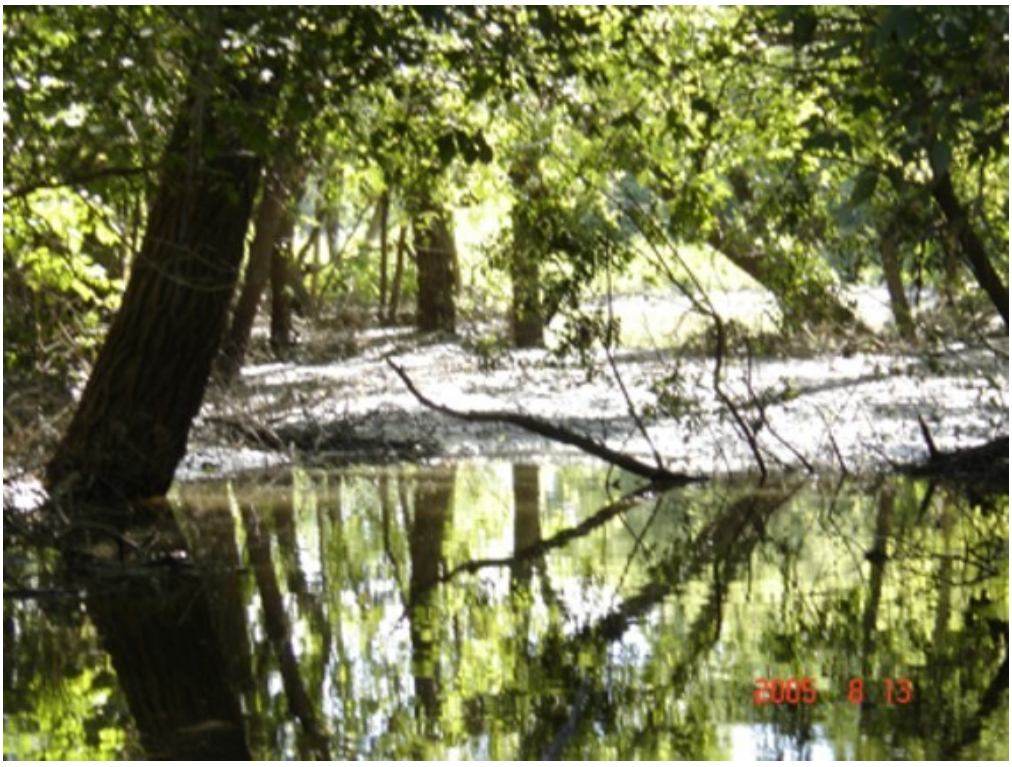


Figure 1 Connection between Lake 1 and Assiniboine River

Problem Description

For the last seven years, as the RM Council described it, Oxbow Lake has been intensely blooming during the summer. Speaking generally, algae blooms are viewed as an indicator of euthrophication. This must also be assumed for Oxbow Lake – a central point of the community, which was causing aesthetic and odour problems. One should add that algae bloom could be a health hazard when it involves species that produce toxins. The main focus of this study was

1. to identify the cause of euthrophication of the lake water, and
2. to recommend possible ways to minimise or eliminate the algae bloom problem.

The term "algae bloom" implies the presence of algae, which in fact is usually not the case. Until a few decades ago, cyanobacteria were named "blue-green algae", because they were believed to be algae, and not bacteria. This report will abstain from using the obsolete term "blue-green algae", and will use the term "cyanobacteria bloom" instead of "algae bloom", although neither bacteria nor algae technically "bloom".

a **b**

Figure 2 Connections between Lake 2 and Assiniboine River: a. natural depression, b. pipe

Scope of Work

The scope of work planned for the research season (June - October 2005):

(a) Determination of the chemical composition of the lake water,

(b) distribution of the chemical elements in the water to identify the essential factors that can potentially contribute to the problem (Ammonia - NH_4, Nitrates – NO_3, Phosphates – PO_4, Iron – Fe, Silicate - SiO_3, Sulfates – SO_4),

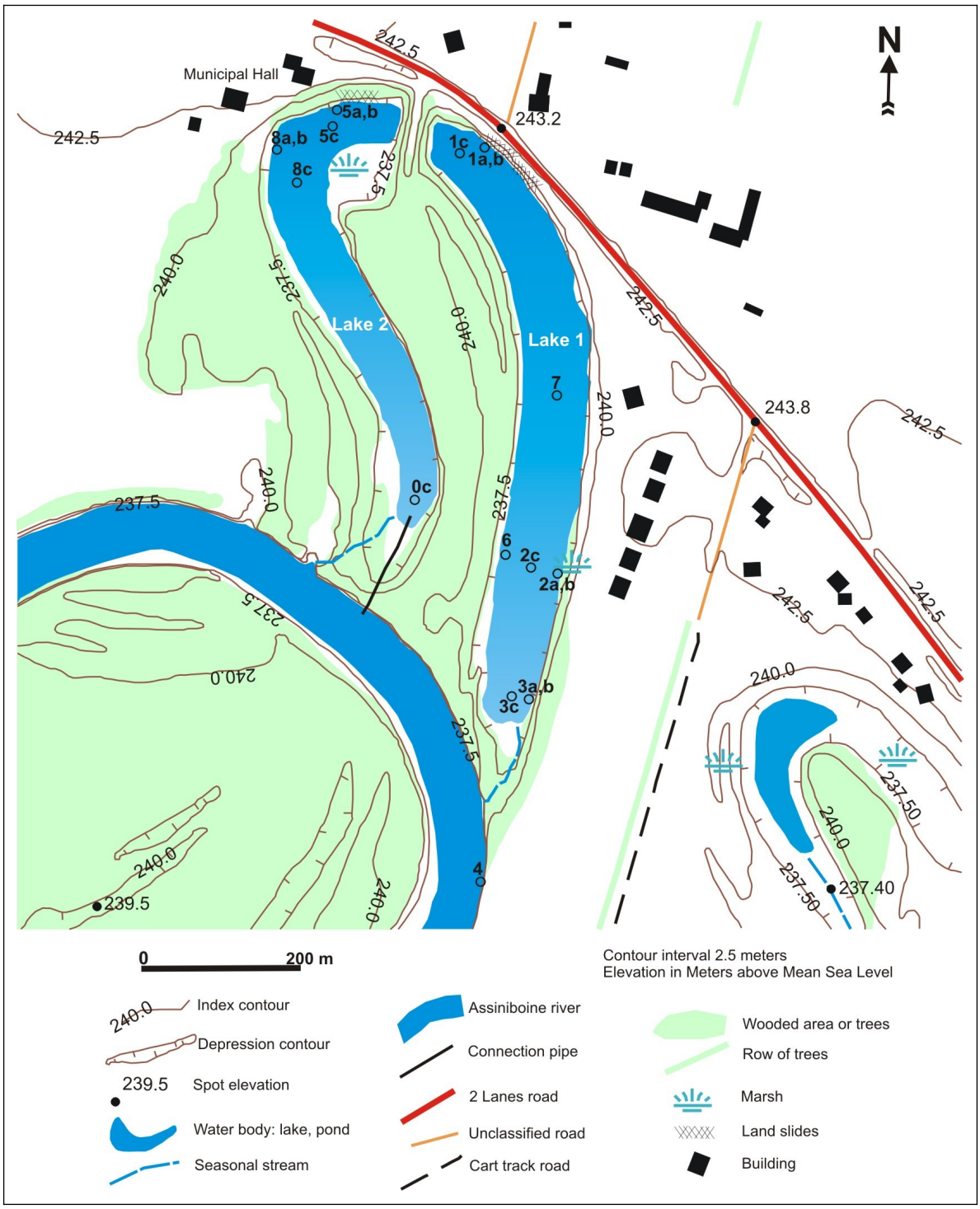


Figure 3 Map of Oxbow Lake with sampling locations [1]

[1] Map drawn on the basis of the Province Manitoba Map AK33, White Plains (Province of Manitoba, Survey and Mapping Branch. 1991).

(c) determination of temperature, pH, DO and ORP gradient that may vary in the water,

(d) determination of the microbiological composition of the lake water, i.e. a study of the microbiological assemblages regarding their population density and their biodiversity (certain assemblages are rather descriptive for the water type),

(e) determination of the potential problems regarding the microbiological composition, for example potential problems through the presence of coliform bacteria, certain flagellated, protozoa, or cyanobacteria,

(f) hydrological investigation of the water supply to the lake to verify influence of the Assiniboine River or other sources of the water on the Oxbow Lake ecosystem.

All these factors were studied in the seasonal change from beginning of the summer until mid-autumn (first frost).

Testing Procedure

The research team drove to the site every week for observation, sampling, and testing. Sampling points were in the central parts of the lake, at lake banks, and at interconnecting points with the Assiniboine River. For the proper data comparison, river water was included in the testing program.

Prior to beginning the field research, the sampling points at each lake were selected. As research progressed, additional sampling points were assigned. Towards the end of the testing period, the number of the sampling points was 19 (Figure 3).

The testing procedures are either standard methods, and are identified as such in this report, or special methods, which are described in greater detail.

Because some parameters of the water are unique and change very rapidly once the sample is drawn, these measurements were done on-site immediately after sampling. These tests included the following analyses: ORP, DO, pH and T for all sampling points selected as described above.

Parameters that change not as rapidly were determined immediately after the water samples arrived at the Water Test Lab of the Avalon Institute, i.e. within 2 hours of sampling. The testing procedure consisted of following analyses: NH_4, NO_3, PO_4, colour, Fe, SiO_3, SO_4, total and faecal coliforms.

Micro-organisms were studied at the Miscroscopy Lab of the Avalon Institute, beginning on the day after the samples were taken. These studies are usually carried on for several days in order to see the development of the natural biota of the water.

Field Observations

Field observations started on June 4, 2005 and concluded on October 29, 2005. Table 1 shows the dates of the field observation and sampling.

Field observations included some geodesic, geological and hydrological investigations of the relief, soil, and lake bottom sediments, water levels and lake depth. This additional investigation allowed a better understanding of local water and soil dynamics, and of the influence of the connection between the Assiniboine River and Oxbow Lake. The sampling was done in parallel on all three bodies of water.

Table 1 Field observation dates

04-Jun-05	23-Jul-05	10-Sep-05
11-Jun-05	30-Jul-05	17-Sep-05
18-Jun-05	06-Aug-05	24-Sep-05
25-Jun-05	13-Aug-05	01-Oct-05
02-Jul-05	20-Aug-05	08-Oct-05
09-Jul-05	27-Aug-05	15-Oct-05
16-Jul-05	03-Sep-05	29-Oct-05

River – Lake Connections

There are some significant observations that are pointed out below.

June 04 - When the observation started, Lake 1 and Lake 2 were not yet connected to the river (Figure 4**a**), but water levels were already high.

June 11 - Due to intense rain, the water level increased by more then one meter, and there was a connection between Assiniboine River and both lakes (Figure 4**b**).

June 25 - The micro-algae community in Lake 1 changed. The cyanobacteria *Aphanizomenon flos aquae* (Figure 29) and *Anabaena* (Figure 29) were present, and were not seen before.

July 09 – The water level dropped by approx. 20-30 cm, Figure 4**c**). Cyanobacteria (mainly *Aphanizomenon flos aquae*) were widely spread on the Lake 1. Green algae like *Chara* are growing along the lake banks (stations #2 and #3). At Lake 2, very intense growths of green algae (*Chara*) and duck weed was found to cover almost the whole lake surface.

July 16 – Growth of cyanobacteria intensifies and now dominates in Lake 1. Cyanobacteria forms slimy layers and round flocs close to the water surface and at the bottom at the shallow areas. accumulating into green balls and layers.

July 23 – Lake 1 now contains a high amount of the small catfish (about 3 cm). Some big fish was seen, appears to be either catfish or carp.

July 30 – Connection between the river and Lake 1 stopped. Lake 2 likely still exchanges water with the river via the pipe (Figure 4**d**).

August 06 – Lake 1 water has greenish colour and is void of *Cyclopes*, *Daphnia* and young fish (Figure 28**c**). Duck weed almost gone.

August 13 – River level dropped. Lake 1 level dropped to the extent that *Chara* is exposed to the surface. Cyanobacteria amount increased. Fish stick heads out of the water.

August 20 – Lake 1 void of cyanobacteria and covered with goose down. Fish is not seen. At Lake 2, the micro-algae population is dominated by flagellates (mainly *Euglenida)*.

August 27 – Water levels dropped further in the river and lakes (Figure 4**e**). Lake 1 - Cyanobacteria is blooming (eutrophication). Lake 2 – Water has greenish colour, high amount of flagellates (*Euglenida*) (continuation after Figure 4).

a. June 04, 2005, no connection between the river and lakes. Water dripping out of the pipe meaning that Lake 2 level is higher than the river level

b. June 11, 2005, water level rises (week of raining), connection between river and lakes established

c. July 09, 2005, water levels dropped, lakes still connected with the river

d. July 30, no connection between the river and Lake 1, limited connection via pipe with Lake 2

e. August 27, no connection between river and lakes, water levels dropped

f. October 29, no connection between river and lakes, water levels dropped, end of the field observation

Figure 4 River water connections with Lake 1 and Lake 2

September 03 – Water level dropped. At Lake 1, cyanobacteria continue to bloom (blue colour), can cleary be noticed from the road. No sign of fish. Land sliding occurred along the roadside near station #1. At Lake 2, water is clear and appears to not contain micro-organisms. Significant landslide behind the Fire Station, near station #5.

September 10 – River and lake levels dropped significantly. Geese arrived at the Lake area. At Lake 1, cyanobacteria bloom ended, but they are still present in the water, the smell of the rotting biomass stands in the air. Amount of *Daphnia* increased. Station #3 area completely dry. Duckweed developed at both lakes.

September 24 – Geese stay in the area. Water is quite clear in both Lakes, almost no micro-organisms. At Lake 1, fish larvae (1-1.5 cm) were spotted.

October 08 – Outside temperature decreased sharply to freezing conditions. Water levels dropped more. Assemblages of micro-organisms in the lakes became similar and consist mainly of diatoms and bacteria floc. Water from Lake 2 has some flagellated and non-flagellated filamentous algae. In the Assiniboine River, the density of micro-algae increased. Geese prefer to stay at Lake 1.

October 29 – Geese left. Land sliding continues at the North side of the both Lakes (Figure 4**f**).

Testing Results

pH Value

pH – Background

Natural waters are hardly ever at the neutral pH of 7. With few exceptions, natural bodies of water are pH-buffered by hydrogencarbonate, which sets the pH to approximately 8.2. If additional carbon dioxide is dissolved in the water - this is very likely -, the pH will be lower. Not just water, but all life depends on this mechanism: the human blood is pH-buffered by hydrogencarbonate, with a pH of about 7.4; the carbon dioxide that is transported by the blood and finally exhaled through breathing, lowers the pH from the typical hydrogencarbonate value of 8.2 down to 7.4. Likewise, most surface waters have a pH between 7.0 and 7.5.

The production of oxygen by plant life in water reduces the amount of carbon dioxide in the water, i.e. increases the pH value slightly. Likewise, fish and other animals consume oxygen and produce carbon dioxide, i.e. they lower the pH. For example, aquarium owners with their typically high fish density use aeration to drive the excess carbon dioxide out of the water.

pH – Assiniboine River

The measured pH fluctuations of the Assiniboine River are shown in Figure 5. The observed pH interval was from 7.5 to 8.7. It is remarkable that the "normal" pH range for a surface water is met only during the period of high water levels. However, high pH values during regular water levels may be an indication of infiltration of alkaline groundwater into the river. Because the pH of the Assiniboine River water is only used for comparison and as an indicator of water exchange with the two parts of Oxbow Lake, the source(s) of pH changes in the Assiniboine River water are outside of the scope of this study.

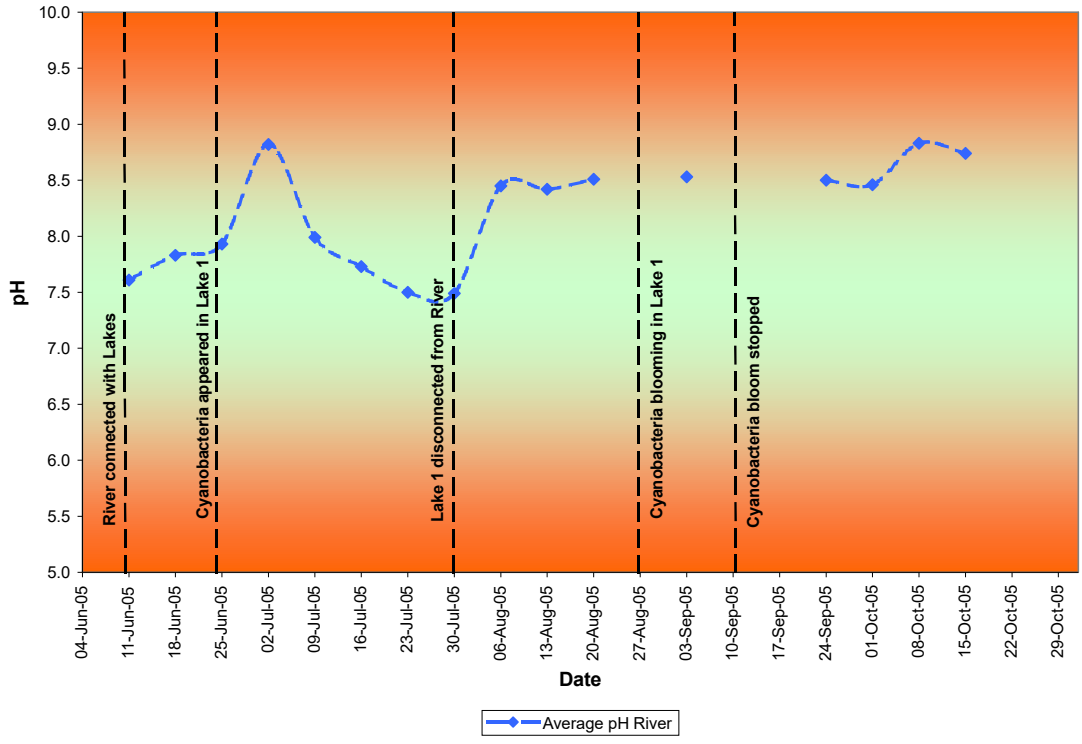


Figure 5 pH fluctuations of the Assiniboine River

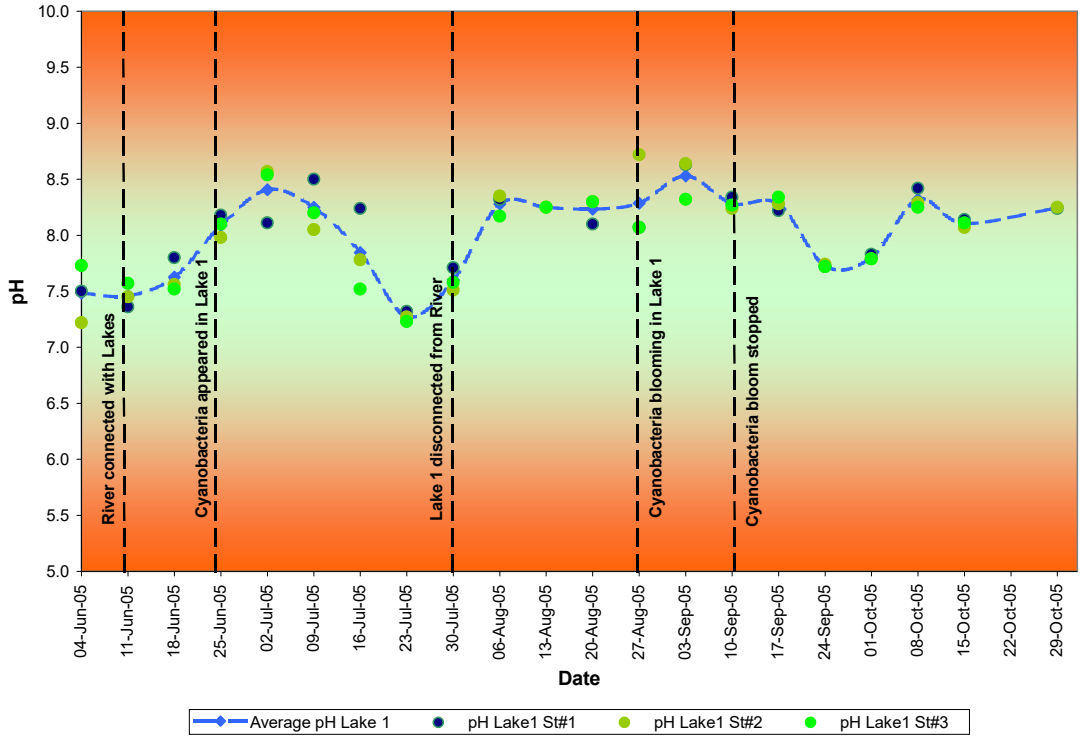


Figure 6 The pH fluctuations of Lake 1

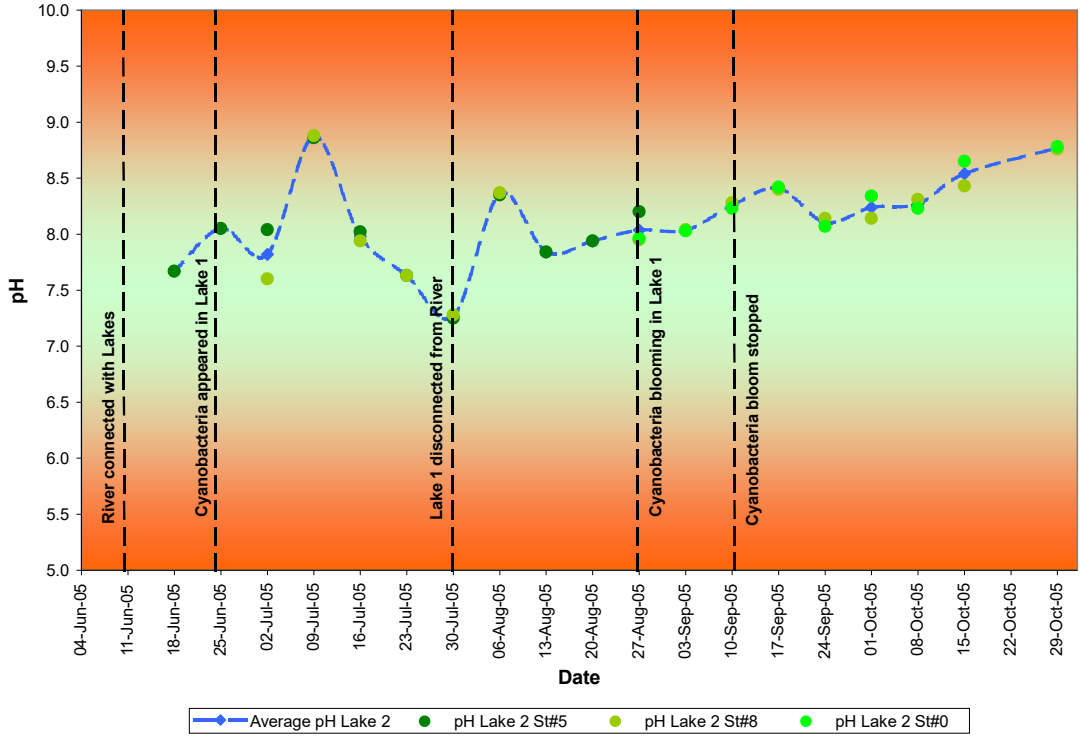


Figure 7 pH fluctuations of Lake 2

pH – Lake 1

The pH fluctuations of Lake 1 are shown in Figure 6; the observed pH interval is slightly lower (7.25 – 8.4) than the values for the Assiniboine River. It is not surprising that initially the pH changes of Lake 1 are synchronised with the pH changes of the Assiniboine River, given the fact that water exchange takes place. Towards the end of the observation season one can see that the pH of Lake 1 is much lower than the pH of the Assiniboine River. This indicates that the separation of the waters is complete, once the overflow into Lake 1 ends.

pH – Lake 2

The pH fluctuations of Lake 2 cover a slightly wider range pH = 7.25 – 8.9 (Figure 7). This may not appear as significant, however, for most aquatic organisms, this span can mean the difference between life and death. Further, the pH of Lake 2 was creeping upwards during the second half of the observation period, while the pH of Lake 1 and the pH of the Assiniboine River both stayed within a narrow, relatively high pH band. This means that the factors increasing the pH reach Lake 2 rather slowly.

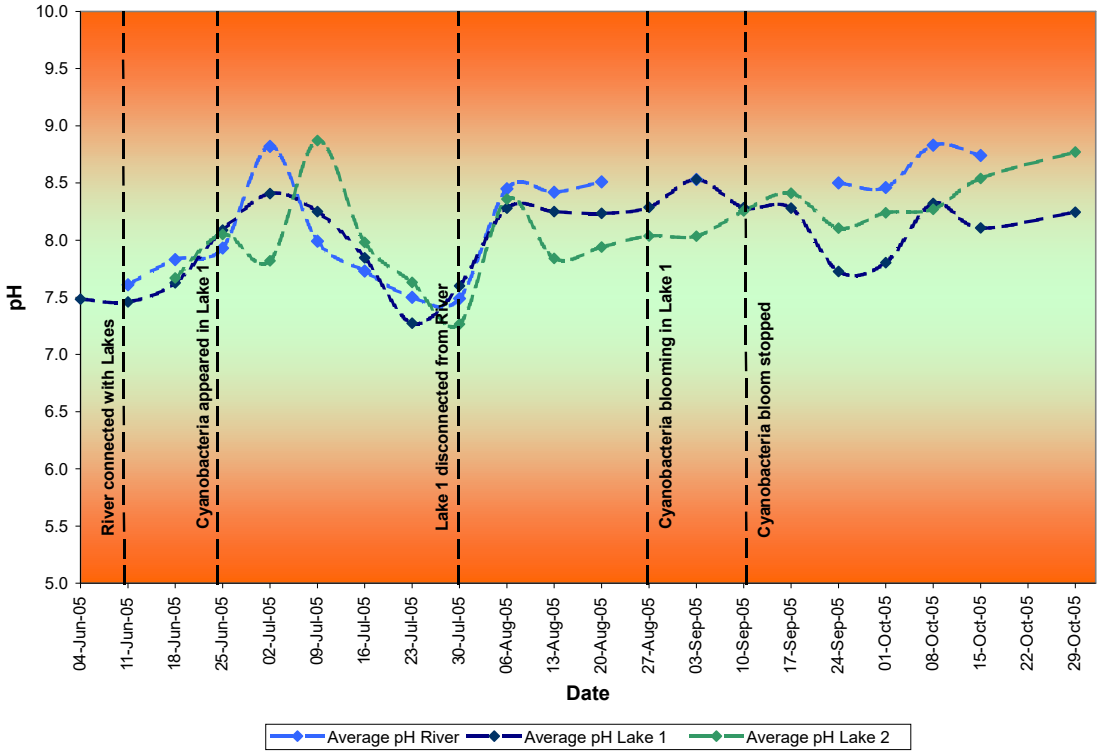


Figure 8. Comparison of pH analyses of the Assiniboine river and both Lakes

The pH values of the Assiniboine River and both lakes were higher than 7.4 during the whole investigation period (Figure 8), with the exception of the short timespan when the connection between the Assiniboine River and the lakes was severed. At this time, also the oscillations of parameters began. In both cases water levels were similar and at the mid-point between highest and lowest levels.

Oxidation-Reduction Potential (ORP)

ORP - Background

ORP values are seldom used in North America in the characterisation of water, however, they are widely used elsewhere, and they are also routinely measured by the Avalon Institute.

Without going to deep into scientific detail, ORP values are indicative of the chemical reactions in the water, such as the consumption of dissolved oxygen (DO) through metabolic processes, while the direct DO measurement only povides the value of its availability, and not its usage by biota or its reaction with dissolved minerals in the water. In fact, ORP values are a very sensitive measurement – so sensitive, that deviations of ± 10 mV can easily be discarded as likely being insignificant.

A guideline to the interpretation of ORP values is as follows:

- +120 to +160 mV: Properly treated wastewater prior to discharge. With excellent treatment, this value is closer to 180 – 200 mV.
- +20 to +100 mV: Discharge of a lagoon in spring in a climate such as Manitoba's, where the lagoon is frozen in the winter, limiting oxygen availability.
- +40 to +70 mV: Threshold level below which iron in the water exists mainly as Fe(II). At the pH of most waters, no bio-available oxygen is present, because the oxidation of iron is kinetically preferred. For all practical purposes, water below this range is anoxic or anaerobic.
- -10 to +30 mV: Threshold level below which sulfate in the water is reduced by bacteria to hydrogen sulfide (rotten egg smell).

For a detail interpretation, it is necessary to interpret the pH-Eh-diagrams (Pourbaix diagrams), which have been omitted here in the interest of the readability of this report.

Lowered ORP levels are usually accompanied by biological reactions that lower the pH, owing to the preference of acidifying metabolic reactions, as oxygen becomes less available. As a secondary consequence, the solubility of phosphate in water increases, and the ability to ammonia oxidation decreases (example: lagoon discharge). As can be seen, ORP measurements take a central role in the proper interpretation of the situation of a given body of water.

ORP – Assiniboine River

The measured ORP range covers 75 - 275 mV, the latter being a good value for surface water (Figure 9). Unfortunately, this good value was only reached once during the whole season, and for the most part, the relatively low ORP values indicate a problematic water quality. At several occasions, the ORP values were so that if this were not river water but wastewater, one would speak of insufficient treatment. The low values for several weeks during the high river water levels may be caused by runoff that perhaps carries high loads of degradable, and degrading, organic material into the river. With receding flood waters, the ORP peaks to an excellent value, followed shortly with a minimum valley. This is an indication that with the availability of oxygen in the water, biota develops rapidly, thus consuming the limited supply of dissolved oxygen, and eventually dying off because of insufficient oxygen supply. The oxygen demand of these decay processes is unfortunately a self-accelerating mechanism in oxygen depletion. The recovery period has not been measured because in the interest of personnel safety, there was no access to river water for some time.

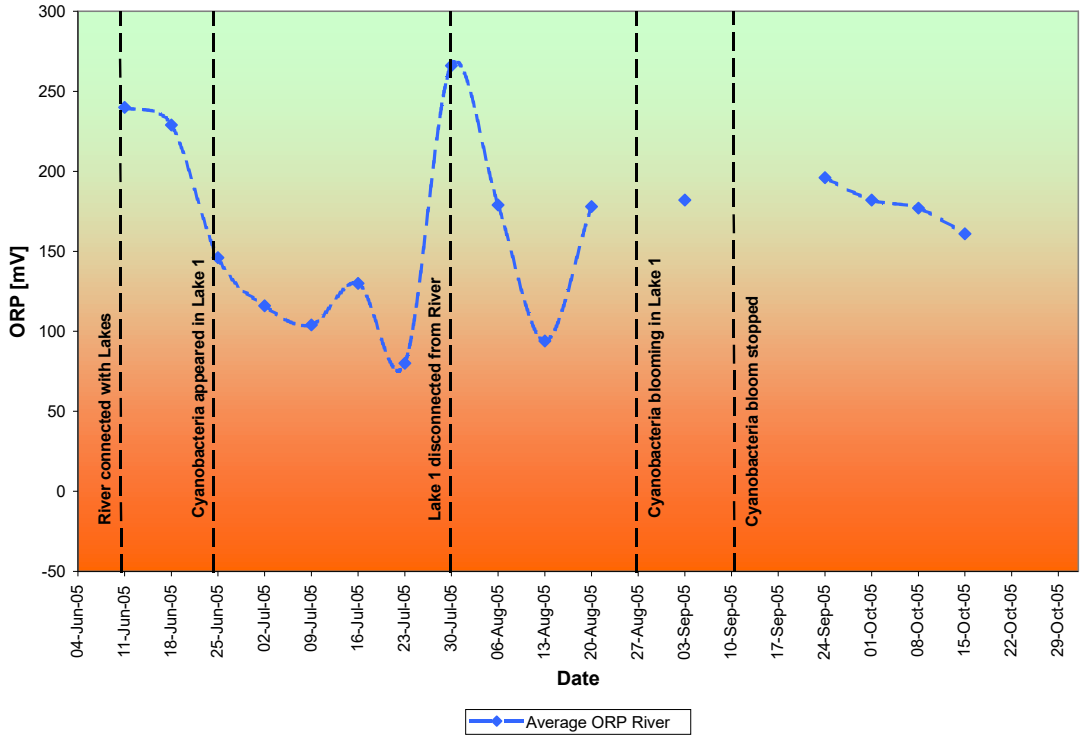


Figure 9 ORP of the Assiniboine River

ORP – Lake 1

The ORP range of Lake 1 is from 10 – 250 mV. The low point of 10 mV is an indication of a serious anaerobic condition (Figure 10). One can clearly see the oscillations of ORP values: the maximum oxidation potential is reached during the separation of Lake 1 from the Assiniboine river, followed by several oscillations of decreasing amplitude, until toward the first frost, a slow decline without noticeable oscillations has been reached. Using the terms of control theory, the flooding was a step function, to which the system responded with the oscillations that any common PID controller shows. This interesting phenomenon deserves further research. Since inorganic chemicals (minerals, dissolved solids) do not show this behaviour, the oscillations must be attributed to a fluctuating biological system. In other words, these oscillations are the projections of a trajectory of the function showing the population density of biological assemblages against pH and ORP. This is scientifically fascinating, and may provide the first clue about the appearance and disappearance of cyanobacteria, which after all are part of the stable (more precisely: temporarily stable) assemblages.

We believe that this type of measurement in conjunction with biological assemblages is new in Manitoba, and has not been used before. The period (= cycle time in changing biota) is about five weeks. This indicates that this cycle is driven by micro-organisms, because the cycle time of higher lifeforms such as fish is much longer.

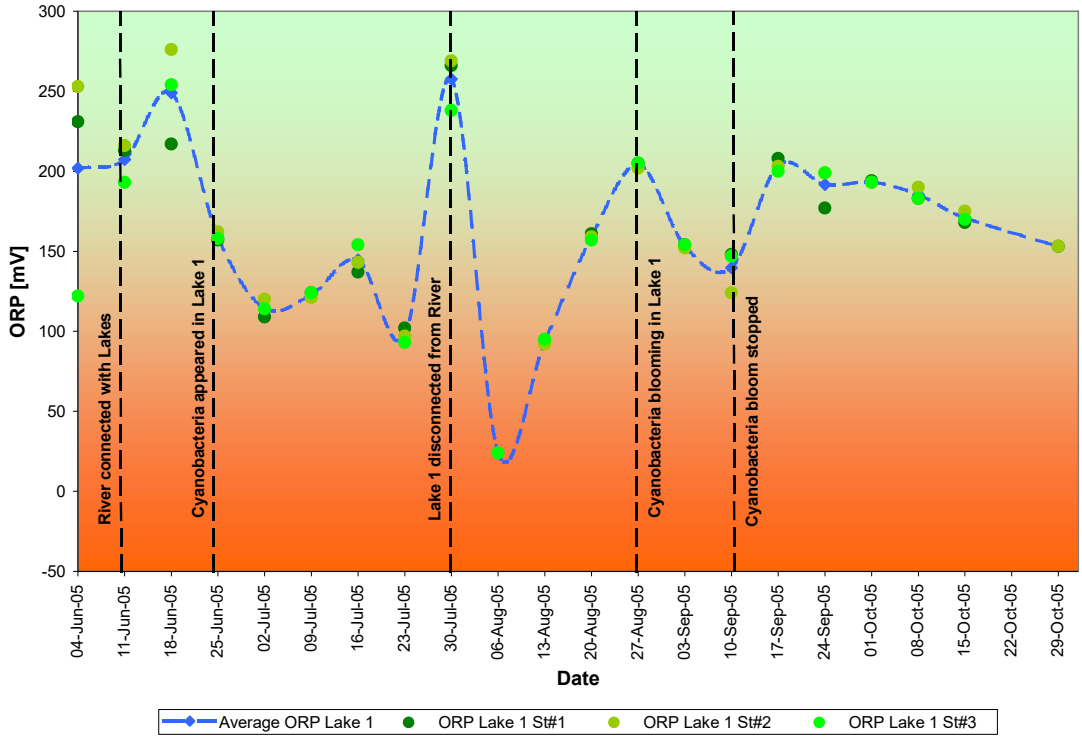


Figure 10 ORP range of Lake 1

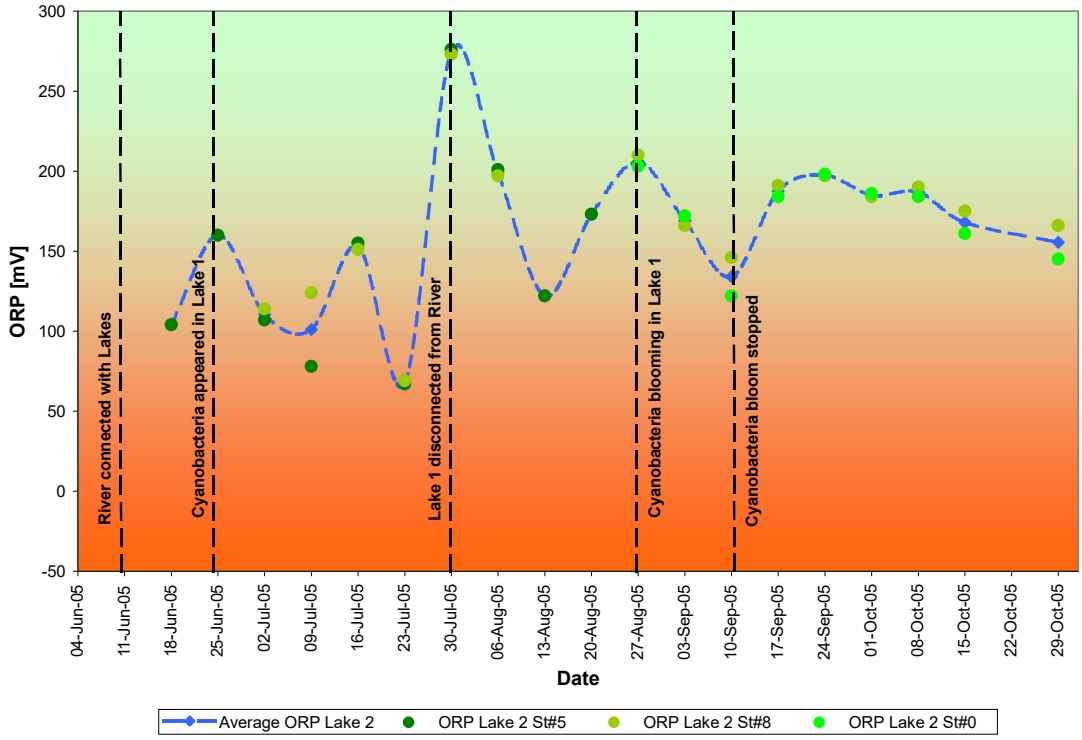


Figure 11 ORP of the Lake 2

ORP – Lake 2

The ORP covers the range 60 - 275 mV in Lake 2 (Figure 11). The general picture – a high amplitude following the separation from the Assiniboine River – is very similar in amplitude and frequency, compared with Lake 1. However, the flood phase ends at a noticeably higher ORP than in Lake 1, which is perhaps the reason that the dip to low ORP values after the maximum is not as pronounced as for Lake 1.

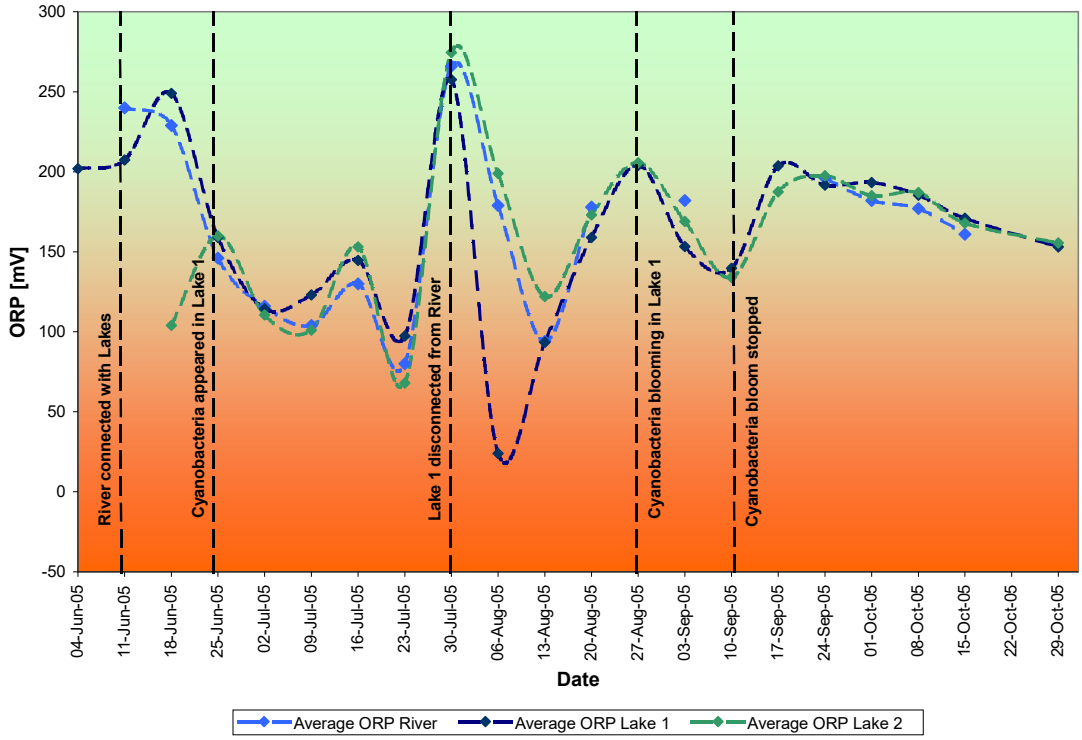


Figure 12. Comparison of ORP data of the Assiniboine river and both Lakes

ORP values during most of the investigation are below 200 mV, which speaks for the low water quality that is comparable to a quality of treated wastewater (Figure 12). The only data confirming good water quality were measured at the beginning of the investigations and during short period when River and Lakes were disconnecting. In both cases, water levels were similar and at the mid-point between highest and lowest levels.

ORP values for all three water bodies oscillated similarly, but with different amplitude. A significant difference was observed after the Lake-River separation. This is perhaps caused by the higher population density in Lake 1 at macro- and micro-levels.

Water Temperature (T)

The comparative analyses of the water temperatures (Figure 13) showed slight differences in the water temperatures of the lakes and the river. ***All water temperature data can be divided into two periods, clearly seen before and after July 30th*** (Figure 13)***, which is the date when the water level dropped and the connection between the river and lakes was interrupted.*** When the river

was **connected** to the lakes, the temperatures were higher (21 – 32 ºC), were steadily increasing, and river temperatures were slightly lower than lake temperatures. When river and lakes were **disconnected**, the temperature dropped (from 21 to 11 ºC), were steadily decreasing, and temperatures between the lakes were quite similar. One should note that the temperature of Lake 1 is almost the same as the river temperature, while the temperature of Lake 2 is mostly higher in the second half of the season, perhaps because Lake 2 is more shallow.

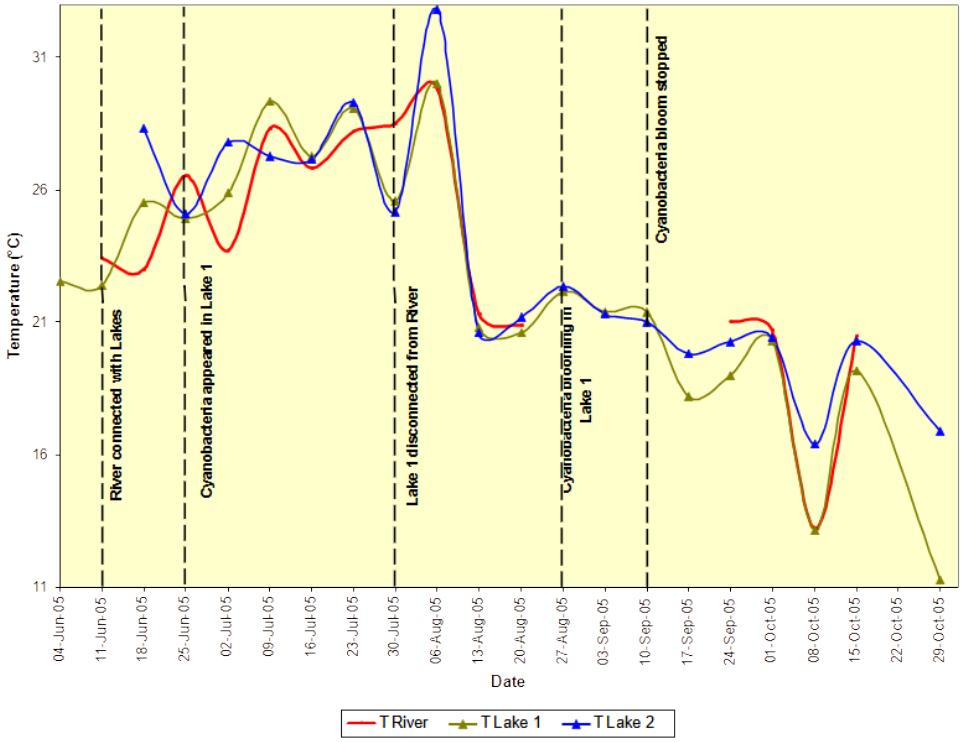


Figure 13 Temperature comparison for Assiniboine River, Lake 1 and Lake 2

Dissolved Oxygen (DO)

Oxygen is slightly soluble in water, but with a strong dependence on the temperature of the water, as shown in Table 2**.**

Table 2 Oxygen solubility (based on Vesilind, P., 1996).

Temperature (°C)	Dissolved Oxygen (mg/L)
0	14.60
10	11.27
20	9.07
30	7.54
40	6.50

It is therefore not surprising that the most anaerobic condition in the river and lakes as seen in the ORP data above, coincide with the highest water temperature. For clarity and ease of

interpretation, Figure 15 shows the percent oxygen saturation, as per tabulated values, in addition to the measured DO data (Figure 14), thus taking into account the changes in temperature.

The DO measurements started on July 23rd when differences between the water levels of the lakes became noticeable (Figure 14). After July 30th, the DO was fluctuating - increasing and decreasing in both Lakes. It is not surprising that the DO value is almost synchronised with the ORP data with respect to the timing of maxima and minima. However, the amplitudes of DO and ORP differ, because the former indicates the ***presence***, the latter the ***utilisation*** of oxygen.

Between July 30th and September 3rd (when Lake 1 started blooming, (Figure 14) phases of the DO fluctuations were coinciding, and after September 3rd they shifted at the beginning half phase and then inverted. On October 29th the DO in both lakes returned to the value of 4 mg/l, which is believed to be the "stable" DO level of the lakes. DO fluctuations were more significant in the Lake 1; dissolved oxygen varied between 1.8 and 8.8 mg/l, while in the Lake 2 DO varied in the interval between 4.1 and 6.9 mg/l.

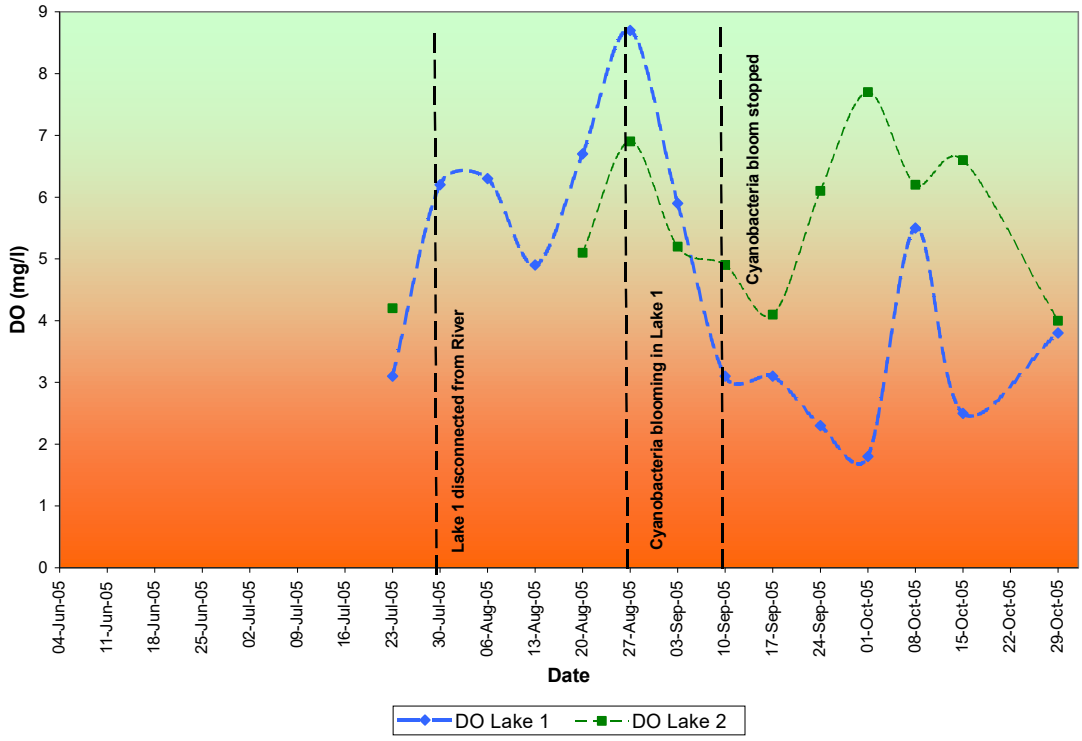


Figure 14 Dissolved Oxygen data in the Lake 1 and Lake 2

Oxygen saturation (Figure 15) of the water was calculated based on DO (Figure 14) and temperature data (Figure 13). The calculation were done for the Lake 1 (St#2) and Lake 2 (St#8). Oxygen saturation O_2 was calculated as the ratio between measured DO results and the maximum DO concentration possible for a given temperature (Vesilind, P., 1996 [2]):

$$O_{2\ saturation}\ (\%) = DO_{exp} / DO_{max} \times 100$$

The results are shown in Figure 14. The water in Lake 2 was more than 50 % oxygen saturated during the course of the investigation. It is remarkable that in the Lake 1 the saturation level

[2] Vesilind, P. A., 1996. Introduction to Environmental Engineering, PWS Publishing Company, Boston, 468 pp.

dropped below 50 % oxygen after the cyanobacteria bloom oxygen, down to an average saturation level of approximately only 30%. ***This high demand for oxygen can be explained by the high amount of decaying organic matter after the Lake 1 euthrophication. Organic matter will include the macro- and micro-populations of the Lake 1 that have been killed by the cyanobacteria bloom and by the release of their toxins.***

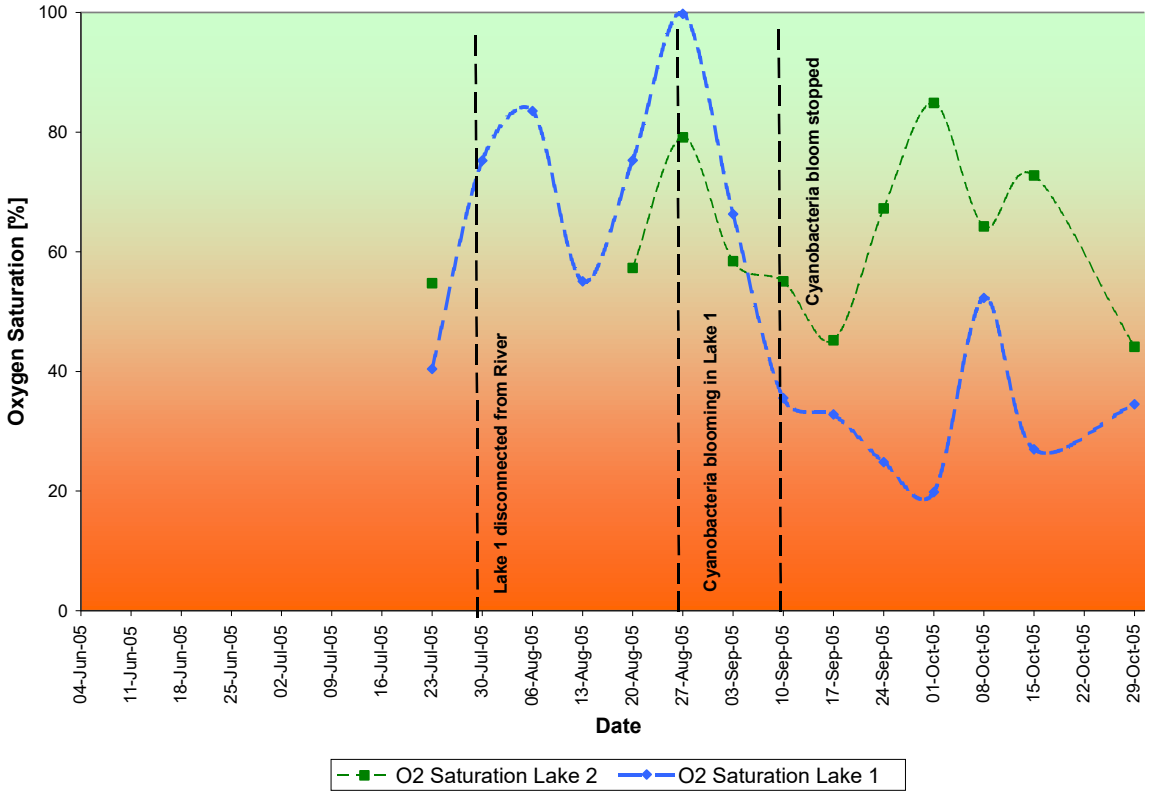


Figure 15 O_2 saturation in the Lake 1 and Lake 2

Chemical analyses

Phosphates (PO_4)

It is common knowledge that cyanobacteria bloom is directly related to the presence of phosphate. This was experimentally confirmed during a several years investigation at the Experimental Lakes Area (EAL) [3]. Within the three macro-nutrients carbon (C), nitrogen (N), and phosphorous (P), only the latter is a bottleneck for the development of cyanobacteria, because the air exchange with the water surface brings carbon dioxide as a carbon source into the water, out of which cyanobacteria can photosynthesise carbohydrates and other essential organic material. Likewise, they are able to convert the abundant atmospheric nitrogen into ammonia in a process called nitrogen fixation, by shifting from photosynthesis during daytime, to nitrogen fixation in the night. Therefore, only phosphorous availability can limit the growth of cyanobacteria, or accelerate their growth if phosphorous is abundant.

[3] Schindler, D.W, *et al*. Euthrophication of Lake 227 by Addition of Phosphate and Nitrate: the Second, Third, and Fourth Years of Enrichment, 1970, 1971, and 1972. J. Fisheries Research Board Canada 30 (10), 1415-1440 (1973).

Phosphorous was measured as phosphate, following common practice. The phosphate data measured for Lake 1 fluctuate between 0.18 and 3.30 mg/l (Figure 16, Figure 18). The highest values and steepest fluctuations are associated with station #2, less with station #1. Phosphate levels at the station #3 are steady about 1 mg/l with several peaks during the study. In general, phosphates at the Lake 1 are decreasing from June to October. At the end of the study, the phosphate concentration was 0.18 mg/l.

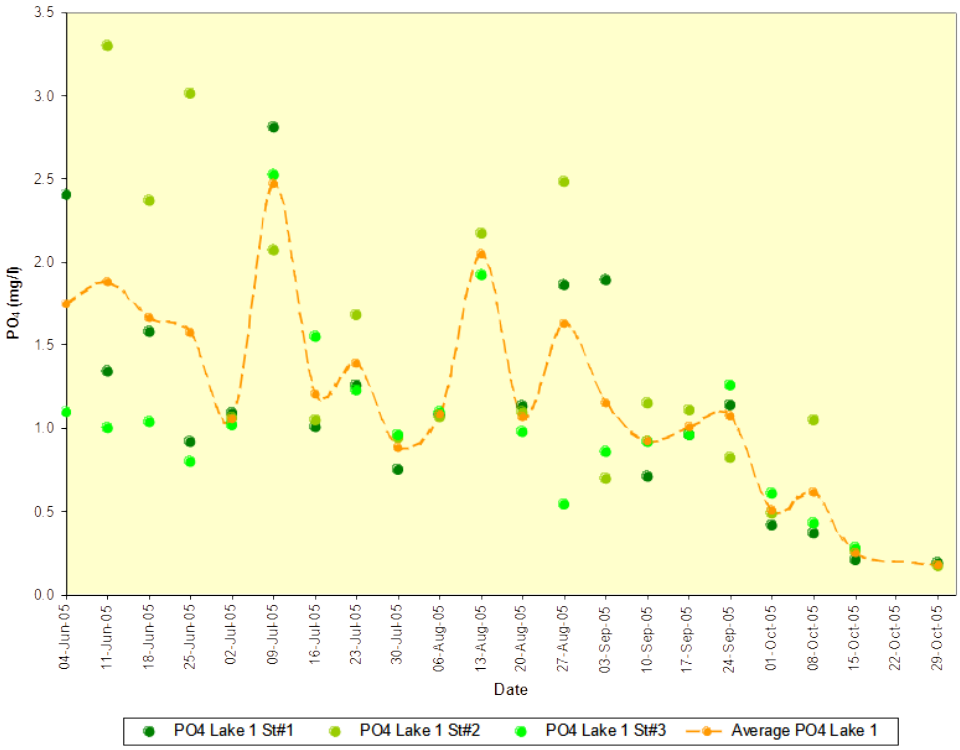


Figure 16 Phosphate test results for Lake 1

It is important to emphasise the following points: a) Stations #1 and #2 are located in the populated part of the Lake 1 (Figure 3); b) The highest values were measured in June before Lake 1 connected to the Assiniboine River; c) The lowest phosphate values were measured when Lake1 had no connection with the River.

The phosphate concentrations at Lake 2 were quite stable between 0.64 and 1.79 mg/l (Figure 17, Figure 18). Only two peak concentrations were recorded during the investigation. Both of these peak values were observed at station #8. On July 2nd phosphate was 4.80 mg/l (lake and river are connected) and on October 15th phosphate was 3.08 mg/l (no connection between lake and river). At the end of the study, the phosphate concentration decreased to 0.2 mg/l.

One should note that station #8 is located in the North part of Lake 2 in front of a farmhouse. This is the only location where direct human influence on the water can be expected.

While both lakes and the river were **connected** with each other, significant changes of the phosphate concentrations were found in the one-week intervals of the observation. This sequence described below (Figure 18):

- June 18th - phosphate decrease in the river, June 25th - in the Lake 2, July 2 – in the Lake 1
- June 25th – phosphate increase in the river, July 2nd – in the Lake 2, July 9th – in the Lake 1

It is important that in the same interval phosphate concentrations in the lakes were always higher than in the river. ***It is obvious that the phosphate concentrations in the lakes during the connection with the river are in fact the sum of river phosphate plus local phosphate contribution.***

When river and lakes are **disconnected**, the phosphate concentrations in all three water bodies have quite comparable average values, but these values fluctuate independently, suggesting that they have different causes, likely of local origin, i.e. being the result of the local human impact. It is important to review the phosphate fluctuation in Lake 1 during the time July 30th – August 27th. The steep increase of the phosphate concentration in one-week from 1.1 mg/l (August 6th) to 2.05 mg/l (August 13th) suggests human influence. The sudden decrease of phosphorous in one week to 1.07 mg/l can be explained by phosphate consumption of the growing organic matter, which coincides with this phosphate loss. One week later, the phosphate concentration increased again (highest value 2.48 mg/l, station #2, August 27th), perhaps because of the release from decaying organic matter, and subsequently started to decrease steadily.

The above phosphate levels need to be reviewed in the context of phosphate levels established and enforced by regulatory agencies elsewhere. In the US, the ten states surrounding the Great Lakes responded to the "Death of Lake Erie" several decades ago by establishing the Ten States Standards, a regularly revised document that has become the *de facto* standard in the United States for discharge of water into surface waters. More than ten years ago, the Ten States Standard[4] already set the maximum phosphorous concentration for discharged water at 1 mg/L. The current trend in the US to lower this limit to 0.5 mg/L, enforced already by the Minnesota Pollution Control Agency. ***In other words, neither the Assiniboine River water, nor the water of Oxbow Lakes, were allowed to be discharged into the watershed if US regulations applied, owing to excessive phosphate concentrations.*** For comparison, research conducted in recent years by *Umweltbundesamt*, the German federal environmental protection agency, shows that already 0.05 mg/L phosphate trigger cyanobacteria bloom. As a result, certain European jurisdictions are limiting the phosphate concentrations in surface waters to 50 µg/L = 0.05 mg/L.

Phosphate concentrations in both lakes depend on two factors: 1) Connection with the river, and 2) local impact, either wash-out of fertiliser, or direct or indirect discharge of sewage or gray water into the lakes. The local impact is much stronger for Lake 1.

[4] Recommended Standards for Wastewater Facilities. A report of the Great Lakes – Upper Mississippi River Board of State Public Health and Environmental Managers. Health Education Services. 1990 edition.

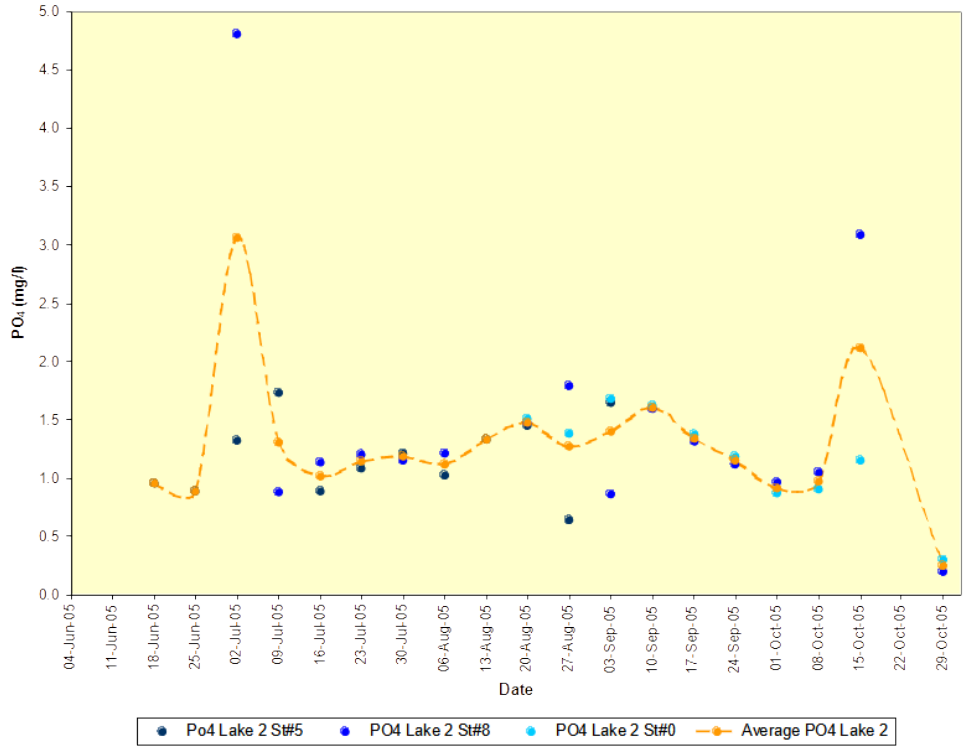


Figure 17 Phosphate test results for Lake 2

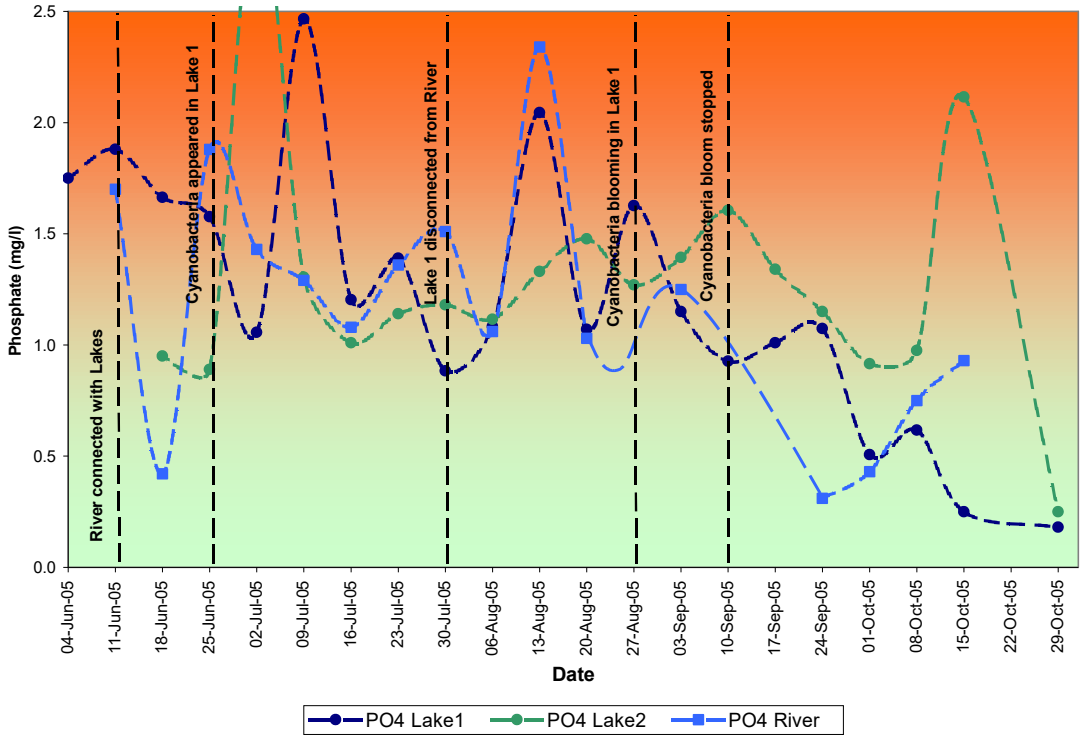


Figure 18 Comparative PO_4 analyses of the Assiniboine River, Lake 1 and Lake 2

Nitrate-Nitrogen

The nitrate-nitrogen concentrations in Lake 1 vary between 0.5 – 1.6 mg/L in average (Figure 19, Figure 21). The highest concentration during the investigation was 2.9 mg/L (station #3), the lowest 0.1 mg/L (station #2). Measurements taken at the same time, but at the different stations, have differences of 0.2 – 1.5 mg/L.

Nitrate-nitrogen concentrations in the Lake 2 vary between 0.2 and 1.1 mg/L in average (Figure 20, Figure 21). The highest concentration during the investigation was 2.7 mg/L (station #2), the lowest 0.15 mg/L (station #8). The difference between the values taken on the same time at the different station is 0.3 – 0.6 mg/L.

The nitrate-nitrogen concentrations in all three water bodies are very similar during the whole testing season. Nitrate fluctuations in all cases parallel with the exception of two intervals June 4th – June 25th and July 30th – August 27th (Figure 21)

1. Between June 4th – June 25th nitrate concentrations in Lake 2 increased, and one week later in Lake 1 and the river.

2. The second interval was between July 30th – August 27th. The nitrate-nitrogen level dropped on August 6th at Lake 2 to an average of 0.23 mg/L, while at Lake 1 it reached a peak concentration of 1.02 mg/L (average between stations). The nitrate concentration increased at Lake 2 a week later, while the nitrate concentration at Lake 1 already was decreasing.

One should note that on August 6th, along with a sudden change of the NO_3 concentration, the water temperatures in all water bodies had a seasonal peak: $T_{Lake\ 1}$ = 30°C and $T_{Lake\ 2}$ = 32.8°C.

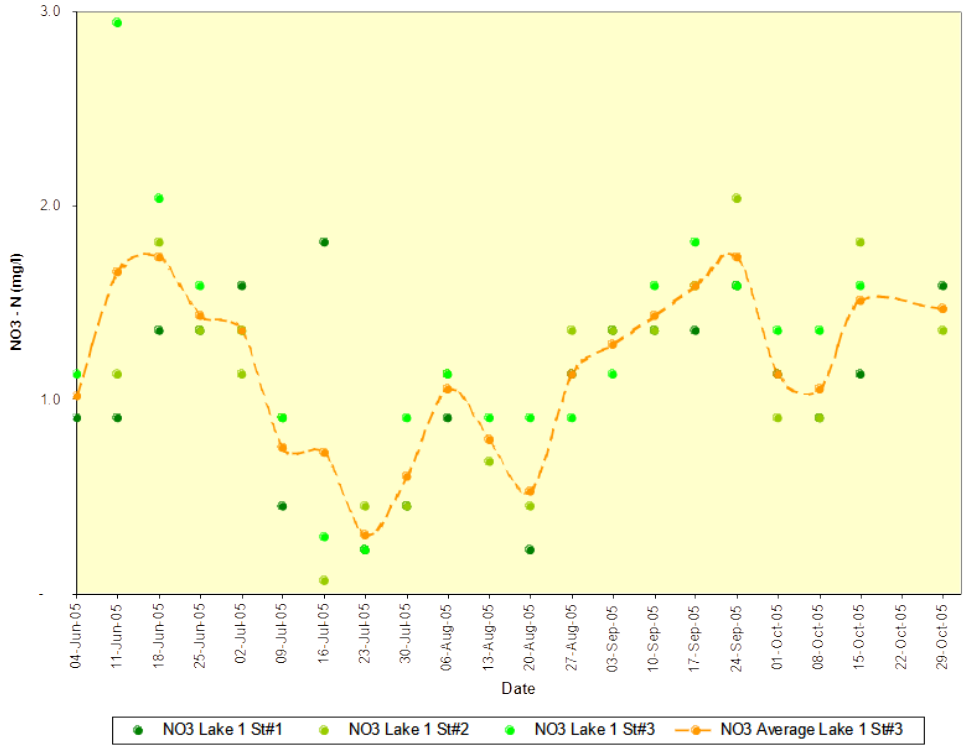


Figure 19 Nitrate - Nitrogen test results for Lake 1

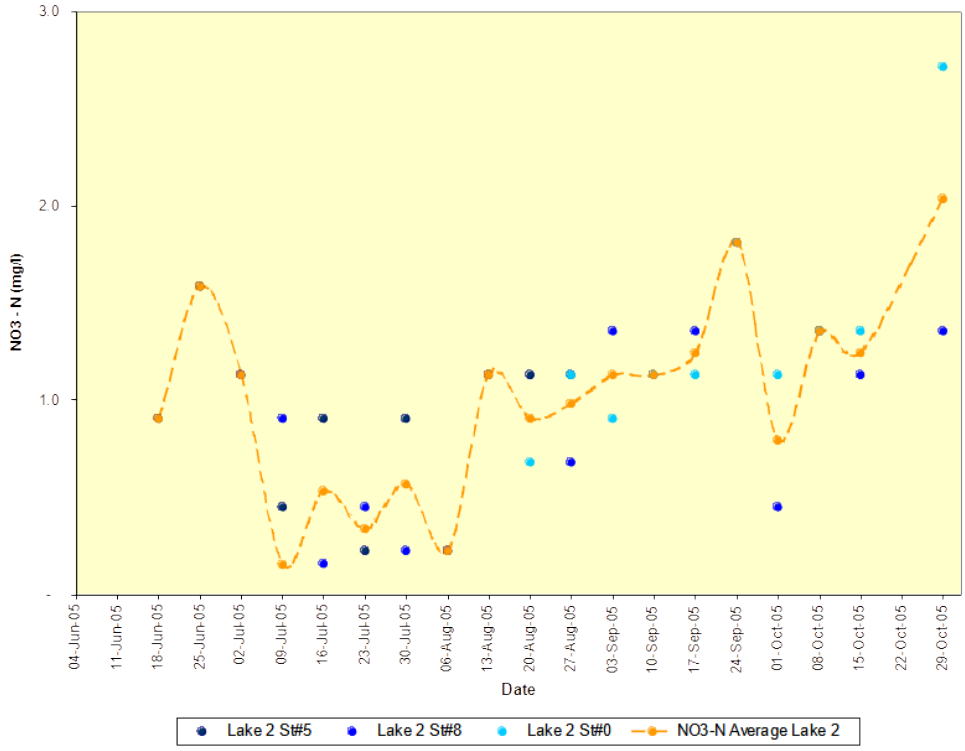


Figure 20 Nitrate - Nitrogen test results for Lake 2

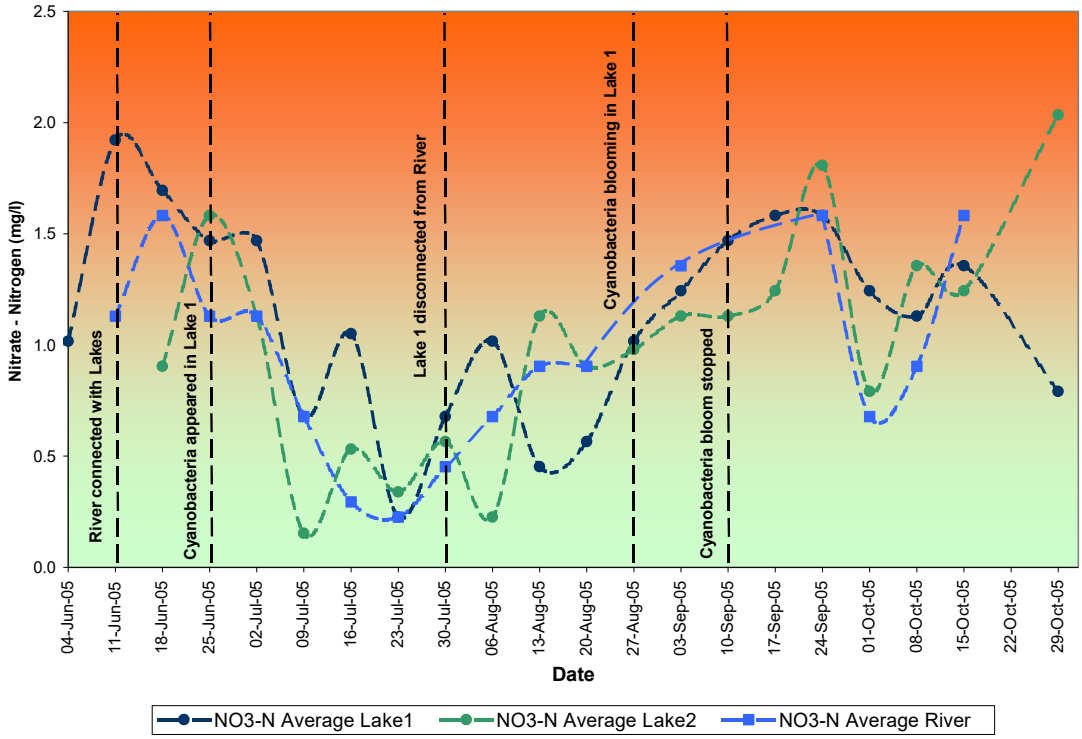


Figure 21 Comparative NO_3 –N analyses of the Assiniboine River, Lake 1 and Lake 2

Ammonia-Nitrogen

Ammonia-Nitrogen concentrations in Lake 1 were relatively constant and fluctuated between 0.1 and 0.7 mg/l until the end of August. By the first days of September, the ammonia concentration started to increase steeply and steadily (Figure 22, Figure 24), and by the end of October, it reached an average concentration of 3.15 mg/l. This increase coincides with the decreasing oxygen saturation of the water, because the oxidation of ammonia to nitrate, a process called nitrification, requires the presence of oxygen. Nitrification is carried out by bacteria that multiply rather slowly.

Ammonia-Nitrogen concentrations in the Lake 2 also fluctuated in the 0.2-0.8 mg/l interval until the end of September (Figure 23, Figure 24). As with Lake 1, ammonia concentrations increased steeply up to an average of 1.64 mg/l (between October 1 and October 8) and dropped to 1 mg/l by the end of October.

A comparison of the ammonia analyses shows that in the June – August interval concentrations are similar and fluctuate in average between 0.2-0.8 mg/l in all three water bodies (Figure 24). In early September, the steep increase of the NH_3–N level in Lake 1 coincides with the cyanobacteria bloom and subsequent decay, which presumably releases much of the nitrogen that the cyanobacteria have accumulated with their nitrogen fixation. The ammonia concentration in Lake 2 increases one month later, but does not reach the ammonia level of the Lake 1, while the ammonia concentration in the river stays almost constant during the whole investigation period.

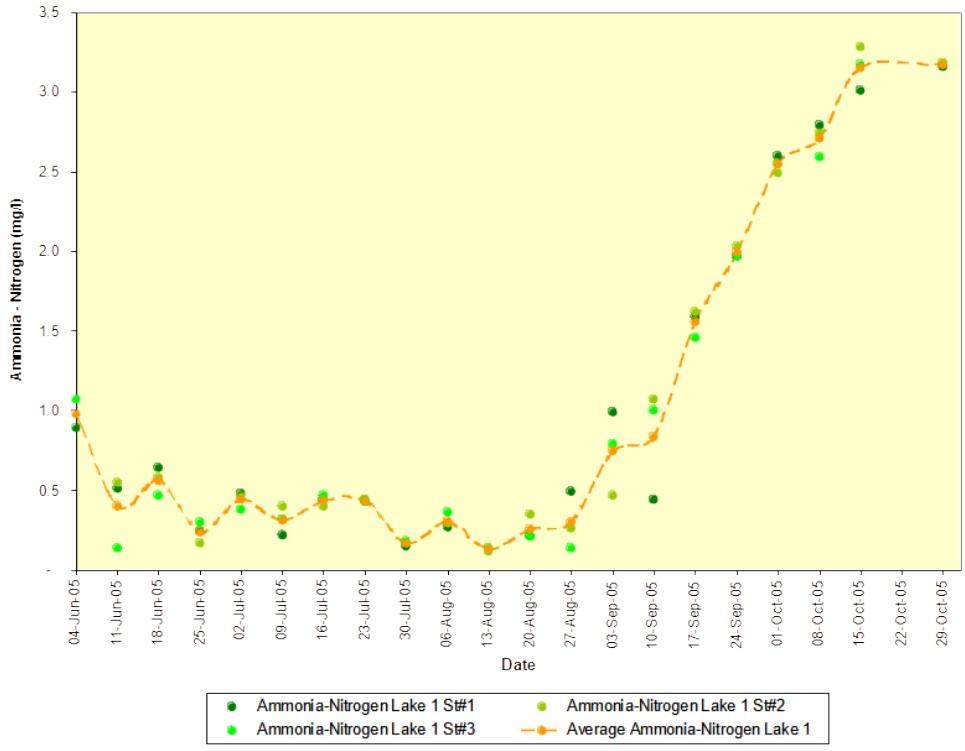


Figure 22 Ammonia-Nitrogen test results for Lake 1

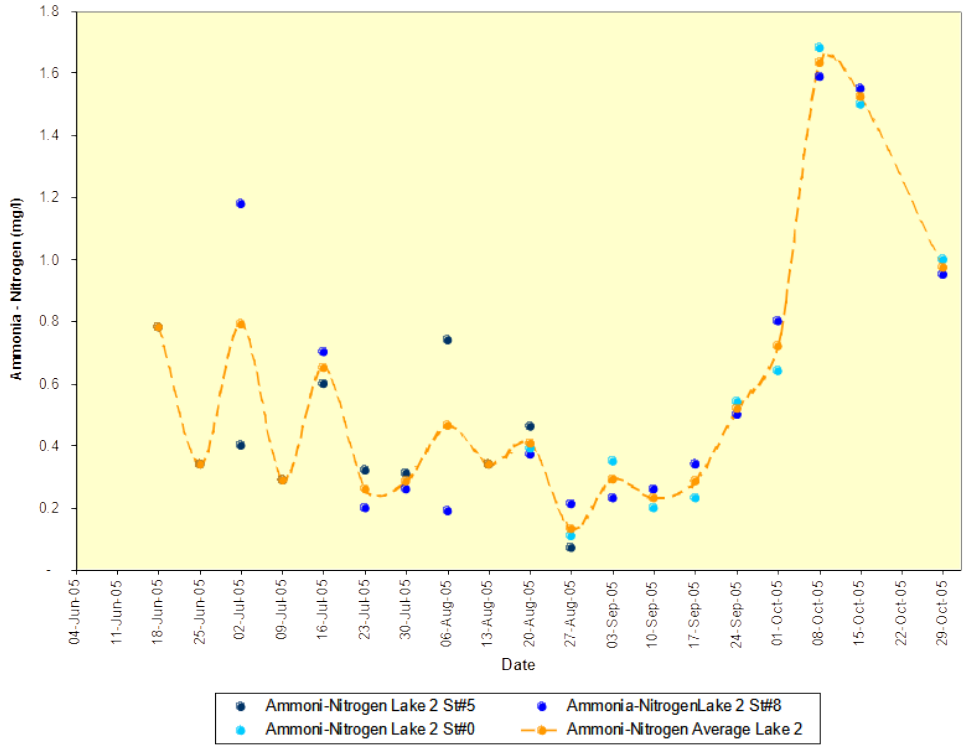


Figure 23 Ammonia-Nitrogen test results for Lake 2

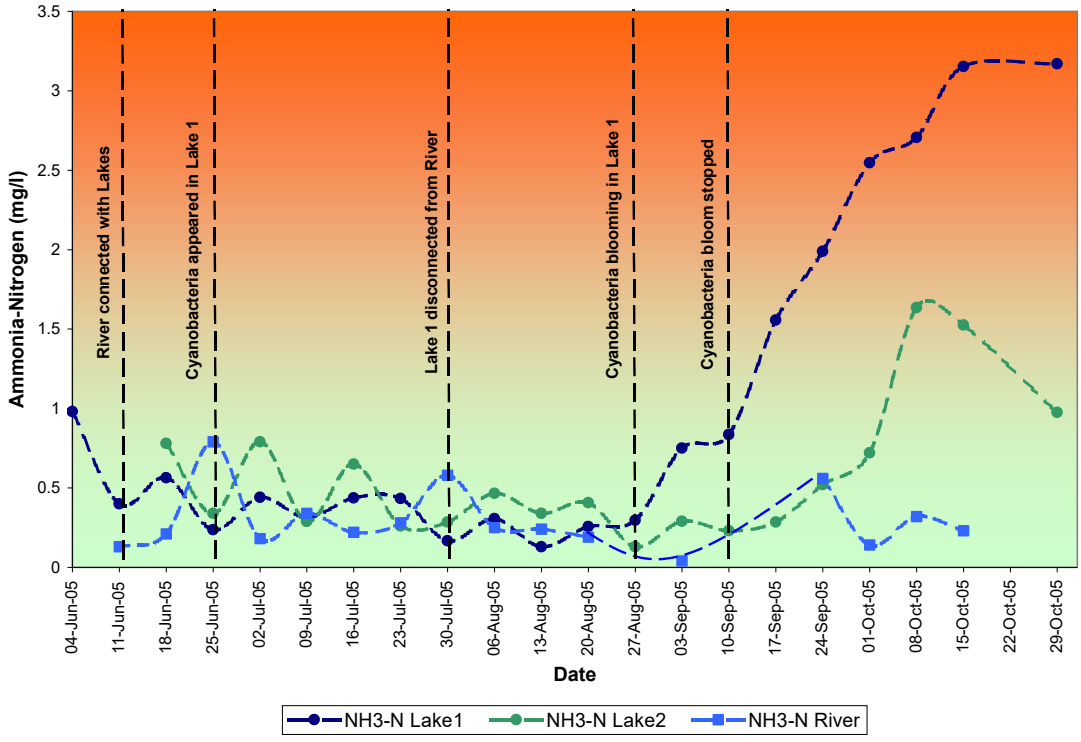


Figure 24 Comparative NH_3–N analyses of the Assiniboine River, Lake 1 and Lake 2

Please note: As with nitrate, ammonia concentrations are ammonia-nitrogen values.

Ammonia is toxic for fish. A level of 1 mg/L is commonly viewed as a threshold level that should not be exceeded, if this were wastewater discharge. Ammonia levels were acceptable, with the exception of the time period after early September, when levels steadily began to increase.

Algae bloom and high ammonia concentrations in Lake 1 are likely related.

Biological Testing

Microscopic Observations

Microscopic observations were performed during the whole investigation period and concentrated on the micro-algae population of all three water bodies. Most of the observed micro-objects were documented, photographed, and in part determined to the genus level, sometimes species level.

Phytoplankton and other organisms in Lake 1

At the beginning of the observation period, the variety of phyto-and zooplankton in the water was wide (high bio-diversity). The algae community consisted of representatives of *Euglenophyta*, filamentous *Chlorophyta* (mainly *Chara*), and diatoms. *Chara* was distributed along the lake banks in shallow spots. The water surface was covered with duckweed. The amount of duckweed was increasing to the end of June and reached the widest distribution in the middle of July (Figure 25). Zooplankton was present by crustaceans like *Daphnia* and *Cyclops*.

Figure 25 Duckweed distribution on the Lake 1 at July 16th, 2005

Cyanobacteria appeared in the second half of the June and then its amount was slowly but steadily increasing, ***which with the rising water level was hardly noticeable***. Initially, elongated flakes of *Aphanizomenon flos aquae* were swimming on the water surface (Figure 26**a**). Subsequently, *Anabaena* flakes appeared and were forming globular colonies that were sinking to the lake bottom at the banks (Figure 26**b**). Together with cyanobacteria, the lake water still contained some diatoms and green algae (Figure 26**c-d**). The crustacean population decreased.

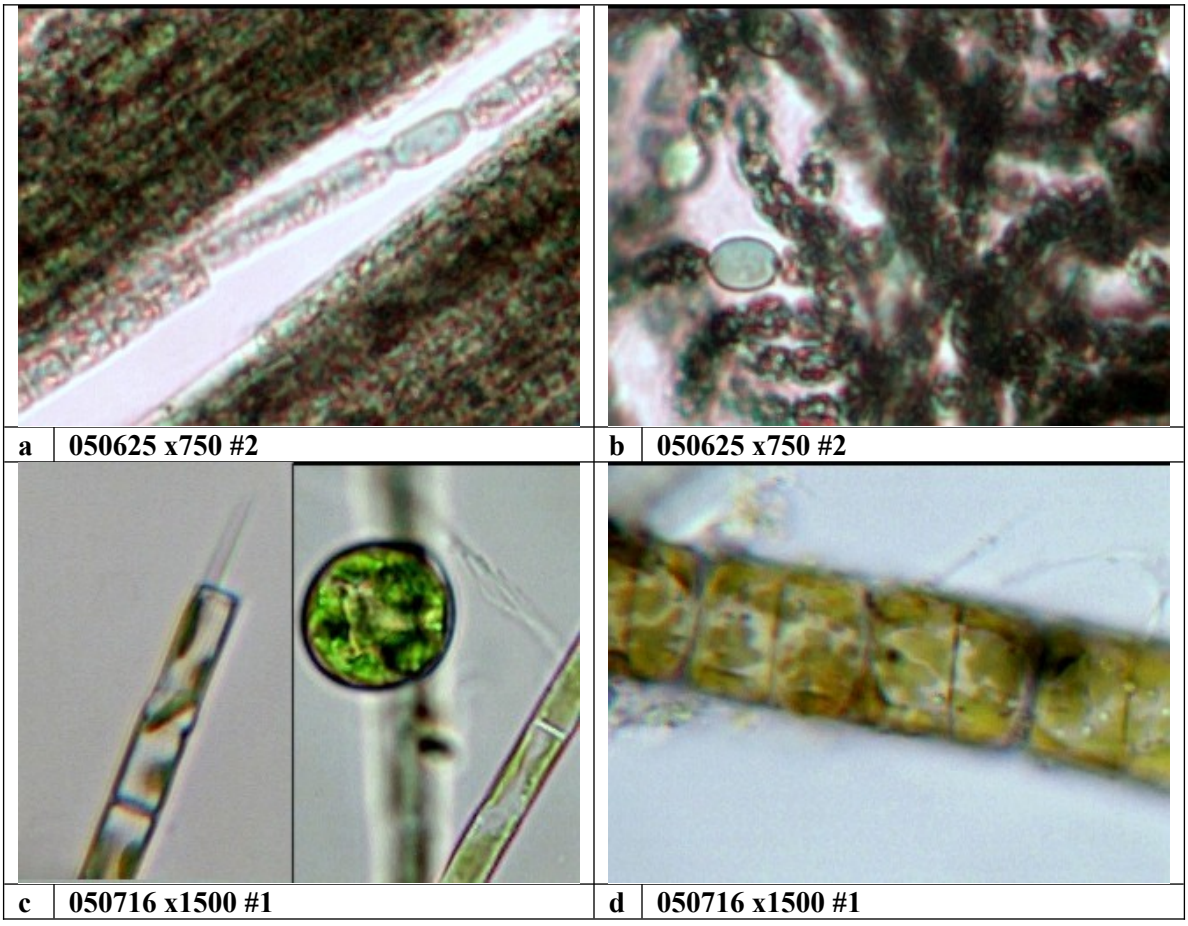


Figure 26 Phytoplankton in Lake 1 prior to blooming: a. *Aphanizomenon flos aquae*; b. *Anabaena sp.*; c. *Aulacoseira granulata* and unidentified *Chlorophyta* (green sphere); d. Unidentified algae (likely diatom)

Significant changes occurred from the second half of July, after the rise of the water level had ended. On July 23rd and July 30th it was found that the amount of duckweed increased, and the growth of *Chara* and other water plants at the shallow points in the lake decreased, while larvae and fry as well as sizeable fish were seen everywhere. The water surface was covered with a greyish film.

On August 6th and 13th, with dropping water level (Figure 27), the water turned greenish. The growth of *Chara* intensified (Figure 28**a**), and the amount of cyanobacteria, crustaceans and fish larvae increased (Figure 28**c**), while duckweed almost disappeared. Fish was appearing at the water surface (Figure 28**b**).

Figure 27 Water level changes: a. August 13, 2005; b. July 9, 2005

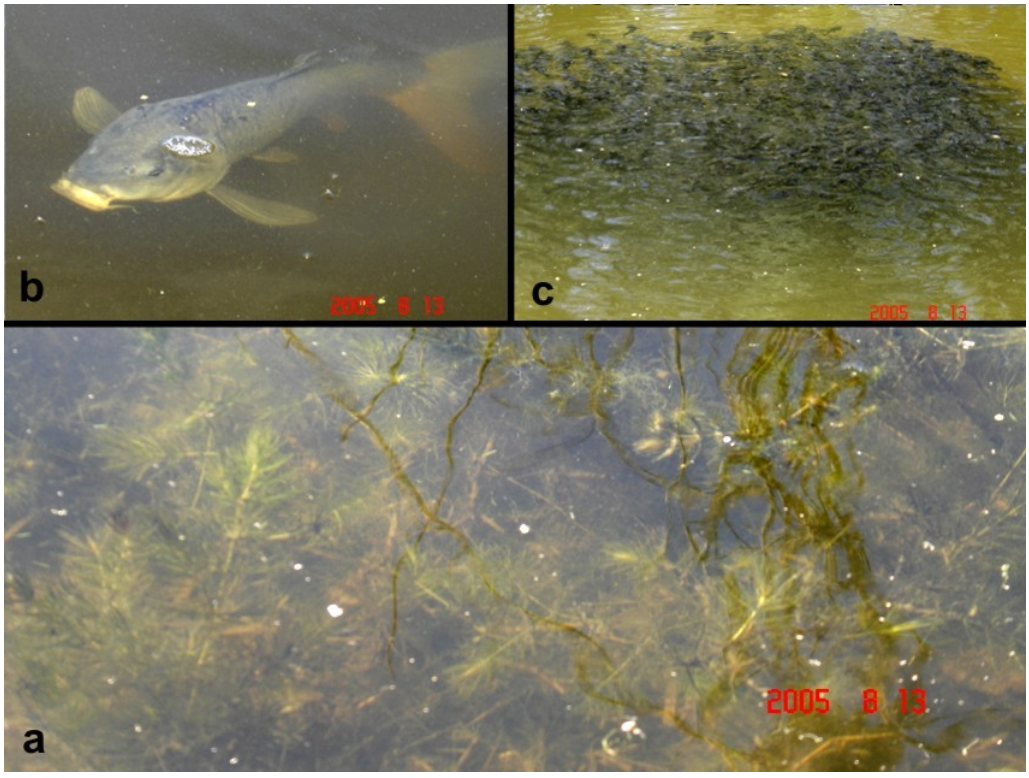


Figure 28 August 13, 2005: a. *Chara*; b. Adult fish; c. Cat fish larvae

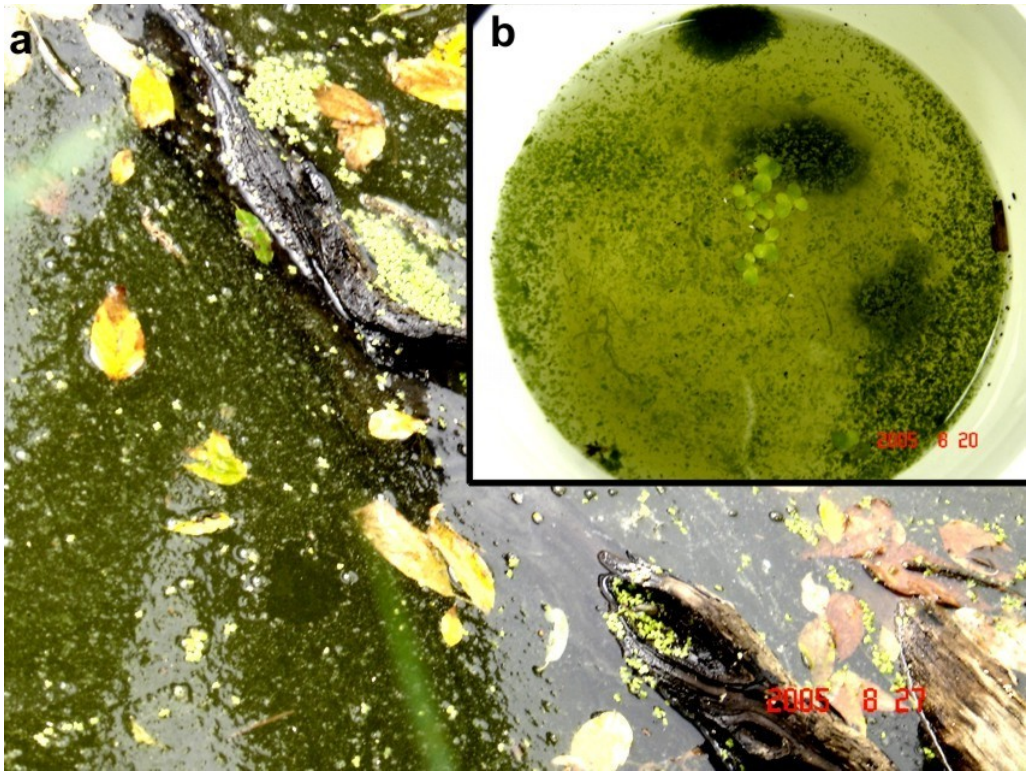


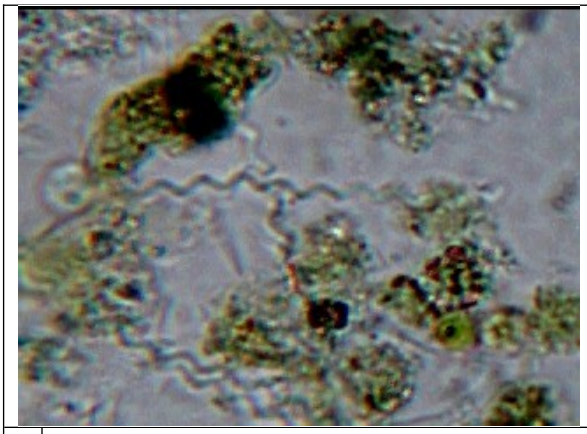
Figure 29 Abundance of cyanobacteria after August 20, 2005: a. Station #3; b. Water sample from the station #2 in a plastic cup

Figure 30 Cyanobacteria bloom at the lake bank not far from station #7

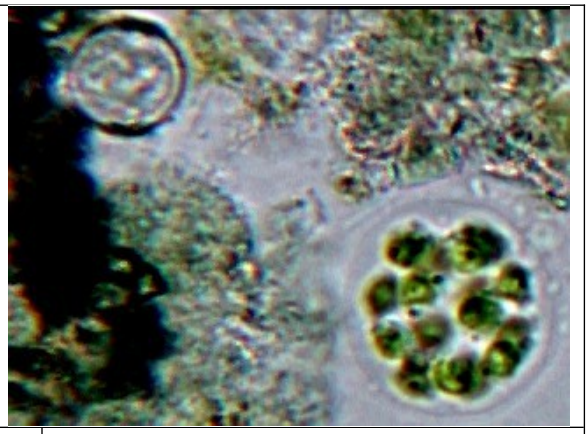
Beginning August 20th, the amount of the cyanobacteria increased dramatically to the extent that the water turned green and looked like "green soup" with bubbles (Figure 29); fish and crustaceans could not be found any longer. The water level had dropped more. Canada geese started to land on the lake and around it. The birds were swimming on and feeding from this water.

On September 3rd, cyanobacteria started to bloom, and the bloom lasted about one week. The bloom turned the water to a turquoise colour, accompanied by a strong unpleasant smell (Figure 30). The amount of geese increased. The birds invaded the lake and stayed there till the end of October.

During the bloom, almost no other organisms have been found in the water, just cyanobacteria, some decaying matter, bacteria, and micro-*Chlorophita* (Figure 31). One week later, the water was re-gaining phyto- and zooplankton. A significant amount of *Daphnia* and *Cyclops* was observed together with some diatoms and bacterial flocs. Figure 32 shows an algal community during the recovery process after the cyanobacteria bloom. One should note that at the end of the investigation the lake phyto- and zooplankton community returned to the type that has been observed at the beginning of the observation, with the exception that fish was now absent.

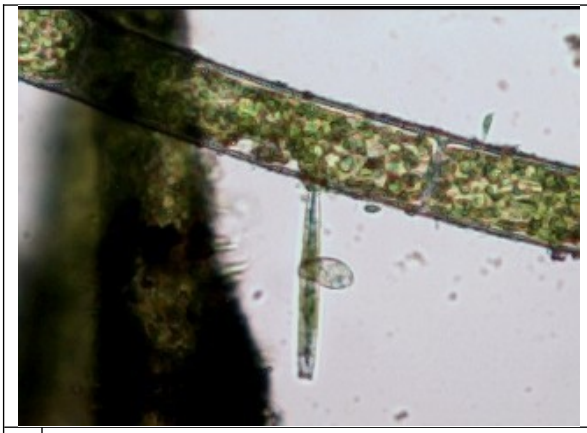


a **050903 x1500 #2**



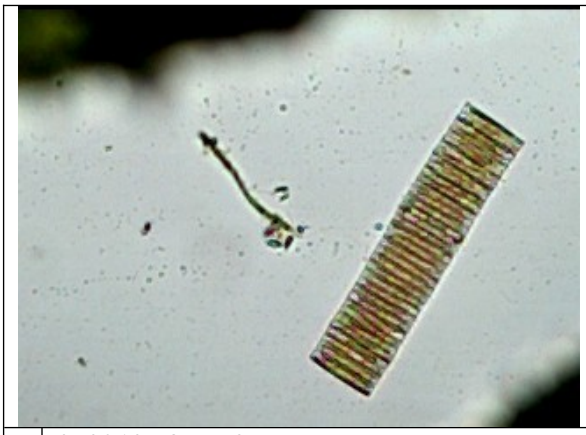
b **050903 x1500 #2**

Figure 31 a. Bacterial background with some algae and decaying matter; b. Unidentified *Chlorophyta* (*Pandorina*?) on the bacterial background

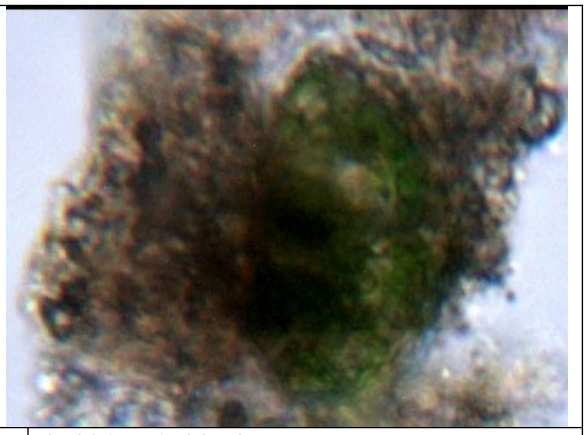


a **050910 x375 #2**

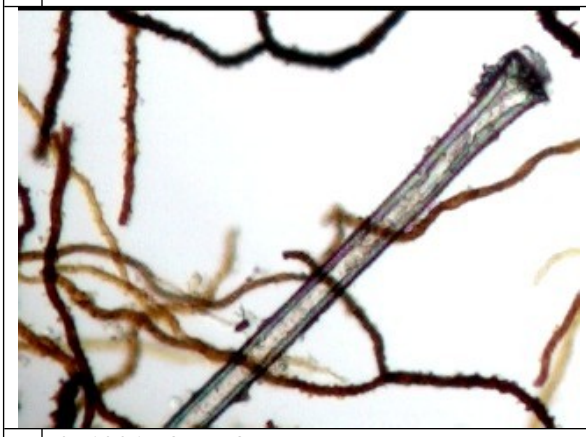
b **050910 x375 #20**



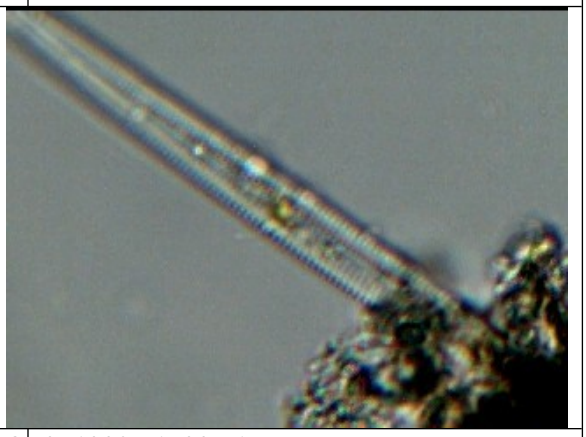
c | **050910 x375 #2**



d | **050917 x1500 #2**

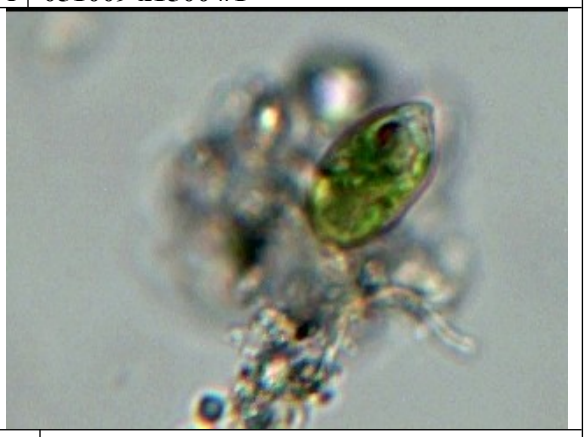


e | **051001 x375 #3**

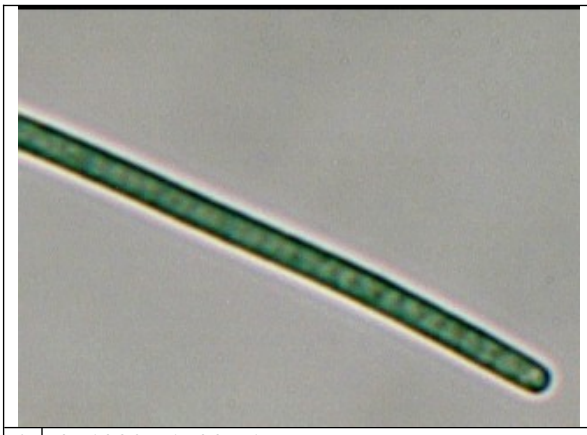


f | **051009 x1500 #1**

g | **051009 x1500 #1**



h | **051009 x1500 #1**



i	051029 x1500 #1	j	051029 x1500 #1

Figure 32 a. *Chlorophyta* (biggest size) with likely diatom attached and protozoa nibbling on diatom; b. ? *Cymbella* (diatom); c. *Fragilaria* (diatom); d. *Euglena sp*. in the bacterial or diatom flake; e. *Asterionella formosa* with unidentified filamentous algae; f. *Cymbella* (diatom); g. *Gomphonema* (diatom); h. *Euglena sp*.; i. Unidentified cyanobacteria; j. ? *Dictyosphaerium*

Cyanobacteria invaded Lake 1 and started to bloom at the beginning of September; this lasted about one week. This bloom caused eutrophication of the Lake 1 and consequently the death of likely the whole population of zooplankton of the lake.

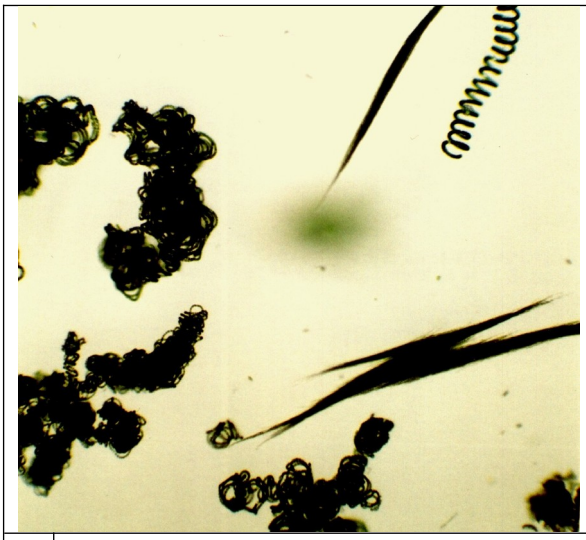
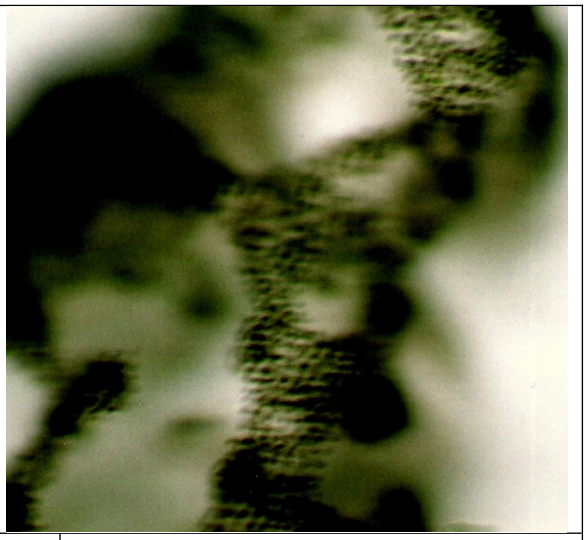
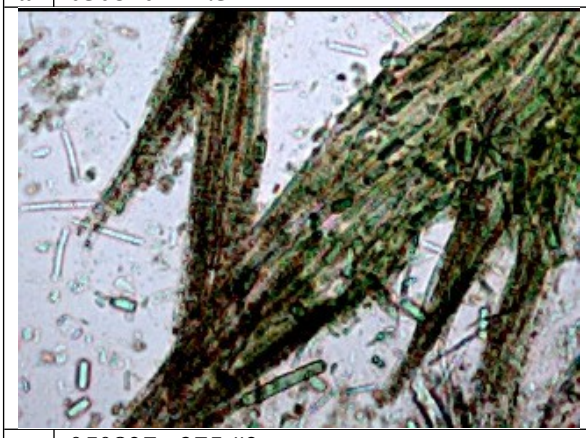
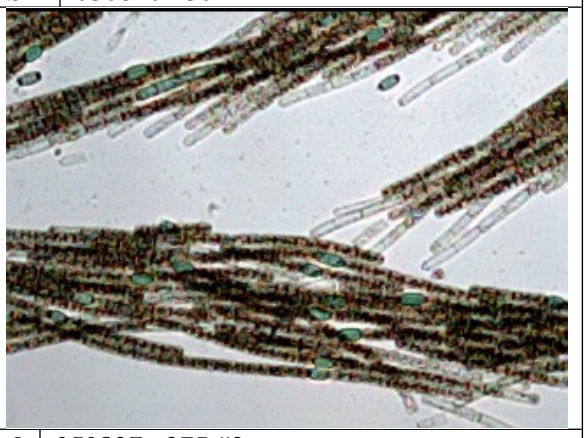
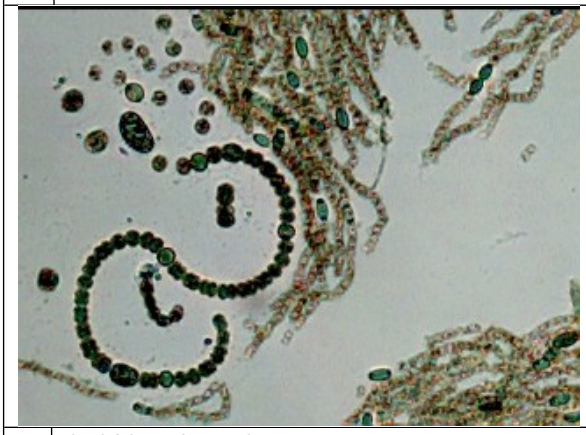
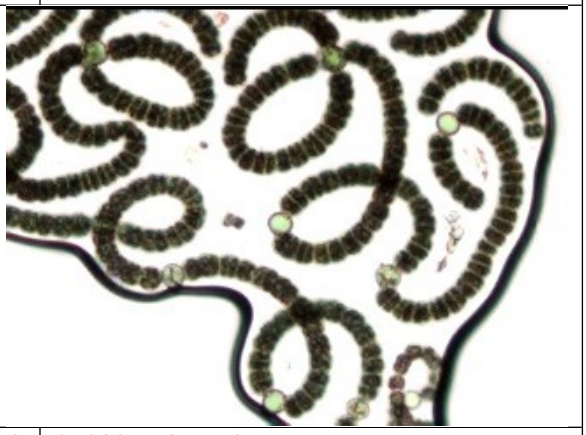
One should note that at the same time, a vast cyanobacteria bloom happened in Lake Winnipeg[5] and Lake of the Woods [6]. Several beaches at these lakes were closed and advisory notes were posted around preventing swimming and use of the water by humans and livestock.

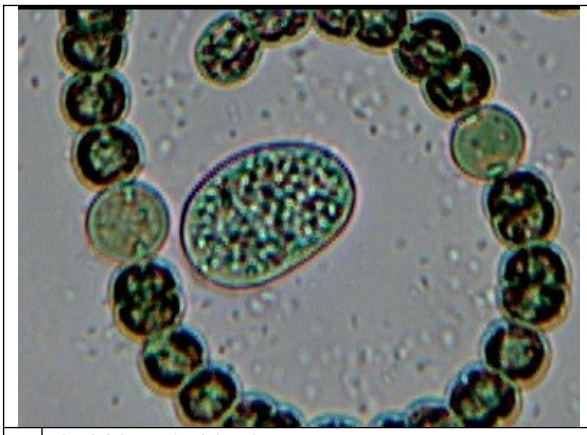
Cyanobacteria

The number of cyanobacteria species that have been found to produce a suite of biologically active compounds, has been ever increasing in recent years. Not surprisingly, many of these compounds have proven to be very toxic to a variety of organisms including humans. Some species of cyanobacteria are capable of producing a variety of toxic compounds, although some toxins appear to be specific for certain cyanobacteria[7].

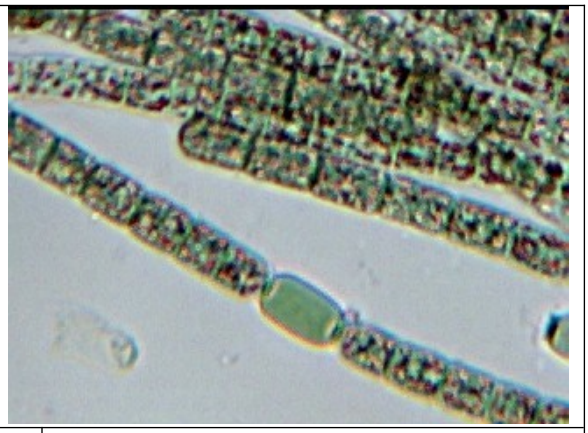
The water containing cyanobacteria can be potentially toxic to wildlife and humans, with animals drinking this water falling ill and eventually dying a painful death. The effect of micro-algal toxins, both in marine and freshwater environments, has increased in severity in recent years, and poisoning episodes are becoming more common and more widespread. For example, in ***the Midwestern United States, the consumption of contaminated water has resulted in the deaths of ducks and geese by the thousands***[8].

[5] Winnipeg Free Press, September 6, 2005 (B1 "Algae bloom covers lake")
[6] Winnipeg Free Press, October 9, 2005 (B1 "Scum of the Earth")
[7] http://www-cyanosite.bio.purdue.edu/cyanotox/toxiccyanos.html
[8] http://www.chm.bris.ac.uk/motm/antx/antxv.htm

	
a 050820 x12.5 #2	**b** 050820 x50 #2
	
c 050827 x375 #2	**d** 050827 x375 #2
	
e 050827 x375 #2	**f** 050827 x375 #2



g	050827 x1500 #2



h	050827 x1500 #2

Figure 33 a. *Aphanizomenon* and *Anabaena*; b. *Microcystis aeruginosa*; c. *Aphanizomenon flos aquae* (partially decomposing); d. *Aphanizomenon flos aquae* (partially decomposing); e. *Aphanizomenon flos aquae* and *Anabaena flos aquae*; f. *Anabaena sp.*; g. *Anabaena sp.*; h. *Aphanizomenon flos aquae*

Anabaena and ***Aphanizomenon*** are genera of filamentous nitrogen-fixing cyanobacteria and are known for producing a suite of cyanotoxins, which range from the ***neurotoxic anatoxin*** and ***saxitoxin*** to the ***hepatotoxic microcystin***.

The **anatoxins** (Figure 34) are a group of neurotoxic alkaloids produced by a number of cyanobacterial genera including *Anabaena*, *Oscillatoria* and *Aphanizomenon* (Figure 33 **a, c-h**). The toxicity of these compounds (LD_{50}) varies from 20 µg/kg (by weight, I.P. mouse) for anatoxin-a (S), to 200-250 µg/kg for anatoxin-a and homoanatoxin-a, making them more toxic than many microcystins. For comparison, the 20 µg/kg correspond to 1.5 mg per adult human, if we assume an average weight of 75 kg for an adult. This makes anatoxin-a 5 to 10 times more toxic than potassium cyanide ("prussic acid") that is the essential ingredient of so many classical crime stories.

The first published report of the potentially lethal effects of micro-organisms known at that time as blue-green algae appeared in *Nature* in 1878. George Francis described an "algal bloom" that had formed in the estuary of the Murray River, in Australia, as "a thick scum like green oil paint, some two to six inches thick"[9].

Concern for wildlife and also issues related to public health led to much investigation into the causes of these mass animal mortalities, and in the 1950s and 1960s Paul Gorham and his co-workers at the National Research Council in Ottawa established cultures of *Anabaena flos-aquae*, which allowed them to isolate the poisonous compounds which it produces. Anatoxin is perhaps one of the most toxic of the cyanobacterial toxins in this group, since the effects of ingestion can be lethal within 4 minutes, depending on the quantity consumed. This led to the compound being dubbed "Very Fast Death Factor." The chemical structure is shown (below); it is an interesting bicyclic alkaloid, and it was hoped that knowledge of this structure would enable scientists to discover the mode of action of the toxin.

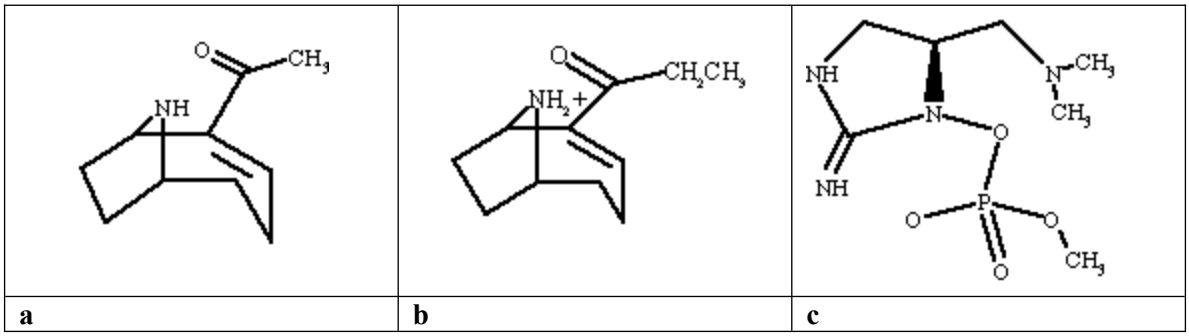
Like anatoxins, the **saxitoxins** (STX, Figure 35) are neurotoxic alkaloids which are also known as PSP's (paralytic shellfish poisons) due to their occurance and association with seafood. They block sodium channels in nerve cells, thus causing their neurotoxic effects. There are a number of STX variants generally divided into groups based on their structure or organism of origin. The

[9]http://www.chm.bris.ac.uk/motm/antx/antxv.htm – University of Bristol, School of Chemistry

single sulphated STX's are known as gonyautoxins (GTX) and the doubly sulphated STX's are known as C-toxins. There are also decarbamyl STX's (dcSTX) and a group of STX variants, so far found only in Lyngbia wollei, known as Lyngbia-wollei-toxins (LWTX).

STX's are highly toxic with LD50's as low as 10 µg kg-1 (i.p.) in mice.

The STX family has the following general structure (Figure 35).



a **b** **c**

Figure 34 a. Anatoxin-A; b. Homoanatoxin-A; c. Anatoxin-A (S)[10]

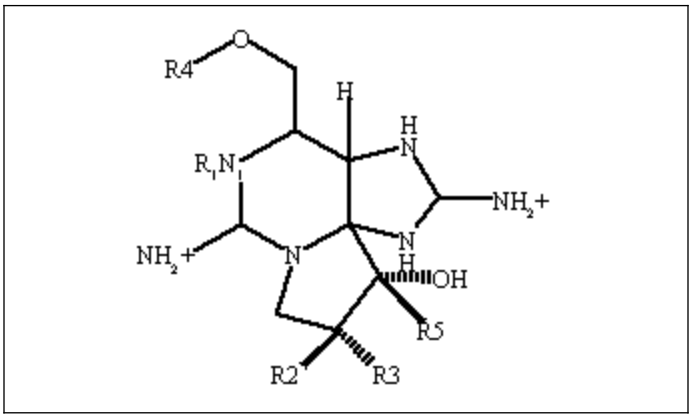


Figure 35 Saxitoxin general structure[Error: Reference source not found]

The **microcystins** (Figure 36) are a group of cyclic heptapeptide (7 amino acids) hepatotoxins (liver toxins) produced by a number of cyanobacterial genera, the most notable of which is the widespread *Microcysts* (Figure 33**b**) from which the toxins take their name. Microcystins have been reported in this organism and other cyanobacteria world-wide. There have been approximately 60 different microcystins identified to date.

Microcystins consist of a seven-membered peptide ring, which is made up of five non-protein amino acids and two protein amino acids. It is these two protein amino acids that distinguish microcystins from one another, while the other amino acids are more or less constant between variant microcystins. Using amino acid single letter code nomenclature, each microcystin is designated a name depending on the variable amino acids which complete their structure. The most common and potently toxic microcystin-LR contains the amino acids leucine (L) and arginine (R) in these variable positions.

Below is the general stucture of microcystins showing the variable amino acid positions "X" and "Z". The amino acids are delineated in this diagram and numbered according to the microcystin standard nomenclature. R1 and R2 are H in demethylated microcystins.

[10] http://www-cyanosite.bio.purdue.edu/cyanotox/toxiccyanos.html

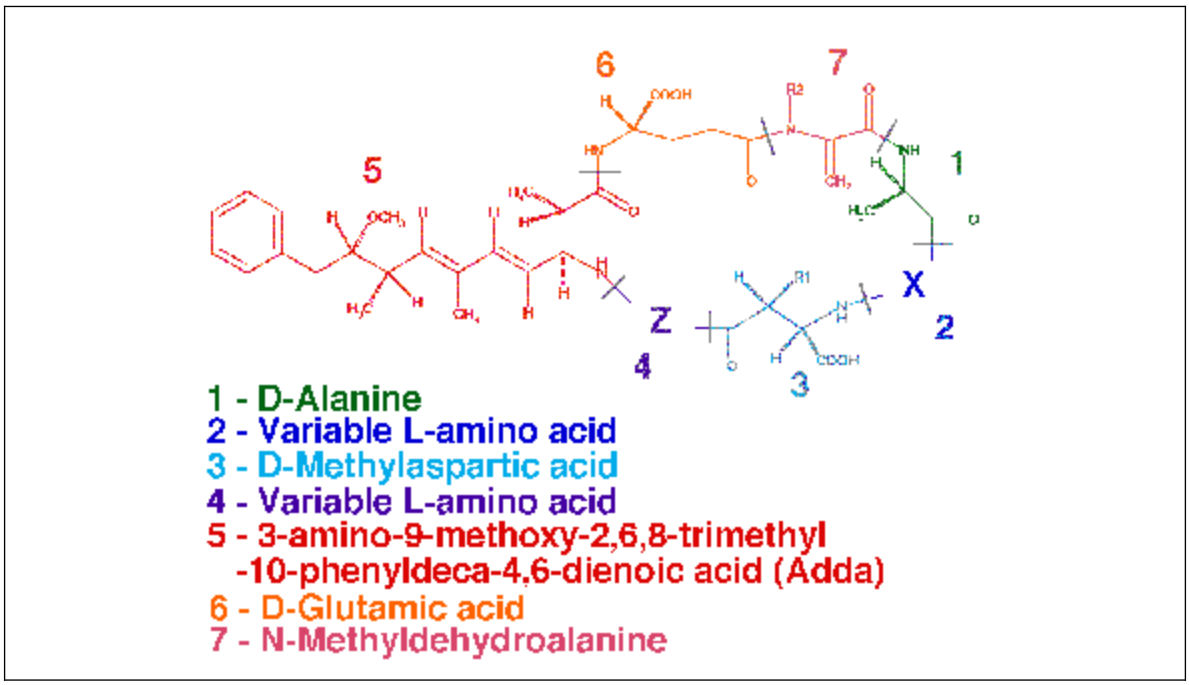


Figure 36 Microcystin general structureError: Reference source not found

Phytoplankton and other organisms in Lake 2

During all field observations, filamentous *Chlorophyta* (mainly *Chara*) were growing along the banks and in some spots in the central parts of the lake. Duck weed was widely spread everywhere parallel to the shoreline. Typically duckweed was growing as a carpet on the top of *Chara*, forming green stripes along the banks (Figure 37). A high quantity of snails was observed in the *Chara* mass. The distribution of the duckweed and *Chara* stayed the same from from June through September. In October, the amount of duckweed and *Chara* started to decrease. Fish was not seen in Lake 2 during this whole study.

Mainly diatoms and some non-filamentous *Chlorophyta* were found at the beginning of the study in June until early August. The phytoplankton population in June – July was not dense. In early August, a small amount of *Euglena* species appeared. In mid-August, the density of *Euglenophyta* increased tremendously. This coincided with a significant drop of the water level. On August 20th, the *Euglena* population was so dense that the water looked like some dispersed solid matter and became greenish (Figure 38**a-b**). One week later, on August 27th the euglenoids still were abundant in the water, the lake level had dropped more, and the water was still greenish from high the amount of *Euglenophyta* (Figure 38**d-e**). Together with euglenoids, some dinoflagellates (Figure 38**c**), non-flagellated green algae (Figure 38**f**) and diatoms were found. During this whole period, an insignificant amount of crustaceans (mainly *Cyclops*) was present.

Euglena is unique because it is in some features like a plant (autotrophic - can make its own food) and also like an animal (heterotrophic - must consume food). It can gain nutrients by absorbing them across their cell membrane, hence they become heterotrophic when light is not available, and they temporarily cannot photosynthesise owing to the lack of light. Euglena can be found in almost any fresh or brackish water. It thrives best where there is an abundance of rich organic waste.

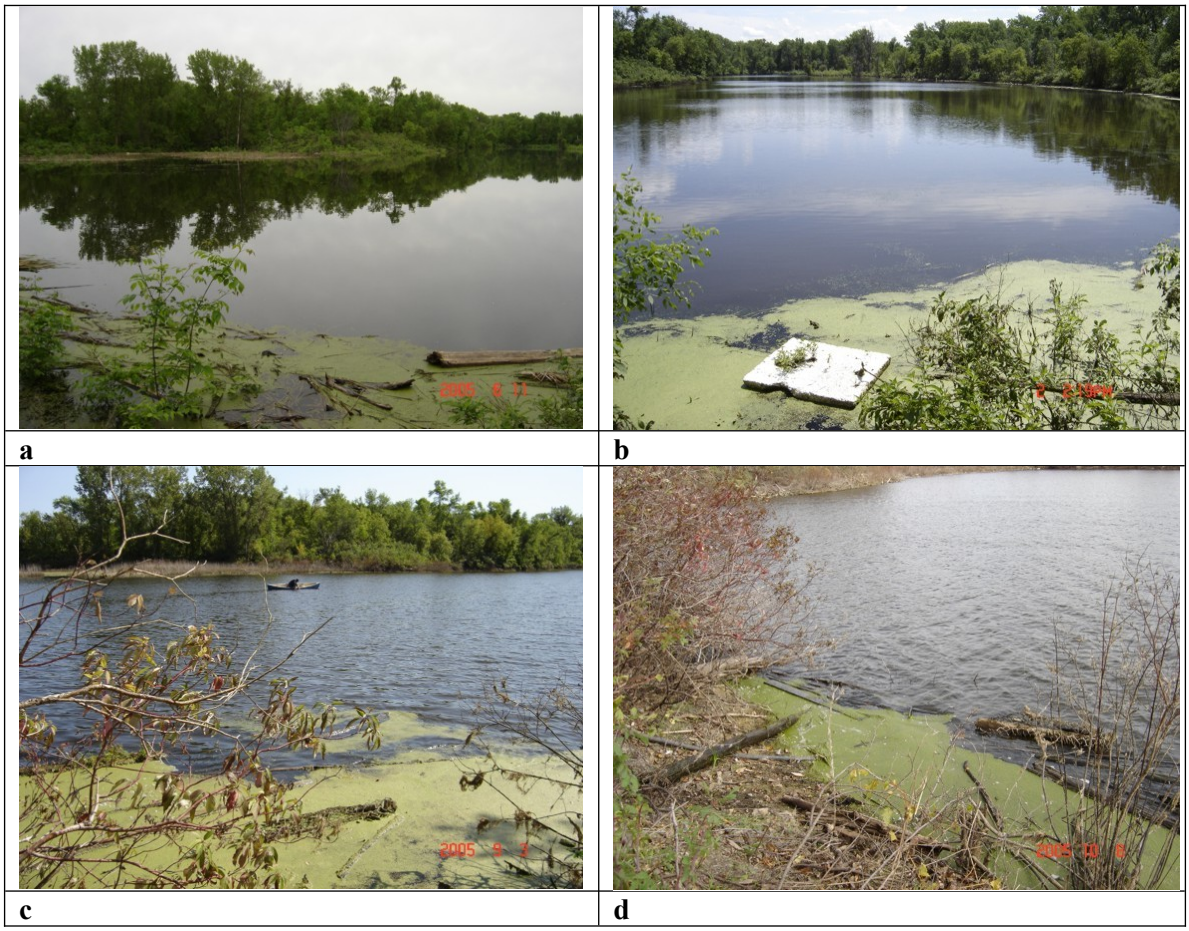


Figure 37 Pictures taken from: a. Station #8 (Jun. 11, 2005); b. Station # 5 (Jul. 02, 2005); c. Station #8 (Sep. 03, 2005); d. Station #8 (Oct. 10, 2005)

On September 3rd, the amount of phytoplankton suddenly dropped to the extent that just occasional organisms were seen. The water became clear again.

On September 10th, diatoms, euglenoids, and dinoflagellates re-appeared again, including some crustacean (*Cyclops*). One week later, the amount of algae started to increase with the dominance of green flagellated algae (Figure 39**a-b**).

"Importance (environmental, commercial, conservation, educational, scientific, social): In general, dinoflagellates are known for producing nasty toxins, particularly when in large numbers, called "red tides" because the cells are so abundant they make the water change colour. Also they can produce non-fatal or fatal amounts of toxins in animals (particularly shellfish) that may be eaten by humans."[11]

The next change happened at the beginning of October, when diatom flocs appeared in the water (Figure 39**c**). From then on, the water was containing mainly diatoms (Figure 39**e-f**) and colonies of green flagellated algae (Figure 39**d**).

One should note that during the period when geese were flying over the lakes area, they mainly stayed at Lake 1. In comparison with Lake 1, Lake 2 was almost unattended by the birds. This

[11] http://gmbis.marinebiodiversity.ca/BayOfFundy/taxListInfo.jsp?taxListInfo=Ceratium%20hirundinella

phenomenon can likely be explained by several factors, the most important of them being the lack of food (fish, plankton).

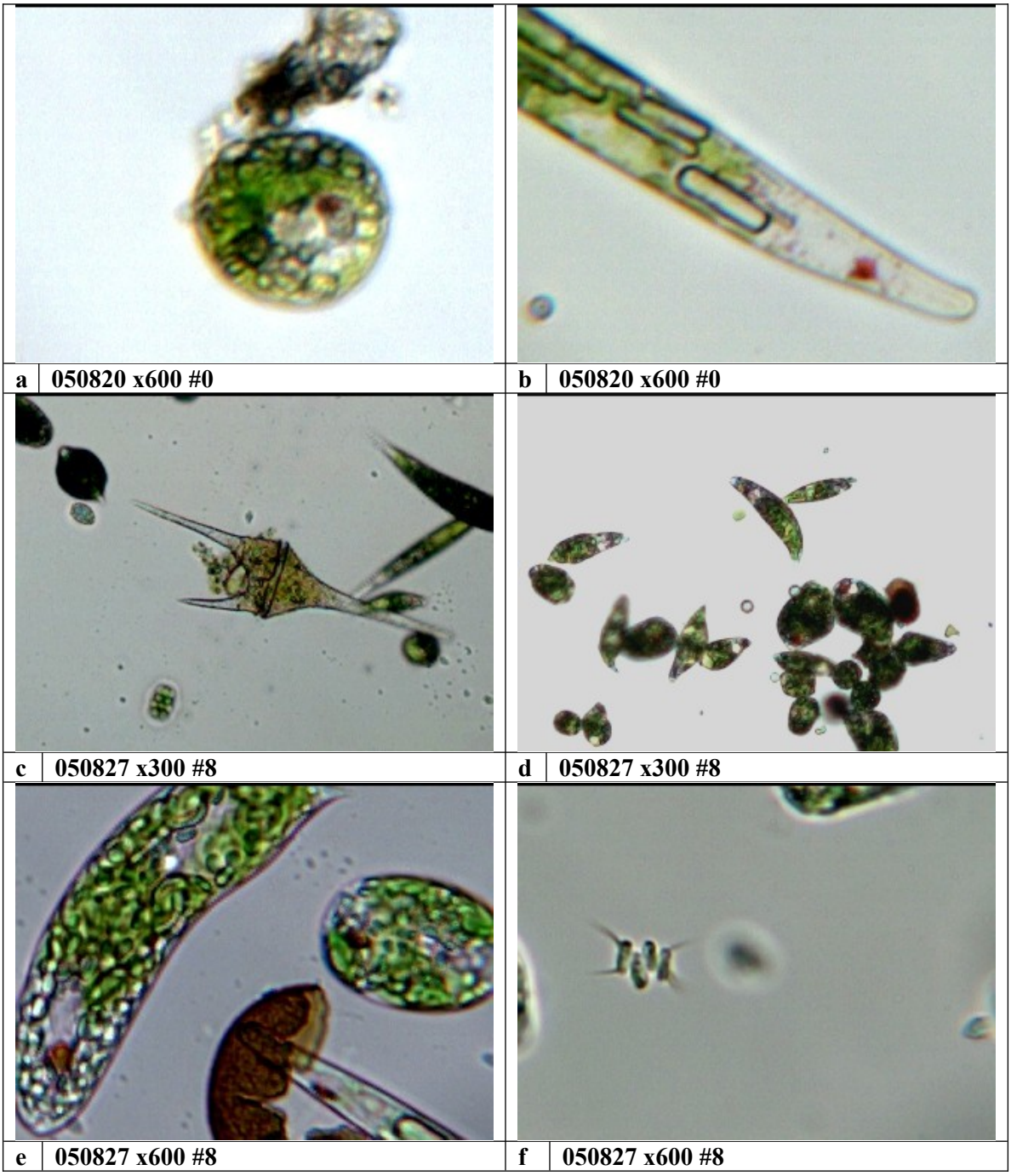


Figure 38 Algae in Lake 2 in August 2005: a – b. *Euglena* sp.; c. *Ceratium* (dinoflagelate) and *Euglena*; d – e. *Euglena*; f. *Scenedesmus*

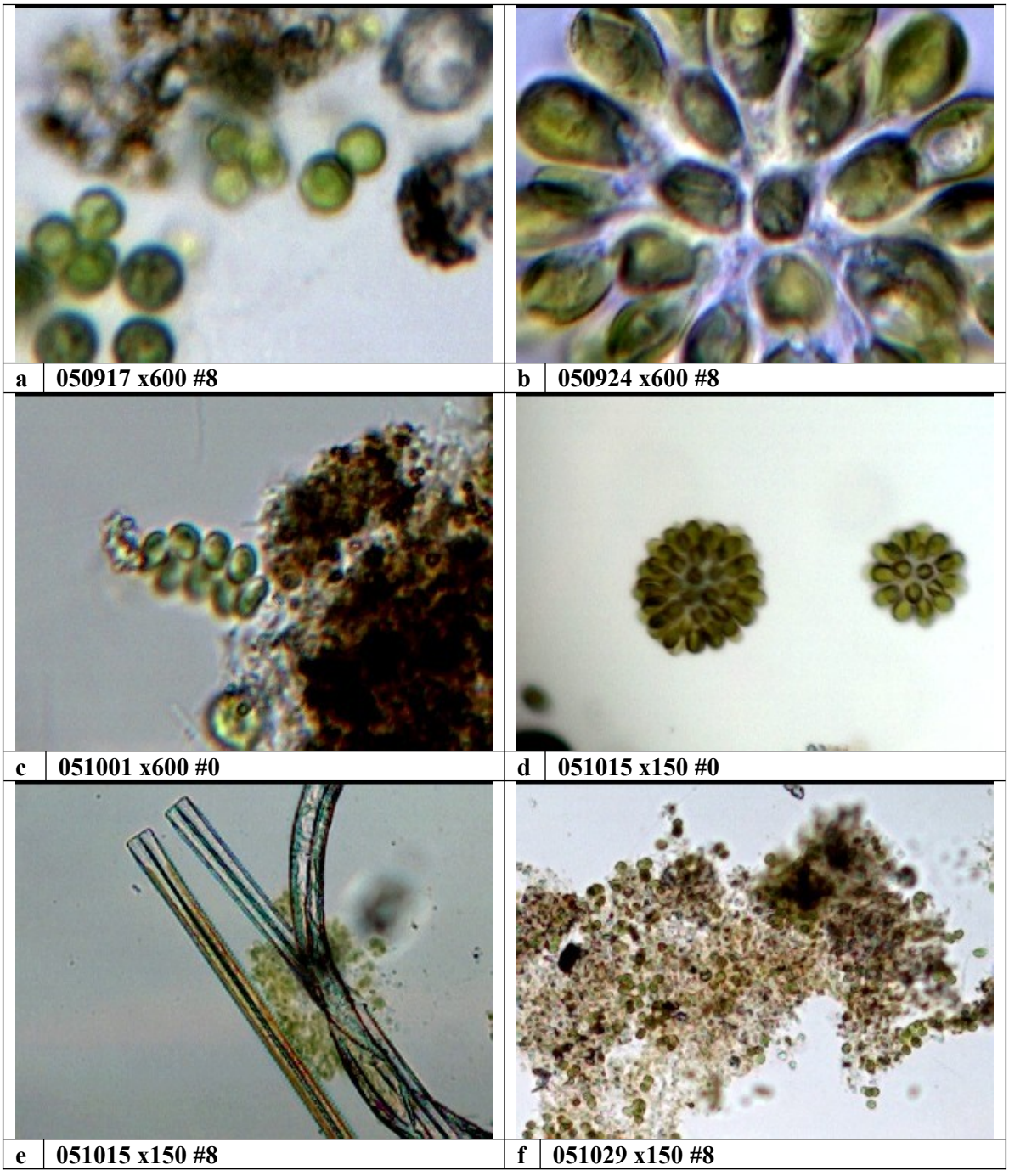


Figure 39 Algae in Lake 2 in September - October 2005: a-b. *Chlorophyta* (unidentified); c. diatoms (unidentified); d. *Chlorophyta* (unidentified) and diatoms; e. diatoms (unidentified); f. diatoms and green algae (unidentified)

While Lake 1 was invaded by cyanobacteria, Lake 2 was densely populated by the Euglenophyta, which do not have a known danger potential. The most dangerous among the observed plankton community can be certain Dinoflagelates.

Phyto- and zooplankton in the Assiniboine River

At the beginning of the study, the river water did not contain a dense population of the micro-organisms. The turbidity of the river water was high (Figure 40**a**) which likely did not allow the development of green algae and cyanobacteria. Phyto- and zooplankton appeared in the samples at the beginning of September, when the water level already decreased (Figure 40**b**).

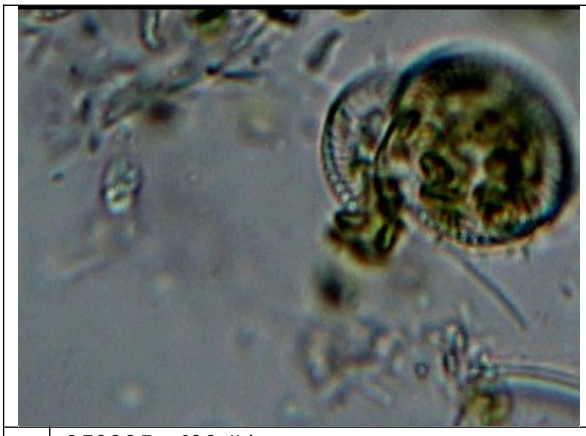
a

b

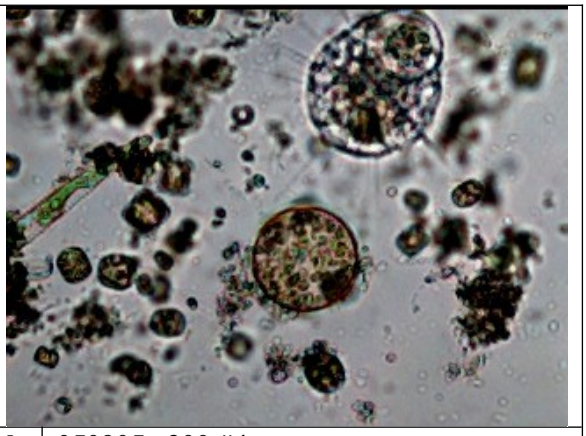
Figure 40 Assiniboine River levels: a. beginning of June; b. beginning of September

Phytoplankton was represented by diatoms (*Chrysophyta*), mainly *Stefanodiscus* (Figure 41**a-c**) and some *Euglenophyta* and *Chlorophyta* (Figure 40**b-c**). Zooplankton was represented by protozoa – *Heliozoa* (spherical amoeboids) and *Vorticella* (Ciliata). *Heliozoa* likely belongs to the order *Actinophryida* which is a small group of heliozoan protists. They are the most common *Heliozoa* in fresh water, and are especially frequent in lakes and rivers [12].

The group of the organisms found in the river water samples allows to preliminary classify this water as mesosuprobic-β – mesopolluted, closer to non-polluted.



a **050905 x600 #4**



b **050905 x300 #4**

[12] http://en.wikipedia.org/wiki/Actinophryid

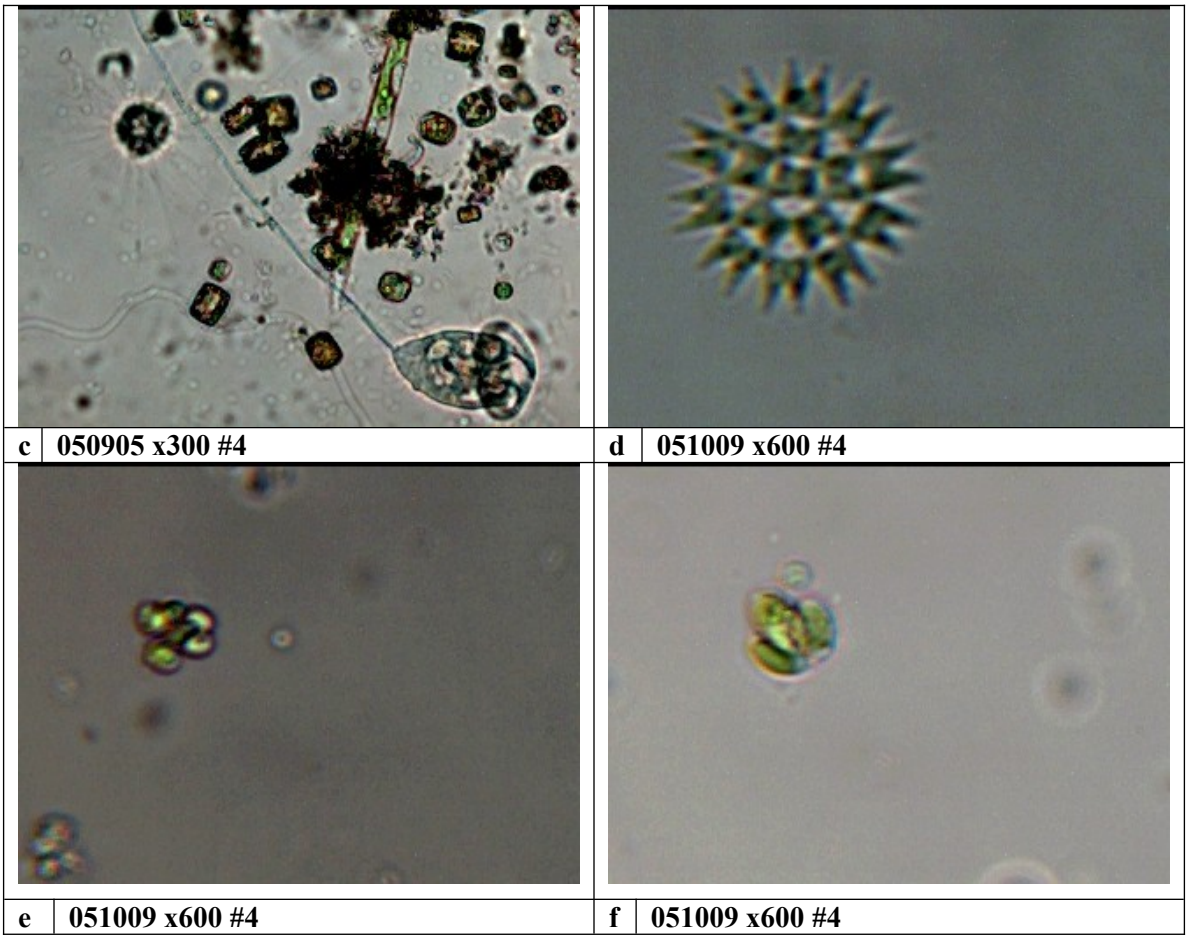


Figure 41 Algae in the River: a. *Stephanodiscus* (diatom); b. bacteria, *Stephanodiscus (*diatoms) and *Heliozoa*; c. *Heliozoa* (protozoa), *Vorticella* (protozoa), diatoms, *Euglena* and bacteria; d. *Chlorophyta* (likely *Pediastrum*); e. *Chlorophyta* (unidentified); f. *Chlorophyta* (unidentified)

According to this investigation, the phyto- and zooplankton groups of the Assiniboine River are not similar to the groups of the organisms from Lakes 1 and 2. In other words, the river is not dominated by cyanobacteria, as Lake 1 is.

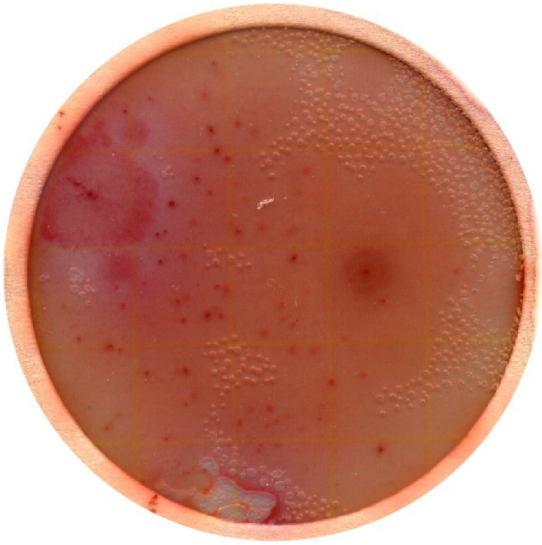
Coliform count

3M Petrifilm™ (Figure 42) was used for the coliform count. This type of analysis allows the count of coliform colonies (cfu – colony forming units), as opposed to the determination of a most probable number (MPN), which is the more popular test. Analyses were done at least twice a month.

At the beginning of the investigation and prior to the lakes – river interconnection (June 4, 2005), the amount of total coliforms in Lakes 1 and 2 was very high. The count was between 800 and 1,400 cfu/100 mL, which in fact exceeds commonly accepted levels for treated sewage by a wide margin. However, the amount of faecal coliforms was below the detection limit (Figure 43).

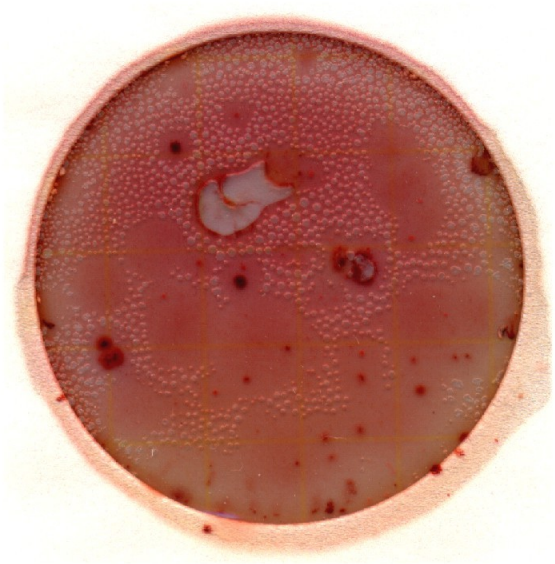
After and during the established connection with the river (June 11 – July 30, 2005, the number of coliforms in the lakes decreased to 100 – 300 cfu/100 mL, and was slightly lower than in the river samples (400 – 500 cfu/100 mL, Figure 43). It is significant that number of

faecal coliforms in the river samples was between 100 and 200 cfu/100 mL, while the count of faecal coliforms in the lakes was below the detection limit. The appearance of faecal coliforms always indicates pollution of the water body with raw sewage, if contamination through migrating waterfowl can be ruled out.



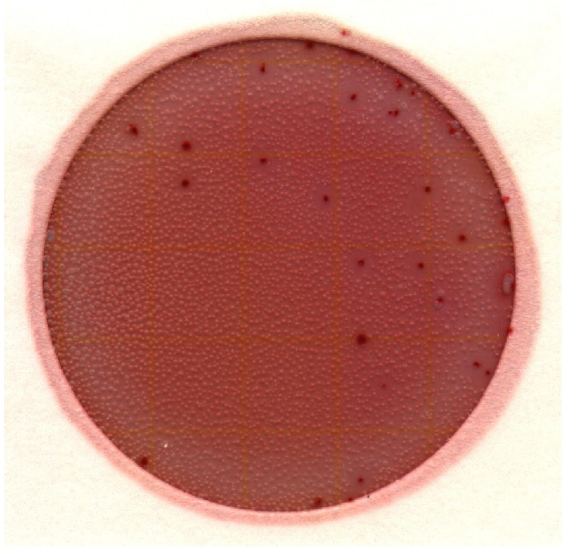
050604 St#1; dilution 1:5

a



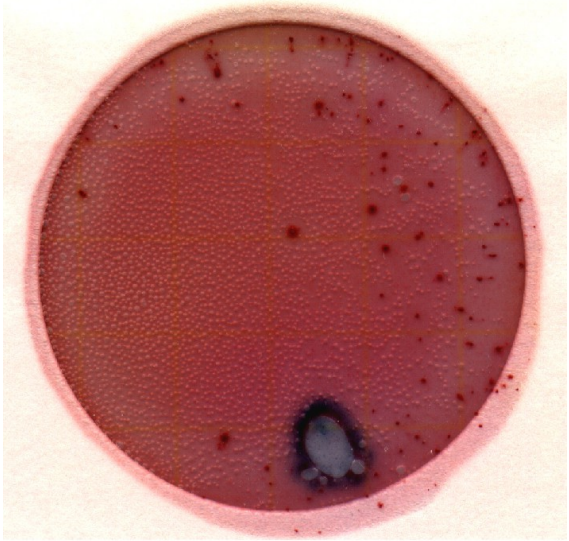
050604 St#3; dilution 1:5

b



050611 St#1 Lake 1; undiluted

c



050611 St#4 River; undiluted

d

Figure 42 Results of the Coliform test with 3M Petrifilm™

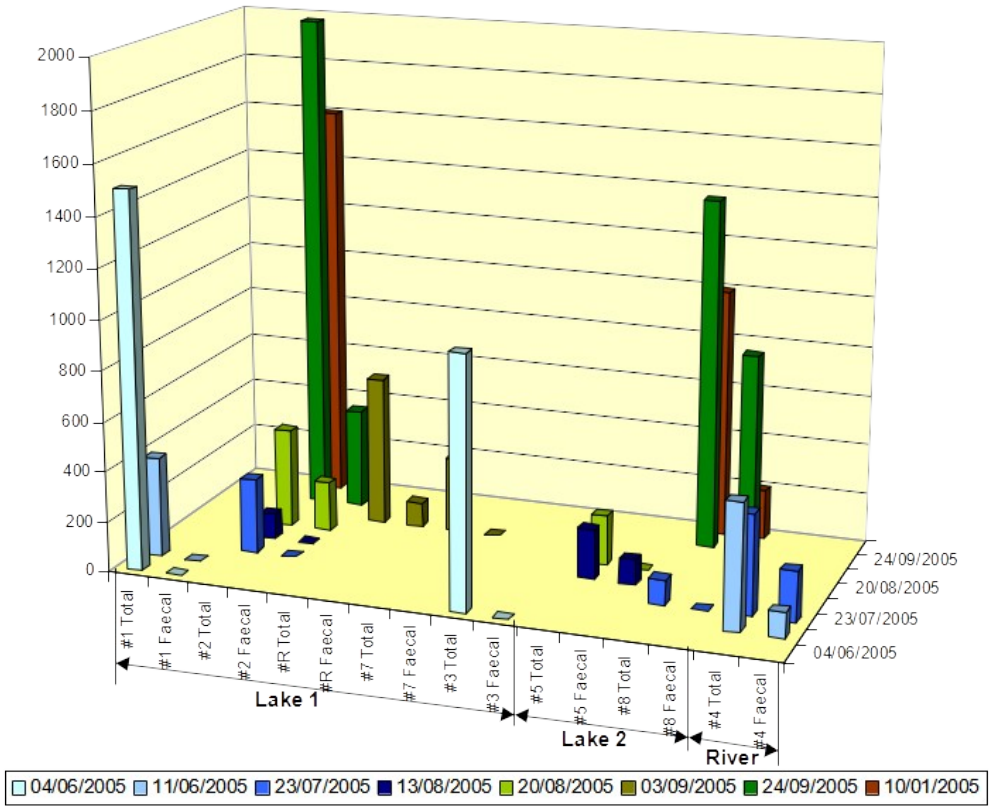


Figure 43 Coliform tests results

After the lakes disconnected from the river (July 30, 2005), the count of coliforms stayed in the previously measured range, however, the count of faecal coliforms interestingly increased sporadically at the locations with lakefront property (August 13, August 20, 2005, Figure 43). ***The available data do not allow pinpointing a possible polluter, and therefore it is recommended to do close monitoring of the situation this year.***

With the migration of waterfowl (Canada geese) at about September 10, 2005, the count of total and especially faecal coliform immediately went up (Figure 43). This type of contamination is natural and normal, and is associated with the infection potential that wildlife inevitably carries. ***The use of, and contact with lake water during this time is discouraged.***

Landslide problem

The research team noticed the sliding of the slopes towards the lakes in certain locations, after the level of the lakes had dropped to the minimum observed in this year. This clay-rich soil saturates with water and therefore expands when the water level is high, and shrinks when the water level drops. This annual volume change causes instability (Figure 45). The Assiniboine River still applies pressure to the meander bends, such as the Oxbow lakes. At the main pressure locations (North part of the lakes, Figure 44), the landslides were observed.

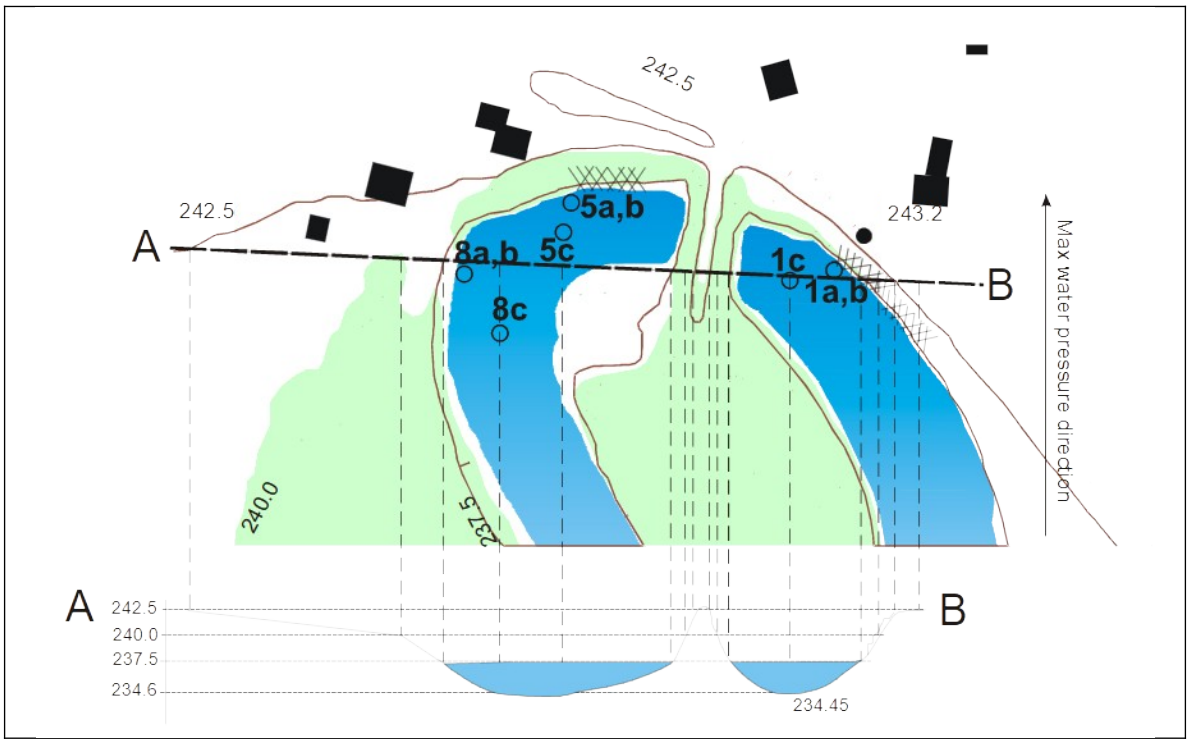


Figure 44 Schematic cross-section through the landslide area

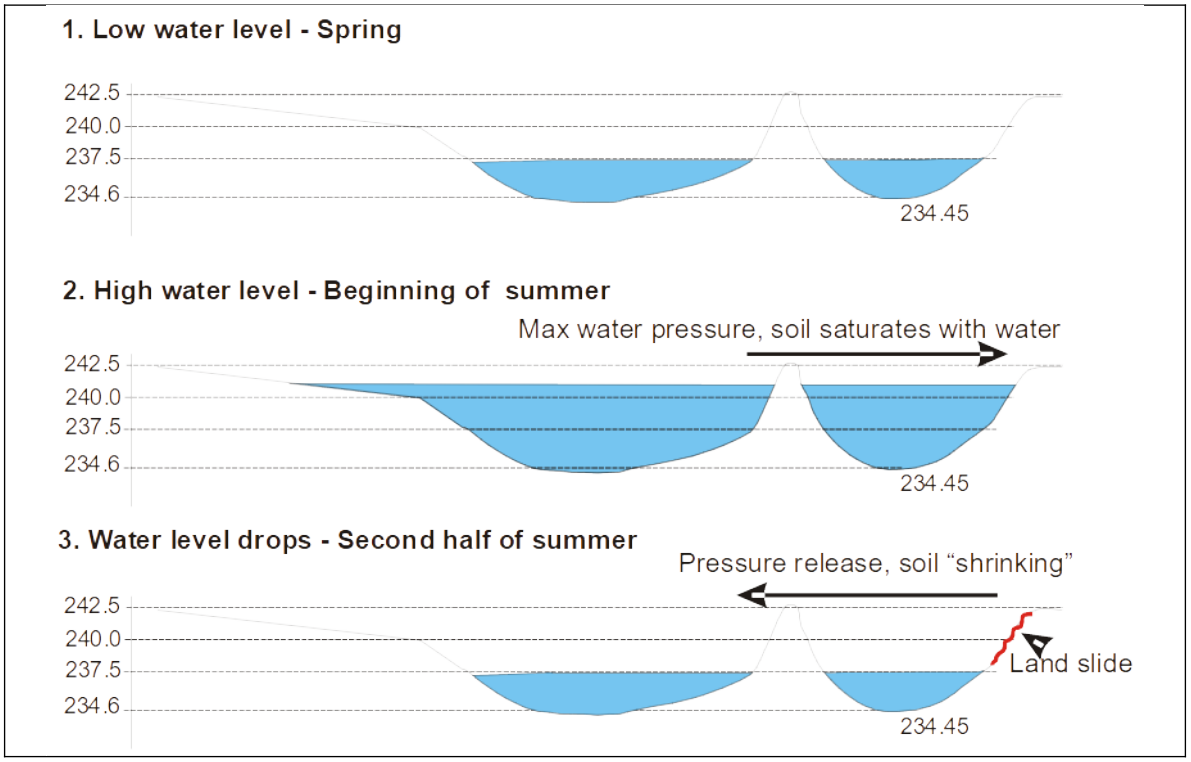


Figure 45 Schematic cross-section through the landslide area during seasonal water level fluctuations and landslide formation

Landslide formation and its prevention are complex engineering work, which requires a geological engineering investigation. In case of the Oxbow Lake problem, this kind of investigation is probably economically not viable, but certain recommendations for the landslide prevention are possible.

It is recommended to stabilise these parts by planting trees and bushes with a widespread root system that can anchor the soil.

An additional option can be the regulation of the lake levels, but it will require proper preparatory work and more observation.

Conclusions

- The pH values of the Assiniboine River and both lakes were higher than 7.4 during the whole investigation period (Figure 8), with the exception of the short timespan when the connection between the Assiniboine River and the lakes was severed. At this time, also the oscillations of parameters began. In both cases water levels were similar and at the mid-point between highest and lowest levels. Factors increasing the pH reach Lake 2 rather slowly.
- ORP values during whole investigation are below 200 mV, which speaks for the low water quality that is comparable to the quality of treated wastewater (Figure 12). The only data confirming good water quality were measured at the beginning of the investigations and during a short period when river and lakes were disconnecting. In both cases, water levels were similar and at the mid-point between highest and lowest mark.
- ORP values for all three water bodies oscillated similarly, but with different amplitude. A significant difference was observed after Lake-River separation. This can be an indication of the higher density of the macro- and micro-population in the Lake 1.
- All water temperature data can be divided into two periods, clearly seen in Figure 13 before and after July 30th, which is the date when the water level dropped, and the connection between the river and lakes was interrupted.
- High demand for oxygen in Lake 1 can be explained by the high amount of decaying organic matter after the Lake 1 euthrophication. Organic matter will include the macro- and micro-populations of the Lake 1, which have been killed by the cyanobacteria bloom and the release of their toxins.
- Phosphate concentrations in both lakes depend on two factors: connection with the river, and local impact, the latter either through wash-out of fertiliser, or direct discharge of sewage or gray water into the lakes. The local impact is much stronger for Lake 1.
- The nitrate-nitrogen concentrations in all three water bodies are very similar during the whole testing season. Nitrate fluctuations in all cases parallel, with the exception of two intervals June 4th – June 25th and July 30th – August 27th (Figure 21)
- On August 6th along with the sudden change of the NO_3-N concentration, the water temperatures in all water bodies had a seasonal peak: $T_{Lake\ 1}$ = 30°C and $T_{Lake\ 2}$ = 32.8°C.
- Algae bloom and high ammonia concentrations in Lake 1 are likely related.
- During the investigation period, Lake 1 was invaded by cyanobacteria, which caused eutrophication of the lake. **The water containing cyanobacteria is potentially toxic to wildlife and humans, with animals drinking this water likely falling ill and eventually dying a painful death.**
- Cyanobacteria bloom in the Lake 1 occurred at the same time when Lake Winnipeg and Lake of the Woods had a cyanobacteria bloom.
- While Lake 1 was invaded by cyanobacteria, Lake 2 was densely populated by Euglenophyta, which have no known danger potential.
- Phyto- and zooplankton groups of the Assiniboine River are not similar to the groups of the organisms from Lakes 1 and 2. In other words, the river is not dominated by cyanobacteria, as Lake 1 is.

- The number of faecal coliforms in the river samples was between 100 and 200 cfu/100 mL, while the count of faecal coliforms in the lakes was below the detection limit. The appearance of faecal coliforms always indicates pollution of the water body with raw sewage, if contamination through migrating waterfowl can be ruled out.
- With the migration of waterfowl (Canada geese) at about September 10, 2005, the count of total and especially faecal coliform immediately went up (Figure 43). This type of contamination is natural and normal, and is associated with the infection potential that wildlife inevitably carries. **The use of, and contact with lake water during this time is discouraged**.
- The available data do not allow pinpointing a possible polluter, and therefore it is recommended to do close monitoring of the coliform bacteria situation this year.
- The clay-rich soil at the lake banks saturates with water when the water level is high and shrinks when the water level drops. This annual volume change causes instability, which causes landslides at the North banks.

Recommendations

- It is well established that phosphate is the main culprit in eutrophication of surface waters, and is the essential ingredient for cyanobacteria bloom [13]. Therefore, measures have to be undertaken to decrease the phosphate concentration by:
  a. limiting the inflow of Assiniboine River water, which carries an undue phosphate concentration,
  b. finding point sources in St François Xavier that contribute to the nutrient load of Lake 1
- The growth of non-fertilised plants (trees or bushes) may serve as a sufficient barrier between the water line and the open area with lawns. Without limiting the inflow of nutrients (mainly phosphate), it will not be possible to control the development of cyanobacteria. The width of the plant stripe should be wide enough to prevent direct washout of lawn fertiliser. The existing tree barrier at Lake 2 is likely a determining factor influencing the significant difference in the micro-organism population between Lakes 1 and 2.
- Besides phosphate, cyanobacteria need stagnant or slowly flowing water; turbidity caused by flow is already detrimental to their development. Therefore, a strategy needs to be devised that allows the re-circulation of water in the lakes, without harming the fish population. This would serve as a sustainable, chemical-free solution in the suppression of cyanobacteria.
- Sunlight is also essential for cyanobacteria. It is advisable to plant trees along the shoreline of Lake 1, as to provide some shadow for the water. The shading effect is likely also the reason for Lake 1 and Lake 2 having so different micro-phytoplankton populations.
- Cultivation of macro-algae such as *Chara* can significantly decrease the population of micro-phytoplankton, according to a scientific investigation[14]. The much higher amount of *Chara* observed at Lake 2 is likely the factor determining the difference between the micro-phytoplankton populations in Lakes 1 and 2.

[13] Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., Smith Nonpoint, V. H. Pollution of Surface Waters with Phosphorus and Nitrogen. Ecological Applications, Vol. 8, No. 3 (Aug., 1998), pp. 559-568

[14] Role of Aquatic Plants in Preventing Algal Booms. Netherlands Institute of Ecology. Progress Report 1999-2000. Ed. Biere, A. *et al*. Nieuwersluis, 2001.

- Landslides at the North parts of both lakes will progress and will eventually damage the road. It is recommended to stabilise these parts by planting trees and bushes with a widespread root system that can anchor the soil.