PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

Prepared by:

BOOJUM RESEARCH LTD

Yong Cao and Margarete Kalin

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

Executive Summary

Mining companies face world-wide environmental challenges in finding sustainable solutions to decommissioning mine waste management areas. Within areas needing restoration (open pits, acidified lakes, tailings and sludge ponds), ecological engineering techniques can be used in mine water bodies in order to retain contaminants, thereby reducing their loads to the receiving aquatic environment. Phytoplankton communities in such water bodies, so-called "Biological Polishing Systems", play significant roles in primary production, metal-removal, overall water quality improvement, and biological monitoring. This study defines the structure of phytoplankton communities and their controlling factors in several mine water bodies. The findings relate water quality to phytoplankton presence, shedding new light on biological monitoring approaches and discusses some implications of eco-toxicity.

The project compiled phytoplankton and water quality data that were collected over periods of up to eighteen years (between 1980 and 1998) from five mine sites in Canada, and analyzed them using univariate and multivariate statistical approaches. Heavy metal contamination, along with low pH (3.5) and other water quality concerns (Ni: 0.03-0.26 mg/L, As: 0.22 mg/L, Zn: 33.0-10.4 mg/L: U: 0.3-1.12 mg/L and Radium^{226}: 0.11-0.41 Bq/L) were among the problems facing these waste management areas.

Water quality problems varied at the different mine sites, and usually more than one variable was involved. The Northern Ontario site combined low pH with high Zn concentrations. At the three sites in Northern Saskatchewan, a flooded open pit with low concentrations of As and Ni, a series of lakes receiving groundwater discharge had intermediate concentrations of Ni (considered a concern to the protection of aquatic life), and a drainage basin receiving seepages from waste rock piles and flux from sediments showed elevated concentrations of U and Ra^{226}. A series of flooded glory-holes in central Newfoundland exhibits strong redox gradients and high Zn concentrations. The water at all of the sites had a circum neutral pH, with the exception of the one in Northern Ontario,

which has a pH value of 3.5.

The total data set consisted of 1,066 water samples, and 210 enumerated phytoplankton samples. The water quality monitoring data typically included comprehensive element analysis and many other parameters, such as pH, conductivity, Eh, acidity and total suspended solid (TSS). The elements/variables that were examined varied from one site to another. Phytoplankton samples were processed and the taxa were identified and enumerated by the same algal taxonomist, resulting in consistent identifications. Algal taxa were identified to the lowest level possible, although in the statistical analyses, genus was used for consistency. For the same reason, it was necessary to use binary information, which retains the majority of community detail, when data from different sites were combined for analysis. Quantitative data were used for detailed analyses of specific sites and time periods. For one mine site, data on 111 water quality and 57 phytoplankton samples from a clean oligotrophic shield lake were available to serve as an uncontaminated control site.

The data were standardization and transformed to enhance the effectiveness of Principal Component Analysis (PCA), Detrended Correspondence Analysis (DCA), Canonical Correspondence Analysis (CCA), Time-Series Analysis (Auto- and Cross-correlation), parametric and non-parametric significance tests. Essentially, these techniques condense data to illustrate spatial-temporal trends, which enabled changes in phytoplankton communities to be related to particular water quality parameters. This allowed the tolerance of given taxa to be evaluated to particular heavy metals. Eco-toxicological considerations are discussed and a perspective on phytoplankton as biological monitoring tools for mining effluents is provided.

It was concluded from the analysis that phytoplankton communities consist of many, widely-tolerant genera and some site- or contaminant-specific genera. The communities can change markedly over time, often because of changes in water quality, or due to factors other than metal contamination. Such factors can vary from one system to another.

In the flooded open pit, for example, TSS and, to a lesser extent As, played major roles in the temporal changes in community structure, but nutrient supply appeared to determine phytoplankton density or biomass. In the low pH lake in Northern Ontario, pH and Zn were more important than other factors in shaping the phytoplankton community structure.

Almost all genera recorded in the wastewater systems were present at the reference site, and most genera found to be common in the former were also common in the latter. It is therefore difficult to define indicator species for mining impacts, unless conditions are extreme. However, the number of genera in the impacted lakes are usually depressed. While phytoplankton community structure may be useful in assessing mining impacts, such assessments are made less than reliable due to the large seasonal and long-term variations and the effects of factors other than metals can strongly influence the results of biological monitoring, as for example TSS fluctuations.

The responses of phytoplankton to metal contamination observed in this study contradict many of the findings published in reports from field surveys and toxicity studies. Phytoplankton taxa found in the mine-site waters showed much greater tolerance to high levels of metals than determined in toxicity tests. This suggests complex protection mechanisms have developed in these taxa. The data also revealed that it is more reliable and useful to define tolerance than sensitivity. When a taxon is not present, or has disappeared, it may be due to water quality variables other than metal concentration - an argument which is supported by the results from the flooded open pit. Similarly, seasonal variation in the phytoplankton community structure between 1993 and 1998 in the series of lakes receiving groundwater discharge from the mine in Northern Saskatchewan, is more significant, than can be accounted for by the decreasing water quality gradient in Ni.

In conclusion, the data documented and analyzed in this report are unique in character and are contributing to a better understanding of aquatic ecosystems in mine water bodies in general.

TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

Acknowledgments

My fascination of aquatic ecosystems of mine waste water bodies started at the Institute of Environmental Studies at the University of Toronto, when my colleague Mary Olaveson identified the first phytoplankton samples I had collected from seepages from uranium tailings sites in Elliot lake, Ontario. According to her understanding of the species identified, a mistake had to have been made, since these species are not tolerant of low pH. I thank Mary Olaveson for revising her accusations of sampling in ecologically unsuitable environments and her dedication in assisting to assemble all the information.

I would like to thank especially Dr. Yong Cao, who joint Boojum specifically for this data analysis. His dedication to data assembly and transformation made the project possible. My limitations in understanding statistical requirements of data sets were apparent in collecting the data. However the world in which data should be collected at the same time, space and with sufficient replication was far different from that of the mine site ecologist. I like to thank Dr. Hans Peterson for his contribution on eco-toxicology and his enthusiasm with which he approached his section on phytoplankton in mine sites and metal toxicity.

I like to thank John Jarrell (Cameco Corporation), George Neary (Buchans Unit, ASARCO) and D. Porter (Talisman Energy Inc.) for their encouragement, financial support and their trust in letting us use the data.

1.0 INTRODUCTION

The mining industry, together with several government agencies, are actively searching for environmentally acceptable approaches to decommission mine waste management areas. Although traditional chemical-physical treatment methods have been well developed and widely used, the uses of these techniques are neither environmentally sustainable nor economically attractive, because they typically require maintenance in perpetuity.

Ecological engineering is an emerging field dedicated to the design and construction of sustainable ecosystems that provide a balance of natural and human values (Mitsch 1994). In applying ecological engineering, the mineral sector is seeking to reduce or resolve environmental issues through measures that create ecological restorations. In the case of mine waste management, ecological engineering aims to utilise, enhance or create ecosystem capacity to remove contaminants from the water column and relegate metal contaminants to the sediment environment, where they are prone to biomineralisation.

Boojum Research Limited has collaborated with CANMET Biotechnology, the Canadian mining industry and academic organizations, to develop a variety of ecological engineering techniques which retain contaminants inside waste management areas and, as a result, protect the receiving aquatic environments (Kalin 1986, Kalin 1989, Kalin et al. 1991, Kalin 1998a, b, Kalin et al. 1998). The techniques are different from traditional methods in that they involve low capital and maintenance costs and require no chemical agent input once the ecosystem has been brought to a state of self-sustenance. Sludge generation, and disposal associated with physical-chemical treatments, are also not a problem here. Ecological engineering is, therefore, regarded as a sustainable technology for wastewater treatment for mine decommissioning and ecosystem recovery.

Ecological engineering applied as part of the decommissioning process involves the establishment of ecological functions of different ecosystem components within the physical-chemical environment determined by the mineralogy of the ore mined and the wastes created. On the other hand, water quality, nutrient supply and other management activities can strongly influence these biological components. For example, phytoplankton and aquatic plants in the ecosystem can remove contaminants (Sigg 1987, Slauenwhite & Wangersky 1991) through biological adsorption and absorption, as well as complexation of metals by algal extrudates, inducing pH changes in the water (Campbell & Stokes 1985, Schenck et al. 1988). On the other hand, water quality, nutrient supply and other management activities can strongly influence these biological components. In mine site decommissioning, phytoplankton and attached periphyton play further an important role in providing organic matter to microbial communities in the sediments or biomineralisation.

Comprehending the functions of biological components in the mine waste water and their interactions within the waste site is critical to the design and implementation of ecological engineering measures, to establish the " biological polishing systems" - the term generally used for such systems. Lakes and ponds receiving mine waste effluent, as well as flooded pits, are the main forms of aquatic ecosystems in post-mining landscapes. In these ecosystems, phytoplankton and periphyton are the major biological components and organic matter producers.

However, our knowledge about phytoplankton in these unique water bodies is limited. The majority of research reported in the literature has concentrated on the taxonomy and ecology of phytoplankton in natural waters, ecological impact assessment concerning species diversity and sensitive species, or the toxicology of certain "model species", which may not be representative of the species behaviour adapted to the waste site ecosystem.

In addition to the lack of information on phytoplankton in mine water bodies, phytoplankton communities in mine wastewater sites are subject to much stronger temporal (seasonal, annual and long-term) and spatial (local, regional and vertical) variations in water chemistry than in natural systems. Although site specific conclusions about phytoplankton populations and their interactions with the waste water are difficult to generalize, it is even more challenging to examine the interactions between phytoplankton communities and a wide range of environmental factors, because these aquatic ecosystems are highly dynamic, both chemically and ecologically. On the other hand, due to chemical reactivity of the mine water bodies, it is extremely difficult to simulate the dynamics of the water bodies in a laboratory, in order to define the response of biological systems to environmental factors. This in part explains the paucity of information on the subject.

Boojum Research, together with CANMET Biotechnology and the Canadian mining industry, has collected both phytoplankton and water quality data from a number of mining sites, each of which covers many sampling locations, over a long time period. The monitoring data are managed using a QA/QC system in a Paradox database, which are updated on a continuous basis. As the phytoplankton populations are generally limited in mine waste water, an assessment was carried out to determine if picoplankton, considered an essential component of ecosystem development, might not be present in the water bodies (Kalin and Olaveson, 1997). Picoplankton was not absent from the mine water bodies but abundant at some sites. The phytoplankton information assembled during this study was considered suitable to be analysed further, using sophisticated multivariate statistics to elucidate water quality-phytoplankton relationships.

The rapid development of advanced statistical techniques (Gauch 1982, ter Braak 1987, Belbin 1992, Clarke & Warwick 1994) and the wide use of powerful computers now allow for the analysis of large data sets to detect and quantify complex patterns and trends in both phytoplankton communities and water quality, and to relate them to each other. Multivariate approaches have been used widely in environmental and ecological studies and have started to gain acceptance in the environmental industry (e.g., Conor Pacific 1998) and environmental agency (Wright et al. 1992).

The data set of phytoplankton and water chemistry was large enough to sustain the required data reductions in order to prepare acceptable data sets prior to the statistical analysis. This provided therefore an opportunity to address the topic of phytoplankton communities in mine wastewater management areas. The study results will also provide useful information regarding the sensitivity of phytoplankton taxa to a particular metal contaminant, or, more importantly, to the combination of environmental stress factors in mine water bodies. In addition, information will be become available, which assists in defining the role of phytoplankton as monitoring tools in the protection of aquatic habitat.

1.1 Objectives

Three objectives, outlined below, were addressed with the existing data assembly. Those have both practical importance in restoration of mine sites, but, at the same time, are of interest from an ecological perspective. The data set is suitable to define phytoplankton community structure in mine wastewater systems where Zinc, Arsenic, Nickel and Radium and Uranium are the contaminants of concern.

The first objective is to define the water quality factors which control phytoplankton assemblages in wastewater on a site specific basis. The second objective is to compare the responses of the phytoplankton communities between the sites, with their different environmental stress factors. As the third objective, the eco-toxicological consequences of the findings are discussed.

In Section 2, the methodologies are described, addressing sampling and data management along with the multivariate statistical techniques employed in the analysis.

In Section 3, the results are presented for each site individually, highlighting the main findings with the detailed results presented in appendix form. In Section 4, the third objective is addressed, identifying the key environmental factors based on a analysis of all sites. The eco-toxicological implications are presented in Sections 5 and 6 and presents the conclusions and recommendation of the work.

2.0 METHODS AND SAMPLING LOCATION OVERVIEWS

2.1 Mine Site Description

The sample set was derived from five mining areas, located in northern Saskatchewan and northern Ontario and central Newfoundland (Map 1). Detailed site description are given with the individual data analysis along with the sampling locations. Both in northern Ontario, at the South Bay mine, north of Dryden and in central Newfoundland at Buchans, the mining operations are shut down for a least a decade and closure plans are being implemented. The South Bay mine was a copper zinc concentrator, which generated close to 1 million tonnes of tailings, containing 41 % pyrite and 4% pyrrhotite. The main contaminant of concern is zinc and acid mine drainage.

The Buchans operation was a polymetallic ore body, which started mining operations in the late eighteen hundreds. Here flooded gloryholes and underground workings discharge acid mine drainage, which is neutralized with ground water rich in carbonates. The main ores milled were Au, Ba ,Pb and Zn. Zinc remains the major environmental concern.

In northern Saskatchewan uranium mines are operating. The Link Lakes, an Upper and Lower Lake, located at the southern end of a drainage basin, receive run-off from waste rock piles located in the headwaters of this drainage. Contaminants of concern are uranium and Radium 226. The waste rock piles are from the first ore body mined, the Rabbit Lake are, located on the Harrison peninsula in Wollaston Lake. During mine development, Rabbit Lake was drained, which altered the lake level of Upper Link Lake and mine slimes added additional contaminant sources to the lake.

On the same peninsula, further ore bodies were mined for feed of the Rabbit Lake mill, the A ,B and D zone ore bodies and Eagle point. A, B and D-zone were open pits which are flooded after they were mined out. The B-zone pit contains about 5 million m^3 with a depth of 45 m at its deepest point. The pit stratifies over the season and turns over completely in the winter. The contaminants of concern are low levels of As and Ni in this pit.

The mining of the Key Lake ore required extensive de-watering due to the location of the ore body. At the onset of the operation in the early eighties, about 70 million m^3 were released from surface and shallow ground water. Pumping was initiated at a rate of 0.8 m^3/sec to release the deeper ground water, while a pumping rate of 0.4 m^3/sec is maintained throughout the operational life of the mine. Such activities will induce physical and hydrological changes in lakes receiving the ground water discharge. The contaminants of concern here are low levels of Ni.

2.2 Sample Collection and Analysis

2.2.1 Field and Chemical Data Collection

In B-Zone, Link Lakes, and Key Lake group, CAMECO's environmental staff routinely collected water samples at biweekly, monthly or seasonal intervals, depending on the particular sampling location. Dissolved Oxygen, Conductivity, pH, Redox (Eh), and water temperature were measured on site. Water samples were then filtered through 0.45µm filters and were acidified, also on site. Chemical analysis was carried out by the Saskatchewan Research Council using standard methods.

At Buchans, the environmental staff of ASARCO Inc. conducted routine sampling from the water bodies in the mine wastewater management areas, including the pit-lakes, the focus of the present study. All sample processing and analyses were as described above. In South Bay, Boojum Research staff and trained local personnel conducted water sampling in all wastewater bodies, as well as the reference site, Confederation Lake. Sampling frequency varied somewhat between years and locations, but was mostly quarterly. Fewer samples were collected in winter.

2.2.2 Phytoplankton Data Collection

Phytoplankton samples were collected from the five study areas, but less frequently than the water samples. They were all processed by the same taxonomist (Mary Olaveson) of Algatax Consulting. This ensured consistency in sample processing, taxon identification and enumeration. The samples (500-2000 ml) were fixed with Lugol's (IKI) Preservative immediately upon collection and shipped to Algatax.

Sample Concentration: All water samples were left to settle for 1 week in the dark, to allow dead algal cells to settle to the bottom of the sampling bottle. A final concentrated sample of 20 ml, containing all of the phytoplankton organisms from the original sample, was obtained after several runs of settling and concentrating. This 20 ml concentrated sample was transferred to a 25-ml glass scintillation vial with plastic lined caps for storage. Additional Lugol's Preservative was added whenever the sample colour had faded.

Enumeration: Not all the samples in the data sets were subject to enumeration. In many cases, only the presence-absence of species/taxon was determined. Depending on the density of algae cells and the amount of debris (inorganic clays, chemical precipitates, organic detritus, etc.), an aliquot of the concentrated sample was either diluted with distilled water or further concentrated using the Utermohl Counting Chamber (with the appropriate settling column (e.g., 5 or 10 ml). The amount of sample concentrate that was examined was always related to the original sample volume so that the results from the computerized enumeration program, PHYCOUNT (Jackson *et al.*, 1984) were complete and required no further calculations.

When taxa were enumerated, the Utermohl Counting Chamber was used, which was designed for use with an inverted microscope (Utermohl 1958; Hasle 1978). After an appropriate settling time (2-8 hours depending on volume), each sample was examined with a Zeiss Axiovert 35 Inverted Microscope equipped with phase contrast illumination, and counted at three different magnifications (100X, 200X, and 400X). The method, described in detail by Nauwerck (1963), Vollenweider (1969), and Hasle (1978), is well established as a standard for phytoplankton enumeration from a variety of environments.

At 100X magnification, at least 5 transects were examined, and large (usually > 50 µm) or rarer taxa were identified and enumerated. The counting program, through statistical tests which compared counts among transects, indicated whether a random distribution of cells had been achieved or whether excessive clumping had occurred. Counts were completed on randomly distributed samples; clumped samples were re-sampled from the sample concentrate and re-settled.

The predominant phytoplankton (10-50 µm size range) were enumerated at 200X, where at least 3 transects were examined. At this magnification, the medium-sized and/or the most common taxa were enumerated. At the highest magnification (400X), smaller taxa (2-10 µm) were enumerated. The smallest taxa were generally clumped by Class (e.g. small unidentified Cyanophyte spp., small unidentified Chlorophyte spp., small unidentified diatoms, etc.). No attempt was made to identify them further, as this identification would have required a use of oil immersion and high magnification

(1000X), which is not possible with the Utermohl Counting Chamber. Electron microscopy (SEM / TEM) is the only way to confirm identification to species, since many of these taxa are very small (in the picoplankton size range) and are difficult to identify even with oil immersion.

In each enumeration, a minimum of 100 units (e.g. cells, colonies, and filaments) were enumerated for the most abundant taxa, and at least 400 units in total were enumerated from each sample. These quantities have been shown statistically to give final cell density estimates which are within 10% of the mean for the sample (Lund et al., 1958; Venrick, 1978). This precision was considered reasonable given the many potential sources of error from:

1) the phytoplankton patchiness in the environment; the sampling techniques in the field (e.g. a very small volume of water is sampled relative to the volume of the lake);the various steps in concentrating and sub-sampling the water sample and;
2) the examination of a portion of the counting chamber.

2.3 Data Refining, Transformation and Standardization

This study largely explores data which are, in part, historical in nature. As such, the data set is not the result from a study designed to address water quality-phytoplankton interactions in mining wastewater. While these data were initially collected in order to describe/document the mining effluents, they can also serve to explore the water quality-phytoplankton relationship, as long as proper refinement, transformation and standardization is performed before carrying out statistical analysis. Many statistical methods are sensitive to erroneous records, outliers, or a non-normal distribution.

2.3.1 Data Refining

Water Quality: The first involved detecting erroneous records in the data set, which usually occur during data entry, or from an incorrect measurement unit or measurement error. Such errors are often obvious. For example, a record in µg/L entered into the database in mg/L, or a pH less than 0, clearly represent data entry error. If one record is much higher than all others, it should usually be regarded as suspicious, but for certain variables, this rule does not apply, since they naturally vary very widely. For example, Total Suspended Solid (TSS) can fluctuate considerably due to severe storms or pit wall slides. Similarly, extreme values should be retained if they are followed by several relatively lower records, forming a continuous series.

Some records are not clearly erroneous, even though they are much higher than the majority at the same sampling location and in the same period. While they may be "outliers", they are not necessarily errors, since they may be actual occurrences due to the specific conditions of the water body, such as algal blooms, reflected in TSS values. We minimised the removal of "outliers" to one or a number of extremely different records. In all cases, deletions were only made after the raw data files and field notes were examined.
Each variable was evaluated individually before extreme values were disregarded.

Frequency of Sampling: At some sampling locations, the sampling frequency varied from month to month. If more than one sample was collected at one location during a month while only monthly data were collected at the other locations, the extra or "redundant records" were removed. Similarly, samples collected from the same location, but at different depths, were deleted when only the surface water samples were consistently available.

Consistency of Variables Determined: Within data sets, some variables were measured only occasionally, and some samples contained few variables, which is not uncommon for long-term, large-scale monitoring data. The percentage of missing data for each variable was calculated against the total number of samples collected. Any variable with more than 15% missing data was regarded as incomplete and removed from the data sets (Hirsch et al., 1991), unless that variable was particularly important, such as nutrients.

Phytoplankton Samples: The phytoplankton were identified generally to a taxon as low as possible, frequently species level. Most genera contain only one species, but a few include more than one. While it is important to ensure the reliability and consistency of species-level identification when samples from different seasons, years, locations and study areas are compared, it is not always possible to differentiate species of the same genus with full confidence. The morphology of a species can vary significantly, depending on seasons, nutrients, sample processing and other factors. Consequently, we trimmed the data at genus level with the few exceptions where we were confident of the species level and knew that the biology of the species of the same genus were different (e.g., *Euglena*). For these exceptions, we retained the species level and more details were given in the relevant appendices.

The refined data sets of both water quality and phytoplankton from different sites are summarized below:

Sites	Systems	Sampling Periods	Water Samples	Algae Samples
South Bay	Boomerang Lake	1986-1997	157	56
	Confederation Lake	1986-1997	111	57
B-Zone	Pit & Collins Bay	1992-1998	230	48
Rabbit Lake	Upper-Lower Link Lake	1980-1998	521	21
Buchans	OWP & OEP	1989-1996	120	17
Key Lake	Six lakes	1993-94, 98	70	82

Some of these data sets were further manipulated to provide composite samples which better represented a system, or to make water and algae samples match each other. The number of variables retained differed among the sites, and between different types of analyses. Details are given for each sample set with the site specific analysis in the respective appendixes.

2.3.2 Data Transformation and Standardization

Data transformation and standardization are frequently undertaken before statistical analyses are carried out. The purposes of such manipulations include:

1) improving the normality of variables;
2) reducing the effects of extremely high value and;
3) adjusting the weights of different variables in multivariate analysis (Gauch, 182, Cao et al., 1999).

There are several models for these data manipulations. According to both data analysis and literature reviews, we standardized data to 0 mean and 1 variance for multivariate analyses. It was calculated for each variable as follows: Y = (X-mean)/StDev, where X= raw data, Y = the standardized value, mean = the average of the variable over all samples and StDev = the standard Deviation of the variable. This model weights each variable equally, ignoring the units of measurement, absolute values and the scales. This approach widely used in environmental and ecological studies. Since log transformation can effectively reduce the effect of very abundant species/taxa (Gauch, 1982, ter Braak, 1987), it was adopted for phytoplankton community analyses in this project.

2.4 Statistical Techniques

Ecosystems are typical multivariate systems, which involve many species and physical-chemical factors. The relationships among different components of the systems are complex and not necessarily linear, often making conventional, univariate statistics inadequate or ineffective. To characterize water quality and biological communities, monitoring programs involve collecting water and biological samples from many sampling locations over a period at certain intervals (weekly, monthly, quarterly, or yearly). In this project, the sampling periods spanned from 7 to 18 years at a frequency ranging from one month to a quarter. Each water sample was measured for many elements, compounds or other water quality variables - more than 30 variables were typically measured. This produced a sample - variable matrix, which could be in the order of 200 x 40 for each study site - far larger than what most conventional statistics were designed for.

Similarly, there are usually many species in each phytoplankton sample. When cross-location/sites comparisons are made, the total number of species/taxa can easily reach a few hundred. The sample-species matrix can reach a size of 200 x 500. In the present study, most locations in the mine wastewater management areas contained fewer species/taxa than most natural waters, but still more than 100 species/genera in total.

Using these large data matrices, the following questions were addressed for each site:

1. do water quality and phytoplankton communities show any spatial-temporal patterns/trends?
2. what are the relationships between water quality patterns and phytoplankton community changes?
3. how do particular species/taxa or their assemblage responds to a given water quality variable or its combination with some others?

These large data matrices are, however, usually highly "noisy" - i.e. the potential spatial-temporal patterns/trend or interactions are compounded by sampling errors, random, non-defined variation, and non-interested variation. For example, a record at a certain time and location may contain:

1) daily, seasonal, and annual variation,
2) random variation,
3) the effects of micro-habitats,
4) real spatial gradients,
5) sampling and measuring errors.

If the aim of the analysis is to determine spatial or long-term patterns/trends, all other sources of variation become "noise". Of course, the definition of "noise" is relative, depending on the objective of a research project.

Monitoring data are collected generally for a specific purpose, but they rarely meet the requirements of conventional statistical methods, such as normal distribution and replicates. This is particularly true for long-term monitoring programs. In applying statistical procedures to the data, a very large proportion of the information contained in the data set is therefore lost for statistical analysis. This may lead to erroneous conclusions when conventional statistics are used in pattern or trend analysis, hence it is better to work with smaller but valid data sets.

It is known that many water quality variables co-vary over space or time. Phytoplankton species also respond to water quality changes in similar ways than does water quality, which may make a large proportion of the variation in data sets redundant. Multivariate analysis removes the redundant information to reveal the underlying pattern, trend or relationship. These techniques also accommodate multiple patterns/trends well, as more than one dimension can be used to present the result. However, the first two or three dimensions usually explain the majority of the variance within a data set.

In the past two decades, great progress has been made in multivariate approaches (Hill, 1979, Gauch, 1982, Digby and Kempton, 1987, ter Braak, 1987, Belbin, 1992, Clarke and Warwick, 1994, Pieleu, 1984). The techniques are largely divided into two categories: ordination and classification. The former includes Principal Component Analysis (PCA), Detrended Correspondence Analysis (DCA) and Canonical Correspondence analysis (CCA) while the later includes hierarchical/non-hierarchical cluster analysis, and TWINSPAN. Each of these techniques has its own strengths and weaknesses, but they are very powerful in condensing environmental data, detecting spatial-temporal patterns/trends and relating biological data to water quality data.

A brief description of the methods is given to elucidate the choice of analytical techniques.

PCA is a multivariate technique with a long history. There are several variants of it, including Centred and Non-centred PCA, and Standard PCA, which are different in data handling. In this study, as data were standardized first, we conducted non-centred PCA. The assumption associated with the technique is that species respond to each gradient linearly. This is not true in most cases, since species abundance responses approximately follow a Gaussian curve, bell-shaped. Therefore, we applied PCA to chemical-physical data and binary phytoplankton data, where species abundance was not involved - only its presence or absence.

DCA overcomes the problem of linear relationship in PCA, but assumes that species abundance responds to an environmental gradient in Gaussian curve. This technique has gained popularity since 1979 in environmental and ecological studies (Hill, 1979). Both DCA and PCA are indirect ordination methods, which means they define theoretical gradients but cannot tell exactly what the gradients are. However, the relationship between a gradient (ordination axis) and other environmental variables can well suggest the nature of the gradient. For example, a theoretical gradient may be strongly correlated with time, or contaminant concentration, and we can infer that it is a time-trend or a contamination gradient.

A more recent technique is CCA (ter Braak, 1987), which combines indirect ordination with multiple regression. When PCA or DCA is used, the first two or three dimensions are usually examined. However, some environmental variables, such as contaminant or management activities, may not be the most important variable and are related to only the fourth or fifth dimension, but they may be important in a particular study. In this case, PCA or DCA may well ignore these variables. CCA is a version of DCA that is constrained by a set of environmental variables. In other words, it seeks an ordination solution that is best explained by a particular set of variables (e.g., water quality). In this way, the variables we are concerned with will not be ignored. CCA has been widely used in environmental and ecological studies, and here was used to analyse the relationships between phytoplankton species/taxa and different water quality variables.

In all three ordination techniques, each sample and species/variables is given a score for each axis based on their relationships. The distance between any two samples in a ordination plot indicates their similarity, i.e., the more similar are two samples, the closer they are located to each other in the data plot. The positioning of a species/taxa or water quality variable with respect to a sample reflects its relative abundance/value in the sample. For example, if species A or variable A is the closest to a sample, its abundance or value is the highest in the sample. The positioning of two species or variables also reflects their correlation. If two variables project into the same direction and are close to each other, they are highly likely to co-vary. In fact, the cosine of the angle between two variables in a PCA plot is their correlation coefficient.

Classification techniques group different objects (samples, species, or lakes) based on their similarities. The members of a cluster are more similar to each other than to the member of other groups. 'Cluster analysis' involves a large family of clustering methods. We used Average Euclidean Distance and Ward Linkage for water samples in this study with standardized data, as it is strongly recommended by many researchers (Orloci, 1967, Milligan and Copper, 1987, Cao et al., 1997).

The statistical techniques used vary from one site to another, depending on the nature of the data sets. Statistical approaches used for the five mine sites are summarised below:

Statistics	South Bay	B-Zone	Link Lake	Buchans	Key
PCA	Yes	Yes	Yes	Yes	Yes
DCA	No	No	No	No	Yes
CCA	Yes	Yes	No	No	Yes
Cluster Analysis	Yes	No	No	No	No
Trend Analysis	Yes	Yes	Yes	Yes	No
Significance Test	No	No	Yes	No	No

Many terms are used in this report which are common in statistical analysis but may not be clear to the non-statistical reader. These are defined below:

1. "species composition" and "species-environment correlation" are standard terms in numeric community analysis, although taxonomic unit is the genus, as is the case in this study;
2. community structure includes two elements: specie genus composition and abundance;

3.0 RESULTS

Mining affected surface waters represent aquatic habitats in which ecosystem functions are restricted by the presence of chemical conditions which allow many ecological tolerances to be expressed. Those are necessary to be understood to develop effective restoration technology. As phytoplankton is one of the most important component of aquatic ecosystems forming the foundation of the food chain, its development is essential in restoring mine water bodies. The phytoplankton data set has been collected over a decade to describe the ecological status of the mine waste waters and to define its role in ecosystem recovery. The analysis of site specific patterns in phytoplankton community structure and the chemical conditions of the mine water body is necessary in understanding the ecosystem recovery. The site specific data analysis is presented in detail separately in the appendixes, for those readers which are interested in the statistical details (Appendix 3 to 7). The key findings for each site are summarized prior to the cross site comparison in section 3.1 presented below. In addition to the site specific analysis of the relationships of phytoplankton community and environmental factors, the sites with their phytoplankton communities are compared and a discussion is presented of the toxicological and ecological implications arising from the presence or absence of these communities.

3.1 Site specific highlights

The water bodies of three uranium mines have been analyzed where the elements of concern partly overlap, in that Ni is of concern, both in the Key Lake area and in the B-zone pit, however in the pit, As and Ni receive environmental attention. In the Link Lake system, Radium and Uranium are the elements of concern. The detailed site descriptions and the sampling locations, for chemical and phytoplankton are presented in Appendix 2. All uranium mine sites are located in northern Saskatchewan.

The data set of the B-zone pit is the most extensive and complete data set, fulfilling sampling requirements for the statistical analysis to the greatest degree, compared to the other mine sites. Water quality and the phytoplankton samples were often collected at the same time. 17 water quality parameters were used in time trend analysis followed by PCA (Principal Component Analysis) covering the time when the pit was flooded in 1992 to 1998. These water quality parameters were accompanied by a set of 46 phytoplankton samples, which consisted of both, a binary data set (presence and absence) and a quantitative set of data where the species are enumerated, covering the time period of 1995 to 1998.

The phytoplankton community in the flooded pit experienced rapid and substantial changes between 1995 to 1998. The change was characterized by replacement of the dominant species and an increase in species richness. The species composition in the pit, did not resemble that of the lake-water which was used for flooding and in does not show any resemblance to that of the adjacent oligotrophic lake. This is likely due to the different nutrient status of the pit, discussed in detail in Appendix 3.

Environmental relationships imposed on the results of the PCA analysis carried out on the community composition indicated, that As, Eh, NO_3 and phosphorous concentrations were strongly associated with the populations in the early part after the flooding (1992 - 1995), whereas TSS showed a very high affinity with the 1992 and 1993 samples. All other water quality parameters were not strongly related with community composition. Of particular interest is that Ni belongs to those factors, which are not of major importance to the community structure although it is considered of environmental concern. A detailed discussion of the pit is given in Appendix 3. As natural aquatic ecosystem are formed over hundreds or thousand of years, the B-Zone pit provides an unique insight into water quality changes and the evolution of biological system in a new born water body.

The findings for the Key Lake site, where Ni is the element of environmental importance, indicated that as the ground water discharge which flows through a series of lakes, downstream, a spacial association is found for Ni, Ca, conductivity and hardness and a second association was found to represent temporal changes for pH Pb and Cu. Relating the environmental factors to the phytoplankton community, only a small proportion of the total variance in the data can be explained by the spacial Ni concentration gradient, leaving the temporal variation to contribute more significantly the control of the phytoplankton community. A detailed discussion of the analysis of the same data set , carried out by others (Rosaasen A. R. 1997 and Conor Pacific 1998) is given in Appendix 7. Although the same data are used and similar statistical analysis, the importance of Ni on the phytoplankton community structure can not be substantiated. The seasonal variance is the data has a similar effect on the phytoplankton community in the lakes into which the ground water is discharged.

The third uranium mine water bodies, addresses an interesting condition, in the that the pH is relatively high and metals are present at low levels. This data set facilitates the analysis of long term trends in water chemistry (1986 - 1998) and their relation ship to the phytoplankton community. However, the chemical data set is much more extensive than the biological data. Seasonal periodicity is noted through auto-correlations in the lake systems, and a detailed discussion of the water chemistry in the system is given in Appendix 5. No relationship of the phytoplankton community to uranium and radium 226 concentrations could be identified. The PCA analysis indicated, that those genera, which are strongly associated with particular samples are either rare or less abundant. This implies that the phytoplankton composition in the two lakes remained stable over the sampling period, covering 1989 to 1995, albeit the environmental concern over Ra 226 and uranium.

The other two sites for which data are available are base metal operations, and both are inactive for at least a decade. In both water bodies Zn is the major concern, one in circum-neutral water at Buchans, and the other in low pH (3.5) at South bay. Although the chemical data set in Buchans is extensive, the phytoplankton data are scarce, and of the community structure with 44 genera present in the pits , only 20 % were considered frequent. The community structure was not related to the time trends which were evident in the chemical data. Details of this site are given in Appendix 6. There is a unique feature to this site, which could not be documented in this study, but is of interest. Several times phytoplankton was sampled in the OEP with dept. In contrast to the B-Zone pit, no phytoplankton was found. This absence was explained by the strong redox thermo-cline in the pit and the resulting low light penetration.

For South Bay, the data set was quite extensive, covering the years 1986 to 1998. Both the time - trend and the multivariate analysis show, that the lake experienced rapid and significant changes in water quality, particularly marked in the last few years, when the seepage from the mine site was diverted into the lake. The changes in phytoplankton community composition over the period of 1986 to 1997 are also very clear. However it is of ecological importance to recognize that some taxa were consistently recorded , albeit the changes in chemistry. The changes in the water chemistry explain the change in phytoplankton. It appears the pH and Zn concentrations are strongly related to the community structure. Therefore during this study, a large scale experiment was initiated, to reduce Zn concentrations and increase pH gradually. These experiments are ongoing in enclosures constructed in the lake. Phytoplankton will be monitored in these enclosures to define the response of the community to the water quality improvements. An extensive discussion of this site is given in Appendix 4.

3.2 Across-Area Comparisons and General Discussion

3.2.1 Water Quality

The five mining sites differ in terms of their limnology, morphology, geology and hydrology, all of which influence their phytoplankton communities. Water quality is the most important factor, since the concentrations of toxic metals or protons often far

Table 1. Summary of five mine sites and water quality characteristics

	Boomerang Lake	Buchans Pits	B-Zone Pit	Little McDonald - Key Lake Area	Link Lakes
Locations	Northern Ontario	Central Newfoundland	Northern Saskatchewan	Northern Saskatchewan	Northern Saskatchewan
Mining Type	Zn, Cu, & Ag	Zn, Cu, Au, & Ag	U	U	U
Mining Period	1977-1981	1928-1984	1984-1991	Since 1980	1972-1984
Surface area (ha)	73	1.95 (OEP) 0.46 (OWP)	24	37.3(LM)	22.7 (ULL) 22.8 (LLL)
Max. Depth (m)	6	7 (OWP) 21(OEP)	57m	11 – average (LM)	3.5
Sampling Period	1986-1997	1988-1996	1992-1998	1993-94, 98	1987-98 (ULL)/-1998 (LLL)
No. of Samples	157	65 (OEP) 55(OWP)	224	15	143 (ULL) 141(LLL)
pH	3.9	6.6 (OEP) 4.27 (OWP)	6.74	6.95 (LM)	6.71 (ULL)) 6.67 (LLL))
Conductivity (μm/s)	453	1839 (OEP) 666 (OWP)	60.78	71.2 (LM)	121 (ULL) 127 (LLL)
Zn (mg/l)	10.53 (0.75-49.71)+	24.3(OEP) 32.66 (OWP)	<0.005	<0.005	<0.005
Ni (mg/l)	0.109	<0.005 (OEP & OWP)	0.26 (0.12-1.2)	0.13	<0.005
As (mg/l)	0.05	0.05 (OEP) 0.185 (OWP)	0.22 (0.05-0.72)	<0.005	<0.005
U (mg/l)	<0.005	<0.005 (OEP & OWP)	<0.005	0.033(HL) 0.015 (LM)	1.003 (ULL)/0.27-3.25 0.301 (LLL)/ 0.06-2.21
Ra^{226} (Bq/l)	<0.005	<0.005 (OEP & OWP)	<0.005	0.015	0.36 (ULL)/0.13-0.80 0.11 (LLL)/0.01-0.60

+. Indicating the minimum and maximum.

exceed the water quality guidelines for aquatic life (Environment Canada, 1995). The multivariate analyses conducted for Boomerang Lake, the B-Zone Pit and Key Lake also show that contaminant metal elements (and low pH in Boomerang Lake) are the most important water quality variables responsible for changes in phytoplankton communities.

Ideally, the difference in water quality among these different systems should be quantified using multivariate analysis to define the characteristics of each of them. However, the data available in this study cover different periods of sampling for each site, and involve varying sampling intensities. To achieve comparability in sampling dates and variable lists would have trimmed large proportions of the samples from each site, which would have resulted in inadequate data to define the water quality characteristics in each system. Moreover, water quality typically changed significantly in the wastewater systems over time. After detailed within-site analysis completed, a qualitative cross-sites comparison based on the long-term data described below, however, can accommodate the objectives of this study.

The water quality parameters vary at the different mine sites, and usually more than one variable is involved (Table 1). In Boomerang Lake, high H^+ concentrations (low pH) is combined with high Zn concentrations while the B-Zone Pit is associated with slightly elevated levels of As and Ni and Key Lake receiving waters with intermediate level of Ni. Link Lakes exhibit elevated U and Ra^{226} concentrations. Buchans Pits are somewhat different in that extensive changes in water chemistry have occurred during the sampling period, except for persistently the high Zn concentrations. Boomerang Lake and Buchans Pits are similar to the extent that both are associated with high levels of Zn (10.5 - 32.7 mg/L), but differ strongly in pH. Little McDonald Lake and McDonald Lake in the Key Lake area have levels of Ni comparable to those in the B-Zone pit, but the latter is also associated with high As levels.

Table 2. Distributions of phytroplankton genera across all five mining sites in terms of occurrence frequencies based on binary data.

Genera/Species	Confederation L.	Boomerang L.	Buchans	B-Zone	Link L.	L. McDonald	No	Means
Oscillatoria	54.4	53.8	5.9	44.9	57.1	12.5	5	34.8
Tabellaria	94.7	53.8	11.8	8.2	61.9	50.0	5	37.1
Nitzschia	77.2	11.5	17.6	42.9	42.9	6.2	5	24.2
Dinobryon	77.2	3.8	29.4	28.6	47.6	100.0	5	41.9
Chlamydomonas	64.9	3.8	41.2	26.5	33.3		4	26.2
Navicula	75.4	3.8	41.2	2.0	42.9	12.5	5	20.5
Pinnularia	61.4	7.7	11.8	12.2	42.9	6.2	5	16.2
Gymnodinium	50.9	30.8	5.9	2.0	28.6	43.7	5	22.2
Melosira	78.9	38.5	5.9	2.0	19.0	6.2	5	14.3
Ochromonas	84.2	7.7	5.9	6.1	38.1	56.2	5	22.8
Cosmarium	49.1	3.8	5.9	4.1	28.6		4	10.6
Rhizosolenia	43.9	7.7	11.8	2.0	9.5	75.0	5	21.2
Scendesmus	47.4		64.7	2.0	85.7	6.2	4	39.7
Euglena	49.1	88.5	29.4	2.0			3	40.0
Cryptomonas	80.7	3.8		46.9	81.0	100.0	4	57.9
Dictyospaherium	19.3	3.8		81.6	19.0	56.2	4	40.2
Ankistrodesmus	63.2	61.5		4.1	38.1	81.2	4	46.2
Trachelomonas	14.0	57.7	23.5		14.3		3	31.8
Asterionella	86.0	23.1		2.0	66.7	12.5	4	26.1
Synedra	94.7	26.9		2.0	47.6	18.7	4	23.8
Achnanthes			52.9	14.3	4.8		3	24.0
Staurastrum	56.1	3.8		2.0	52.4		3	19.4
Chroomonas	15.8	42.3	5.9		9.5		3	19.2
Neidium	5.3	38.5	11.8		4.8		3	18.3
Oosystis	50.9	38.5		2.0	9.5	25.0	4	18.8
Peridinium	80.7	3.8		12.2	33.3	50.0	4	24.9
Stauroneis	40.4	34.6		2.0	9.5		3	15.4
Glenodinium	14.0	11.5	11.8	22.4		12.5	4	14.6
Synura	22.8	11.5	5.9		23.8		3	13.7
Ulothrix	36.8	15.4	23.5	2.0			3	13.6
Cymbella	43.9	11.5		4.1	19.0		3	11.6
Chlorogonium	8.8	3.8	5.9		4.8		3	4.8
Planktosphaeria	3.5		17.6		61.9		2	39.8
Euglena sp-1.	7.0	50.0	23.5		71.4		3	48.3
Netrium	3.5	65.4			4.8		2	35.1
Euglena sp-2.	40.4	26.9	35.3				2	31.1
Mougeotia	63.2	3.8			47.6		2	25.7
Epipyxis	1.8	34.6	5.9			6.3	3	15.6
Pediastrum	24.6		5.9		33.3		2	19.6
Chlorella	36.8	34.6		2.0			2	18.3
Lepocinclis	12.3	26.9	5.9				2	16.4
Mallomonas	33.3	23.1			9.5	37.5	3	23.4
Rhodomonas	50.9	3.8			28.6	18.8	3	17.1
Anabaena				6.1	23.8	12.8	3	14.2
Eunotia	35.1	7.7			19.0		2	13.4
Oedogonium	24.6			6.1	19.0		2	12.6
Temnogametum	8.8		5.9	16.3			2	11.1
Sphaerellopsis	17.5	11.5		10.2			2	10.9
Eunotia	35.1	7.7			19.0		2	13.4

Table 2. - continuation

Genera/Species	Confederation L.	Boomerang L.	Buchans	B-Zone	Link L.	L. McDonald	No	Means
Gomphosphaeria	17.5		5.9		9.5	6.3	3	7.2
Gomphonema	14.0		11.8	2.0			2	6.9
Gleoecystis	5.3	3.8			9.5		2	6.7
Carteria	1.8		5.9		4.8		2	5.3
Chroococcus	31.6	7.7		2.0			2	4.9
Fragilaria	64.9	7.7		2.0			2	4.9
Kephryon	14.0	3.8	5.9			62.5	3	24.1
Bitrichia	12.3			2.0	4.8	75.0	3	27.3
Chromulina	68.4			2.0	4.8	75.0	3	27.3
Merismopedia	26.3			2.0	4.8	6.3	3	4.3
Nephrocytium	3.5			2.0	4.8	6.3	3	4.3
Roya	1.8	57.7					1	57.7
Cryptomonas rostriformis					52.4		1	52.4
Stichococcus			47.1				1	47.1
Aphanizomenon	7.0				42.9		1	42.9
Rhopalodia	28.1	42.3					1	42.3
Elakatothrix	3.5		41.2				1	41.2
Ceratium	22.8				33.3	6.3	2	19.8
Closterium	26.3				28.6		1	28.6
Quadrigula	21.1				28.6		1	28.6
Spirogyra	7.0				28.6		1	28.6
Phacus	7.0				23.8		1	23.8
Coelastrum	7.0				19.0		1	19.0
Amphora	1.8	15.4					1	15.4
Coelosphaerium	33.3				14.3		1	14.3
Pseudokephyrion	17.5			14.3		87.5	2	50.9
Stigeoclonium					14.3		1	14.3
Gonatozygon	1.8			12.2			1	12.2
Microthamnion	1.8		11.8				1	11.8
Tolypothrix	3.5		11.8				1	11.8
Diploneis	10.5	11.5					1	11.5
Eudorina					9.5		1	9.5
Schroederia setigera					9.5		1	9.5
Selenastrum					9.5		1	9.5
Spondylosium	36.8				9.5	12.5	2	11.0
Amphipleura	1.8	7.7					1	7.7
Cyclotella	14.0	7.7					1	7.7
Cocconeis	5.3		5.9				1	5.9
Gloeococcus	1.8		5.9				1	5.9
Amphirora					4.8		1	4.8
Botrycoccus	45.6				4.8	6.3	2	5.5
Cruciegenia	5.3				4.8		1	4.8
Euastrum	3.5				4.8		1	4.8
Frustulia	7.0				4.8		1	4.8
Gonium	1.8				4.8		1	4.8
Hyalotheca dissiliens					4.8		1	4.8
Lagerheimia					4.8		1	4.8
Lyngbya	7.0				4.8		1	4.8
Micractinium					4.8		1	4.8

Table 2. - continuation

Genera/Species	Confederation L.	Boomerang L.	Buchans	B-Zone	Link L.	L. McDonald	No	Means
Microspora	5.3				4.8		1	4.8
Pandorina	1.8				4.8		1	4.8
Zygnema	3.5				4.8		1	4.8
Chlorocloster	1.8	3.8					1	3.8
Surirella	22.8	3.8					1	3.8
Anabaenopsis				2.0			1	2.0
Arthrodesmus	33.3			2.0			1	2.0
Monoraphidium	8.8			2.0		18.7	2	10.4
Stephanodiscus	21.1			2.0			1	2.0
Tetraedron	7.0			2.0			1	2.0
Aphanocapsa	3.5							
Aphanothece	1.8							
Binuclearia	5.3							
Bloeocapsa	3.5							
Bulbochaete	1.8							
Chrysochromulina	5.3							
Chrysoikos	3.5					62.5	1	62.5
Chrysolykos	12.3							
Chrysosphaerella	8.8							
Diatoma	1.8							
Epithemia	15.8							
Eremosphaera	1.8							
Gloeocapsa	21.1					6.3	1	6.2
Hapalosiphon	1.8							
Kiebshormidium	3.5							
Kirschneriella	3.5							
Micrasterias	1.8							
Pleurataenium	3.5							
Rhabdoderma	15.8					6.2	1	6.2
Tabillaria	28.1							
Xanthidium	14.0							

3.2.2 Phytoplankton

With such different and complex water quality contaminants in the five study areas, the following questions arise:

1) How do the phytoplankton communities vary in such different water bodies?
2) Do any phytoplankton taxa associate with particular types of contamination?
3) How does the tolerance of different taxa estimated in this study compare with that determined in toxicity tests?
4) How potentially useful are phytoplankton in the biological monitoring of mining impacts?

Table 2 summarizes the occurrences of phytoplankton genera in the five sites, based on the data used in the present study. As with water quality, this comparison is qualitative. In all five systems, the concentrations of the major contaminants varied over time, but compared with the differences between sites, these variations were minor. Consequently, the temporal changes in phytoplankton communities at each site can be regarded as within-site variation, mainly attributable to other factors (such as changing TSS in B-Zone, and pH in Boomerang Lake), although the contaminant itself might partly contribute to the change (e.g., As in B-Zone). C8 at Confederation Lake, South Bay was used as a reference site, as the phytoplankton data were best documented at this site.

Column 1 in Table 2 shows all genera recorded in the different sites, listed in order of average occurrence frequencies in Boomerang Lake, the B-Zone Pit, Buchans Pits, Link Lakes and Key Lake (Little McDonald). Columns 2-6 show the occurrence frequencies of genera at the above five sites, while Columns 7 and 8 indicate the number of sites where a particular genus was present, and its average frequency. A number of interesting findings are evident:

1) Almost all genera recorded in the biological polishing systems were observed

in the reference site, Confederation Lake, i.e., no genus was unique to these mining impacted systems,

2) Many common genera retained their dominance in one or more biological systems, such as *Oscillatoria* at Boomerang Lake, B-Zone Pit and Link Lakes, *Cryptomonas* at Boomerang Lake, the B-Zone Pit and Link Lakes, *Ankistrodesmus* in Boomerang Lake and Link Lakes, *Chlamydomonas* in Buchans pits, B-Zone Pit and Link Lakes,

3) The first 13 genera were present at all sites, indicating their wide distribution and broad tolerance to low pH, high concentrations of Zn, Ni, As, U and Ra^{226}; these genera appear to be persistent "residents" of the biological polishing systems,

4) Each system supported some genera that were either absent or at low frequencies in the other systems, such as *Dictyosphaerium* in B-Zone Pit, *Netrium* and *Roya* in Boomerang Lake, *Achnanthes* in Buchans pits, and *Planktospheria* in Link Lakes; These genera appear to be specific "residents" in a particular system,

5) Boomerang Lake shared more genera common with Confederation Lake than any other sites, indicating the possible effect of geographical region and that a local reference site should be established,

6) Some genera, listed in the back part of the table, were not recorded at any impacted system, but they are usually rare or at low occurrence frequencies at reference site,

7) Overall, Boomerang Lake and Link Lakes are more similar to each other than to the pits, which lack many genera found in the lakes,

8) In general, the numbers of genera in the biological polishing systems were considerably lower than in Confederation Lake. Boomerang Lake and Link Lakes supported more genera than the B-Zone Pit and Buchans pits, indicating the differences between lakes and pits,

9) Those genera common in the Key Lake group (Little McDonald Lake) all had

high occurrence frequencies in the other biological polishing systems, and in Confederation Lake.

3.3 Phytoplankton community in mine sites and metal toxicology by H. G. Peterson

3.3.1 Field Surveys

Many studies have been carried out in the acidified lakes of Sudbury region, Ontario. Cattaneo (1997) reviewed the phytoplankton occurrences in some of these impacted lakes. In Clearwater Lake, where low pH (5-6) and high Cu (0.09-0.11mg/L) and Ni (0.27-0.29mg/L) existed, *Peridinium* was abundant. This genus was common only in Link Lake in this study, and rarely recorded at the other sites. In Baby and Alice Lakes, with high Ni (>1.0 mg/L) and low pH (4.0-6.3), *Dinobryon* and *Rhizosolenia* were dominant. In the early 1990s, pH was close to neutral, but Ni concentration was still above 1mg/L, *Dinobryon*, *Peridium*, *Chlorella*, and *Kephrion* occurred most commonly. Of these, only *Dinobryon* was common in the mine sites in the present study. Dixit et al. (1989, 1991) surveyed 72 lakes in the Sudbury region and observed that *Pinnularia*, *Eunotia*, and *Frustulia* predominated in most impacted lakes. The first genus was found in all mine sites in this study, but not in very high frequencies.

3.3.2 Phytoplankton Tolerance of Metals Determined by Toxicity Tests

It is important to compare the distribution of phytoplankton genera across mine-site water bodies with the taxon tolerance determined by toxicity tests. A literature study was carried out, which scanned several major citation abstract journals and databases (e.g., Biological Abstract, Current Catalog). The information in the scientific literature with respect to phytoplankton tolerance to metals in mining effluents is limited. However, toxicological information is forming the basis for environmental protection, and guidelines are set for protection of aquatic life. Hence discharge regulations on the metal concentrations which are considered safe for discharge to the receiving environment follow generally our understanding of toxicity.

Rai et al. (1981) and Stokes (1983) who reviewed the responses of phytoplankton species to metal contaminants noted that green algae are generally more tolerant than cyanobacteria and diatoms Voke et al (1980) observed that *Scenedesmus* was most sensitive to Ni. This genus was common in Confederation Lake, Link Lakes and Buchans pits, where Ni concentrations were very low, but it was rarely recorded in the B-Zone Pit only and Little McDonald Lake, where Ni concentrations were intermediately higher. Neither was this genus recorded in Boomerang Lake, where pH is low. Cattaneo (1997) also presented a list of species tolerance according to a literature review. The responses of species in the same genus are frequently reported to be different, but the results are sometimes from different workers. The reported responses of phytoplankton agree with our observations for some genera in this study, but for many others they do not. For instance, *Chlamydomonas*, *Nitzschia* and *Peridium*, listed as tolerant in Cattaneo (1997), are common in all sites except Little McDonald Lake. In contrast, *Ankistrodesmus* and *Tabellaria*, among the most common genera in Boomerang Lake and Link Lakes, were listed as sensitive by the same report.

A comparison was made to the concentration ranges of Zn, Cu, As, and Ni in the waters studied here to those which are derived from toxicity studies in standard growth tests of algae in media with low metal complexing capacity (inorganic media) at a pH of 8. This pH value is considered as the value, where most metals will likely exhibit maximum toxicity if they form inorganic complexes.

Peterson et al. (1996) listed the metal concentration causing 25% inhibition of growth to assess the toxicity of metals to phytoplanktonic algae, cyanobacteria and duckweed in inorganic medium in inorganic medium. The major results are summarised below:

Metals	Green algae	Diatoms	Cyanobacteria	Duckweed
Arsenic (mg/L)	0.3	0.06	>0.5	>0.5
Zinc (mg/L)	0.002	0.025	0.002	0.042
Nickel (mg/L)	0.022	0.02	0.02	0.005

By comparing the results with the metal concentration of metals in the mine-site water bodies in Table 1, the following observations can be made.

1) Arsenic is not present at levels that would cause major inhibition of growth for cyanobacteria and green algae. For diatoms the levels are quite toxic,

2) Copper concentrations are toxic in Buchan's Pit and in Boomerang Lake although the low pH in Boomerang Lake will likely reduce the copper toxicity (Peterson et al. 1985),

3) Zinc would be highly toxic at Buchan's Pit and at Boomerang Lake even after pH adjustment ,

4) Nickel is highly toxic for B-Zone Pit, Buchan's Pit and Little McDonald Lake and likely marginally toxic for Boomerang Lake,

5) The lowest metal levels were encountered in Link Lakes; this water would likely present low toxicity to most phytoplankton.

However, as Table 2 showed, many genera were recorded in all water bodies of the mine sites, including all major groups. One explanation is that when laboratory conditions are different from those prevailing in the mine sites, the results from such toxicity tests are not applicable to the real world. For example, the low pH, multiple metals and dynamic water quality in Boomerang Lake are very different from standard conditions, and would also be difficult to be simulated in laboratory.

However, alternative explanation exists. For some of these sites the algae may have developed a tremendous tolerance for metals present or mechanisms are in place to drastically reduce the toxicity of these metals along with acquired tolerance of metals which can manifest itself through Pollution Induced Community Tolerance (PICT, Blanck and Dahl 1996). The presence of a stressor (for example metal) will change the structure of the communities to a more tolerant species composition resulting in PICT. This has been shown with several metals including tin (Blanck and Dahl 1996) and arsenic (Wangberg et al. 1991). Drastic changes in the community structure in Boomerang Lake are consistent with a move towards a community induced by pollution. Combined with a change in the community the number of species have also been drastically reduced. The reduction in species was particularly severe from 1992 to 1997, as the zinc and copper concentrations increased and the pH decreased.

Mechanisms such as inorganic and organic complexation leading to toxicity reductions are discussed more extensively below.

3.3.3 Effects of pH

Hydrogen and hydroxide concentrations in water will affect the inorganic complexes that metals will form. As the pH increases from acidic levels, the concentration of free metal ions decreases and the quantity of carbonate and hydroxide metal complexes increase for those metals which form these two geochemical forms. Some metals will only form small quantities of these inorganic complexes, such as cadmium, and some dissociate at high pH values requiring each metal to be evaluated separately. As an example, copper will form strong hydroxide and carbonate complexes at basic pH levels, with the free copper ion dropping to levels below 1%. In contrast, cadmium will remain as a free ion, even at basic levels, and its hydroxide and carbonate complexes remain below 10% of the cadmium pool in some inorganic media.

A complicating factor in the determination of metal speciation (geochemical form) in natural water is the definition of "dissolved". It is operationally defined as something passing through a 0.2-1.0 µm filter, which ignores the fact that a large amount of particles are smaller than this, for example colloidal particles. These particles can react with metals decreasing the free metal concentration (Stumm and Morgan 1981). Metal toxicity is therefore composed of a many confounding factors including metal speciation and adsorption, which both will change with the pH and Eh conditions. In addition, competitive actions between metals and hydrogen ions can also explain variations in metal toxicity.

Decreased toxicity of metals at acid compared with basic pH levels has been shown by a host of authors. Peterson *et al.* (1984) and Peterson and Healey (1985) showed that toxicity to the green alga *Scenedesmus quadricauda* was much lower for Cd and Cu at acid pH levels compared with neutral or basic pH levels. This was also shown by Peterson (1991) for another green alga (*Selenastrum capricornutum*). These authors used inhibition of nutrient uptake to determine toxic effects of metals and the reduced toxicity at acid pH levels was interpreted as increased competition by hydrogen ions for surface binding sites leading to an exclusion of metal ions. Several other authors have since shown a similar phenomenon with the blue-green alga (cyanobacterium) *Aphanizomenon gracile* (Luederitz and Scholz, 1989), the green alga *Chlamydomonas reinhardtii* (Macfie *et al.*, 1994) and green alga *Chlorella pyrenidosa* (Parent and Campbell, 1994). These results would be generally applicable to other metals and species. *Scenedesmus, Chlamyodomonas,* and *Chlorrella* were all abundant or common at more than one sites examined in this study.

On the other hand, pH increases the free metal ions present at acidic pH levels will instead start to form inorganic metal complexes, which are less toxic, thereby reducing the toxicity of the metals. Dissolved organic material present in the water will also complex metals and the complexing capacity increases with increasing pH levels also decreasing the toxicity of the metals. Metals are more likely to precipitate at higher pH levels again rendering them less toxic. Some of the "dissolved organic material" will, in nature, in fact, be composed of colloidal material which may react differently to the truly dissolved organic material.

Metal-metal interactions will complicate the picture further. These interactions can be additive, antagonistic and synergistic depending on the metal combination and dose, which will be discussed in more detail later. There are therefore several major factors that need to be taken into consideration when evaluating how pH changes will affect toxicity changes of metals.

3.3.4 Effects of dissolved organic material

Dissolved organic carbon (DOC) present in natural waters will generally act as a complexing agent for metals making the metals less toxic (Campbell and Stokes, 1985; Rhee and Thompson, 1992; Kushner, 1993). At the same time as metals can be made less toxic by combining with naturally occurring dissolved organics this process can also limit the availability of trace minerals causing nutrient limitation for some phytoplankton species (Gensemer et al. 1993). Dissolved organics can, however, also make some minerals more bio-available. Dueker et al (1995) showed that dissolved organics can make colloidal metals more bio-available. Metal chelators, such as EDTA, are added to different media and products to keep metals in solution and in a similar fashion dissolved organics from natural sources will to some extent prevent precipitation of metals. Metal mining liquid effluent discharges can therefore be less toxic if the receiving water is high in DOC compared with waters that are low in DOC. While DOC additions have resulted in reduced metal toxicity for a host of metals and organisms (Bresler and Yanko 1995; Gensemer et al 1993; Winner and Owen 1991) a systematic evaluation of these relationships over a broad DOC range has not been made.

When metal-DOC interactions were evaluated systematically in the laboratory (Peterson in prep.) two different patterns emerged: The first, represented by Cu and Ni, is strongly related to the level of DOC with any increase in the DOC level translating into a decrease in the toxicity of the metals (Type 1 response). For other metals, including cadmium, zinc and chromium, a different pattern emerged where there is a strong relationship between the metals and the dissolved organics up to a set DOC level after which there is no reduction in toxicity of the metals or an increase can even be seen (Type 2 response). Depending on the inhibition level (IC_{25} *vs.* IC_{50}) when metal mining liquid effluent toxicity is examined the pattern can move from a Type 2 response (IC_{25}) to a Type 1 response (IC_{50}). The evaluation of the effects of natural DOC on the toxicity of metals has therefore a host of connected variables including: actual metal, amount of DOC, and inhibition level. In mining waste waters combinations of metals with Type 1 and Type 2 behaviour combined with receiving waters of different chemical composition makes it quite challenging to predict toxic effects. It is possible that Type 1 and Type 2 responses are related to the nature of the DOC and as binding sites are occupied by metals the Type 2 response is elicited.

Winner and Owen (1991) postulated that DOC changes in freshwater change quantitatively and qualitatively with time making it impossible to use DOC concentrations to predict copper-binding affinity and therefore toxicity to aquatic biota. In contrast to this prediction the present work (Peterson in prep.) showed a strong correlation between toxicity of copper in relation to DOC spanning a wide range of DOC concentrations (0-24.5 mg/L). This indicates that the copper binding capacity of DOC from varying freshwater sources can be quite similar per unit DOC. Similar copper binding capacities per unit DOC have also been shown for marine systems (Newell and Sanders 1986). The level of DOC in a receiving water body may therefore be used at least as an indicator of potential metal complexing capacity. Therefore a method of protection of the environment would be to increase the DOC.

3.3.5 Metal-metal interactions

Metal mining wastewater effluents commonly contain several potentially toxic metals, such as Ni and As in B-Zone Pit. Therefore, the interaction between metals in eliciting a toxic response is of concern. Wong *et al.* (1978) showed that in a mixture of 10 metals with each metal at a non-inhibitory concentration inhibited growth of *Scenedesmus quadricauda* 70%. Braek *et al.* (1976) suggested that potential effects of combined metals cannot be predicted on the basis of the toxicity of the individual metals themselves as they demonstrated various metal interactions in the toxicity of copper and zinc. There are three possible scenarios for such interactions: no-interaction (additive), synergistic, or antagonistic (Christensen *et al.* 1979). However there are further complicating factors which must also be considered.

Stratton and Corke (1979) concluded that algal response towards combinations of metal ions was dependent upon the order of metal additions, the metal concentrations involved, and the selected test endpoint. Evidence supports that combined metal effects are species dependent (Wong *et al.* 1978, Stratton and Corke 1979, Braek *et al.* 1980). Another factor to consider is that metals interact in different ways with other media components (such as organic complexing agents and pH).

Individual metal concentrations strongly influenced the response when the metals were tested in combination. Dose dependency was supported by Stratton and Corke (1979), who indicated that the result of metals acting in combination are affected by the actual concentrations of the metals. Results indicate that zinc and copper act synergistically when tested in combination (Bartlett *et al.* 1974, Braek *et al.* 1976). Results in

Peterson's laboratory indicate an additive effect for combined zinc and copper at only one of the combination concentrations tested.

When copper and nickel were combined synergistic effects were shown as was also shown by Hutchinson and Stokes (1975). Bartlett *et al.* (1974) reported that cadmium inhibits copper toxicity, which is in agreement with our experimental results for low concentrations of combined copper and cadmium. At higher levels of cadmium the antagonistic effect is replaced by a weak synergistic effect. Results from Peterson's laboratory indicated a synergistic effect for cadmium and zinc combined, which is in agreement with Braek *et al.* (1980). Peterson's data also showed that this synergism increased at higher concentrations of cadmium and zinc. Stratton *et al.* (1979) found the combination of nickel and cadmium acted antagonistically. On the contrary, our results showed that cadmium and nickel acted synergistically, and this combination was the only one which did not show dose dependency. Most of this work has been carried out at circumneutral or slightly alkaline levels.

Our present understanding , reflected by the literature is in agreement that the interactions of metals when in combination is complex and that many factors influence the response. Species differences, endpoint, test media composition, temperature, pH, number of metals in combination, and mode of action of individual metals will all influence the toxic response.

Further work is needed to understand phytoplankton responses with metals. Methods for accurate experimental design and presentation of results are necessary. The finding that metal-metal interactions can be antagonistic, non-interactive (additive), or synergistic for the same metal pairs depending on the concentration makes it difficult to predict toxic response from chemical data alone.

3.3.6 Zooplankton effects on phytoplankton species composition

Zooplankton will consume phytoplankton that are 2-50 μm in size. Small algae will often have substantially greater rates of growth than colony-forming algae. Rapid growth rates of these smaller algae will, however, be counter-acted by zooplankton predation. It is therefore not common to find blooms of small algae in systems that are not contaminated. For example, if copper sulphate is added to a reservoir (typically to control cyanobacteria), the cyanobacteria will be killed, but in addition most zooplankton will perish. This often results in "man-made" green algal blooms (small green algae within the zooplankton edible range) simply as a result of lack of predation.

High levels of metals may have a similar effect on the zooplankton populations resulting in a competitive advantage for small phytoplankton. In contrast, high zooplankton pressure will encourage non-edible phytoplankton species (larger phytoplankton). Such species are typically forming filaments or colonies with or without production of mucus. In the B-zone pit , zooplankton was monitored each year once. Although this is not sufficient to document the interactions of zooplankton as a predation force, in is interesting to note, that in 1998 rotifers were suddenly quite abundant, but they were not found previously. Thus in the presence of Ni , zooplankton could have an effect on the community structure. If these observations are translated to the B-Zone pit, it would allow us to state, that given that *Dictyospherion* was present in the very beginning as virtually the only species in the pit, and at bloom levels continuously, that this species could have been there due to zooplankton pressure, rather than due to its tolerance to Ni and As. This adds certainly an interesting argument to considerations of community structure in mine waste waters.

3.3.7 Effects of dissolved inorganic carbon

One factor for that data is not available in this study, but probably also strongly influencing phytoplankton communities, particularly their abundance, is Dissolved Inorganic Carbon (DIC). Several evaluations have been carried out in Boomerang Lake to determine the type of nutrient limitation. Phosphate and nitrate have been added to the lake to increase nutrient levels. However, this did not lift the growth limitation. It was suggested that dissolved inorganic carbon concentrations are restricting growth as discussed below.

The pH level not only controls the speciation of metals it also controls the speciation and solubility of an algal nutrient, inorganic carbon. The effects of reducing the pH to low levels results in dissolved inorganic carbon (DIC) levels around 10 µM until the pH of 5.5 or so is reached. It then increases to around 17 μM at pH 6. These are theoretical values and when actual values were determined it was shown that for acidic lakes in the U.S. 6% were below theoretical equilibrium values, 16% were more than 5 times higher than expected and 76% were well above equilibrium values (Titus *et al.* 1990). These results were probably due to the DIC being released from organic sediments.

These low DIC values may limit the rate of photosynthetic production in acidic systems like Boomerang Lake. A tight relationship between DIC and net photosynthesis was shown by Turner et al. 1995 for benthic productivity. From Turner et al's (1995) and Titus et al's (1990) data it is possible to predict benthic increases in algal productivity. Net photosynthesis will not increase until a pH of 6 with the largest gains between 6 and 7 (all based on theoretical equilibrium concentrations). This suggests a productivity of 7.2 mg C/m^2/d, (assuming a 10 h day) which is very oligotrophic.

However, if most of the DIC in Boomerang Lake is coming from organic sediments rather than equilibrium with the air, the greatest beneficiaries would be the benthic vegetation (e.g. *Sphagnum*). We expect an even larger benthic algal/moss component with a more "benign" water column DIC concentration. The acidification of a lake and its effects on periphyton have been studied most recently by Turner et al. (1995). They suggest that as acidification of a lake proceeds, the benthic algae may become DIC limited. This is apparent from the decrease in net photosynthesis, and in increase in compensation irradiances. Highest photosynthetic rates of benthic algae in acid lakes occurred in spring and fall when water temperatures were low, and CO_2 solubilities were highest. Algal respiration also increased with increasing acidification.

Can it then be assumed that increases in photosynthetic rates as the pH is increased to around neutral will also result in increased phytoplankton biomass? There were no increases in phytoplankton biomass and/or biovolumes in acidic lakes around Sudbury. Yan (1979) suggested that phosphorus was a better indicator of community biomass than hydrogen ion concentration. Molot et al. (1990) also suggested that for lakes in the vicinity of Sudbury, neutralization effects on phytoplankton biomass were "not significant", although dramatic changes in composition occurred. Many of the studies which showed either no effect of pH or a negative effect of biomass increases with neutralization suggested that as the lakes became more circum-neutral, they started to develop more stable ecosystems, which included zooplankton. As zooplankton diversity and biomass increase, there must be corresponding decrease in their primary food source - phytoplankton.

Probably the biggest advantage of increasing pH would be the increase in buffering capacity, by adding a carbonate/bicarbonate/CO_2 buffering system to Boomerang Lake. As the pH increases, more species of carbon are found in equilibrium. This will assist in maintaining pH and acidity in the lake.

As carbon levels in the lake increase, we need to maintain the appropriate ratios of carbon to nitrogen and carbon to phosphorus. The phosphorus additions of several years ago will hopefully still be found in the sediments, and that P is being recycled in the sediments and the water column.

In conclusion, it is still evident that Boomerang Lake is carbon limited for production. However, raising the pH to 5 or even 6 will do little to raise the equilibrium concentration of carbon in the lake. It is still far better to raise the level of respiration in the organic sediments. This could produce DIC concentrations in the lake that are far higher than equilibrium DIC levels. A raise in pH to 7 would significantly increase plant productivity, but would also allow zooplankton to colonize the lake. This may lead to a decrease in algal productivity.

3.3.8 Some general considerations

There are therefore several complicating factors when toxicity of metals are evaluated in metal mining polishing ponds. Four of these environmental factors were discussed above (pH, dissolved inorganic carbon, dissolved organic material and metal-metal interactions). A fifth factor, the interaction of colloidal particles and metals, may be important in determining the speciation and toxicity of metals to algae. While it can be stated that in inorganic media as the pH increases, the protective effect of hydrogen ions on the cell surfaces will decrease rendering metals more toxic.

When environmental factors reducing the toxicity of metals have been excluded in laboratory bioassays with micro-algae it is clear that toxicity is elicited at levels that are in the low microgram range. In contrast, metal levels in biological polishing ponds are frequently orders of magnitude higher and still algae will grow under those conditions. The algae may have developed a tremendous tolerance for metals present. This could be attributed to effective exclusion mechanisms, or mechanisms are in place to sequester metals and render them non-toxic. Environmental factors, such as reduced pH, dissolved organic material, or colloidal material will render metals less bio-available and therefore less toxic. As toxic metals become less bio-available so will likely trace nutrients (often metals) and it is possible that these species have low requirements for trace elements. We have poor knowledge of how metals affect phytoplankton in biological polishing ponds. With some of this basic knowledge it may be possible to effectively bio-manipulate these biological polishing ponds.

In situations where the free metal ions remain dominant at different pH levels the hydrogen ions will therefore generally protect the organism from the toxicity of the metal resulting in less toxicity (Peterson and Nyholm 1993). Les and Walker (1984) showed that the amount of metal bound to the cyanobacterium *Chroococcus paris* increased with pH (decreased hydrogen concentrations on the cell surface) for Cu, Cd, and Zn supporting this theory. While decreased toxicity is anticipated at acidic pH levels this may also infer that trace metal deficiencies can occur at acidic pH levels (Gensemer *et al.* 1993). Hydrogen ion protection of cells can explain the growth of algae in acid mine waste streams where metal levels are extremely high as outlined in the present report. Thus toxicity of metals at lower pH values, however, is a complex issue and the manipulation of such systems to increase biological polishing requires a better understanding of algal interactions with the chemistry of mine waste waters.

When working with algae it needs to be recognized that in contrast to animals, algae and aquatic weeds can greatly affect water quality. If this is not taken into consideration when toxicity tests are carried out, the results may not accurately represent the toxic potential of the tested sample. These complications in evaluating toxicity was pointed out by Nyholm and Källqvist (1989); these authors clearly showed that when the U.S. EPA test is carried out according to the established protocol, the pH drift in the test medium was quite severe. When algae are stressed due to exposure to toxicants they often release organic compounds that can in turn form organic metal complexes decreasing the toxicity of the metals . While this is a problem in toxicity testing, it is the founding principle for using algae to treat metal mining waste waters in a bioremediation sense.

4.0 CONCLUSIONS AND RECOMMENDATIONS

The present study examined the phytoplankton communities in the water bodies in five mine sites, where different types of metal contaminants existed in relation to major water quality variables, using data collected over the long term. A variety of univariate and multivariate statistical approaches were applied to describe and quantify trends or patterns in water quality and phytoplankton communities.

A number of conclusions can be drawn from the detailed data analyses and discussions presented in the previous sections:

1) Phytoplankton communities in the biological polishing systems consist of many widely-tolerant genera, and some site- or contaminant-specific genera. The communities themselves could change markedly overtime, often due to changes in water quality factors other than metal contaminants,
2) Control factors vary from one system to another. In the B-Zone Pit, TSS and, to a lesser extent As, play major roles in the temporal changes in community structure, but nutrient supply appears to determine the total phytoplankton density or biomass. In Boomerang Lake, pH and Zn are more important than other control factors in shaping the phytoplankton community structure,
3) Since almost all genera recorded in the wastewater systems are present in the reference site, and most common genera in the former are also common in the latter, it is difficult to define indicator species for mining impacts unless the conditions are extreme (e.g., an *Euglena* species was found to tolerate pH as low as 3-4). The number of genera in the biological polishing systems and impacted lakes, however, are typically depressed. Phytoplankton community structure appears to be more useful in assessing mining impacts, although seasonal and long-term variations and the effects of non-contaminating factors can strongly influence the results of biological monitoring,
4) There are many obvious disagreements between the responses of

phytoplankton to metal contamination observed in this study and those published in reports from field surveys or toxicity studies,

5) Phytoplankton taxa found in the mine-site waters showed much higher tolerance to high level of metals than determined in toxicity tests, suggesting that complex protection mechanisms have been developed in these taxa,

6) It is more reliable and useful to define tolerance than sensitivity. When a taxon is not present or has disappeared, it may be due to water quality variables other than metal concentration - an argument that the results from the B-Zone Pit support,

7) In Key Lake, there is a water quality gradient along Little McDonald - McDonald -other reference lakes, and Nickel concentration decreased in this order. The variation in the phytoplankton community structure over the three sampling years (1993, 1994, 1998), however, is more significant than over the water quality gradient.

A well-designed sampling program is required to better define the differences between phytoplankton communities among different metal-related biological polishing systems; Two or more water bodies that are similar in metal contaminants and other major water quality variables can be used to quantify the within-contamination category variation, and key community components.

More comprehensive investigations of the biology, ecology and toxicology of key genera/species in the biological polishing systems are needed; Mechanism studies are also important. The two key questions that need to be resolved include: i) by which means do algae/aquatic plants most effectively detoxify (bioremediate) metal waste waters? ii) how can we take advantage of those attributes?

Routine monitoring programs provides critical information about water quality changes, but more studies are required to show what factors or processes control phytoplankton community and water quality changes. Maybe the generation of dissolved organic material is a key factor. Maybe the generation of colloidal organic material is another key factor. Resolving the key issues surrounding effective bioremediation of metal mining waste waters will have both environmental and economic benefits. The tools to establish the importance of the above factors are available in Canada even if several of them have not been applied to metal waste waters before. It is now possible to mimic in the laboratory full seasonal cycles (temperature, light conditions, light period etc.) within short time spans (less than one month) using specially designed incubators. Combining ecological assessments with targeted laboratory bioassays will pave the wave for more efficient bioremediation efforts.

PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 1

REFERENCES

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

Bartlett, L., F. Rabe, and W.H. Funk. 1974. Effects of copper, zinc and cadmium on *Selenastrum capricornutum*. *Water Res.*, **8**:179-185.

Belbin, L. 1992. PATN: pattern analysis package - Reference Manual. Division of Wildlife and Rangelands Research, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia.

Boojum. 1993. Ecological engineering of the Rabbit Lake Drainage Basin - 1993 Final Report - submitted to CAMECO.

Boojum 1996. Ecological Engineering of the Rabbit Lake Drainage Basin - 1995 Annual Report. Submitted to CAMECO.

Boojum. 1997. Zinc Removal Strategy - 1996 Final Report, prepared for ASARCO, Inc.

Boojum. 1998. B-Zone Pit: The Fate of As and Ni - proof from the sediment 1998 Final Report, submitted to CAMECO.

Braek, G.S., A. Jensen, and A. Mohus. 1976. Heavy metal tolerance of marine phytoplankton III. Combined effects of copper and zinc ions on cultures of four common species. *J. Exp. Mar. Biol. Ecol.*, **25**:37-50.

Braek, G.S., and D. Malnes, A. Jensen. 1980. Heavy metal tolerance of marine phytoplankton IV. Combined effect of zinc and cadmium on growth and uptake in some marine diatoms. *J. Exp. Mar. Biol. Ecol.*, **42**:39-54.

Bresler, V., and V. Yanko, 1995. Acute toxicity of heavy metals for benthic epiphytic foraminifera *Pararotalia spinigera* (Le Calvez) and influence of seaweed-derived DOC. *Environmental Toxicology and Chemistry*, **14**(10):1687-1695.

CAMECO Corporation 1979. Key Lake Project EIS.

Campbell, P.G.C. and P.M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**:2034-2049.

Cao, Y., W.P. Williams, and A.W. Bark. 1997. A comparison of clustering methods for river benthic community analysis. *Hydrobiologia*. 347: 24-40.

Cao, Y., D.D. Williams and N.E. Williams. 1999. Data standardisation and transformation in multivariate analysis of river water quality. *Ecological Applications*, 9(2): (in press).

Cattaneo, A. 1997. Phytoplankton. In: St- Cyr, L., A. Cattaneo, R. Chasse, and Fraikin, C.G.J. (eds), Aquatic Effects Technology Evaluation Program - Technical evaluation of monitoring methods using macrophyte, phytoplankton and periphyton to assess the impacts of mine effluents on the aquatic environment. CANMET, Natural Resources Canada, Ottawa.

Christensen, E.R., J. Scherfig, and P.S. Dixon. 1979. Effects on manganese, copper and lead on *Selenastrum capricornutum* and *Chlorella stigmatophora*. *Water Res.*, **13**:79-92.

Clarke, K.R. and R.M. Warwick. 1994. Change in marine communities: an approach to statistical analysis and interpretation. UK NERC and Plymouth Marine Laboratory.

Conor Pacific Environmental Technologies, Inc. 1998. Key Lake Operation - McDonald Lake Drainage 1998: Nickel Investigation Report. Submitted to CAMECO Corporation.

Davis, W. S., and T.P. Simon. 1995. Introduction. In W.S. Davis and T.P. Simon (eds), Biological assessment and criteria: tolls for water resources planning and decision-making. Lewis Publisher, London, Page 3-6.

Digby, P.G.N., and R.A. Kempton. 1987. Multivariate analysis of ecological communities. Chapman & Hall, London.

Dixit, S.S., A.S. Dixit, and J.P. Smol. 1989. Relationship between chrysophyte assemblages and environmental variables in seventy-two Sudbury lakes as examined by Canonical Correspondence Analysis (CCA). *Can. J. Fish. Aquat. Sci.* 46:1667-1676.

Dixit, S.S., A.S. Dixit, and J.P. Smol. 1991. Multivariate environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. *Freshwat. Biol.* 26: 251-256.

Dueker, A., A. Ledin, S. Karlsson, and B. Allard, 1995. Adsorption of zinc on colloidal (hydr)oxides of Si, Al and Fe in the presence of a fulvic acid. *Applied Geochemistry*, **10**(2):197-205.

Elliot, R. 1983. Statistical methods for processing benthic invertebrates data. FBA Special publication 25, Windermere, England.

Environment Canada. 1995. Canadian water guidelines - summary of guidelines for water quality in Canada. Evaluation and Interpretation Branch, Environmental Conservation Service, Environmental Canada, Ottawa, Ontario.

Gauch, H.G. 1982. Multivariate analysis in community ecology. Cambridge University Press.

Gensemer, R.W., R.E.H. Smith, H.C. Duthie, and S.L. Schiff, 1993. pH tolerance and metal toxicity in populations of the planktonic diatom *Asterionella*: influences of synthetic and natural dissolved organic carbon. *Canadain Journal of Fisheries and Aquatic Sciences*, **50**(1):121-132.

Hasle, G.R. 1978. The inverted microscope method. In A. Sournia (ed.), Phytoplankton Manual. UNESCO Publication, Paris, France. Pp:88-96.

Hill, M.O. 1979. DECORANA - A FORTRAN program for Detrended correspondence Analysis and Reciprocal averaging. Section of Ecology and Systematics. Cornell University, Ithaca, New York.

Hirsch, R.M., R.B. Alexander, and R.A. Smith. 1991. Selection of methods for the detection and estimation of trends in water quality. *Water Resource Research.* 2: 803-813.

Hutchinson, T.C., and P.M. Stokes. 1975. Heavy metal toxicity and algal bioassays. In: *Water quality parameters*. Am. Soc. Test. Mater. Spec. Tech. Public. **573**:320-343.

Jackson, G.S., P. Hamilton, K. Solomon, and N. Kaushik. 1984. A computerised plankton counter. *Hydrobiologia*. 118: 225-228.

Kalin, M. 1986. Biological polishing of acid seepage creeks. Proceedings of the Third Annual General Meeting of BIOMINET, Toronto, Ontario, August 20-21, pp: 173-174.

Kalin, M. 1989. Ecological engineering applied to base metal and Uranium mining wastes. Proceedings of the 1989 Biohydrometallurgy Conference (CANMET), Jackson Hole, Wyoming, August 13-18. Pp: 363-368.

Kalin, M. 1998a. The role of applied biotechnology in decommissioning mines operations. Proceedings of the 30th Annual Meeting, London, UK, January 20-22. pp: 154-167.

Kalin, M. 1998b. Function of wetlands/muskeg in decommissioning mine waste management areas. Submitted to Canadian Mineral Processors Conference at Toronto, Ontario, September 10-14.

Kalin, M., J. Cairns, and W.N. Wheeler. 1991. Biological alkalinity generation in acid mine drainage. Proceedings of the Second International Symposium on the Biological Processing of Coal, San Diego, California, May 1-3. Pp: 105-112.

Kalin, M. and M. Olaveson. 1997. Controlling factors in the production of extracellular polysaccharides in phytoplankton - Final Report. Prepared for CANMET.

Kalin, M., A. Fyson, and M.P. Smith. 1998. Ecological engineering with carbon amendments to treat acid mine drainage: field and laboratory experiments. *Ecological Engineering* (under review).

Kushner, D.J. 1993. Effects of speciation of toxic metals on their biological activity. *Water Air and Soil Pollution*, **23**(1):111-128.

Les, A. and R.W. Walker 1984. Toxicity and binding of copper, zinc and cadmium by the blue-green alga *Chroococcus paris*. *Water Air and Soil Pollution*, **23**(2):129-139.

Luederitz, V. and F. Scholz 1989. Copper uptake by blue-green algae. *Acta Hydrochimica et Hydrobiologica*, **17**(5):537-542.

Lund, J.W.G., C. Kipling, and E.D. Le Cren. 1958. The inverted microscope method of estimating algal numbers, and the statistical basis of estimation by counting. *Hydrobiologia*. 3: 390-394.

Macfie, S.M., Y. Tarmohamed, P.M. Wellbourn 1994. Effects of cadmium, cobalt, copper, and nickel on growth of the green alga *Chlamydomonas reinhardtii*: the influences of the cell wall and pH. *Archives of Environmental Contamination and Toxicology*, **27**(4):454-458.

McBride, B.B., J.C. Loftis, and N.C. Adkins. 1993. What do significance tests tell us about the environment? *Environment Management* 17: 423-432.

Milligan, G.W. and M.C. Copper. 1987. Methodology review: clustering methods. *Appl. Psychol. Measurement*. 11: 329-345.

Mitsch, W. J. 1994. Ecological engineering and Ecosystem restoration: a study of the use of ecological principles for solving environmental problems. SCOPE Newsletter. No. 46. SCOPE Secretariat, Paris.

Molot, L.A., L. Heintsch, and K.H. Nicholls. 1990. Response of phytoplankton in acidic lakes in Ontario to whole lake neutralisation. Can. J. Fish. Aquat. Sci. 47: 422-431.

Nauwerk, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton in See Erken. *Symb. Bot. Upsal.* 17:1-163.

Newell, A.D. and J.G. Sanders, 1986. Relative copper binding capacities of dissolved organic compounds in a coastal plain estuary. *Environmental Science and Technology*, **20**(8):817-821.

Nyholm, N. and T. Källqvist. 1989. Methods for growth inhibition toxicity tests with freshwater algae. *Environmental Toxicology & Chemistry*, **8**:689-703.

Orloci, L. 1967. An agglomerative method for classification of plant communities. *Journal of Ecology*. 55: 193-206.

Parent, L., P.G.C. Campbell 1994. Aluminum bioavailability to the green alga *Chlorella pyrenoidosa* in acidified synthetic soft water. *Environmental Toxicology and Chemistry*, **13**(4):587-598.

Peterson, H.G., F.P. Healey and R. Wagemann. 1984. Metal toxicity to algae: a highly pH dependent phenomenon. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**:974-979.

Peterson, H.G., F.P. Healey 1985. Comparative pH dependent metal inhibition of nutrient uptake by *Scenedesmus quadricauda* (Chlorophyceae). *Journal of Phycology*, **21**:217-222.

Peterson, H.G., N. Nyholm, N. Ruecker, and I. Cantin (in prep *a*). Development of a fluorimetric multispecies 96-well microplate growth inhibition test for screening toxicity to phytoplankton.

Peterson, H.G., N. Nyholm, and N. Ruecker (in prep *b*). Effects of naturally occuring dissolved organic carbon on the toxicity of metals and metal mining effluents to the green alga *Selenastrum capricornutum.*

Peterson, H.G. 1991. Toxicity testing using a chemostat grown green alga, *Selenastrum capricornutum*. In: *Plants for Toxicity Assessment: Second Volume*, J.W. Gorsuch, W.R. Lower, W. Wang, M.A. Lewis, Eds., ASTM STP 1115, American Society for Testing and Materials, Philadephia. PA, pp. 107-117.

Peterson, H.G. and N. Nyholm. 1993. Algal bioassays for metal toxicity identification. *Water Pollution Research Journal of Canada*, **28**:129-153.

Peterson, H.G., N. Nyholm, M. Nelson, R. Powell, P.M. Huang, and R. Scroggins, 1996. Development of aquatic plant bioassays for rapid screening and interpretive risk assessments of metal mining liquid waste waters. *Water, Science and Technology*, **33**:155-161.

Pielou, E.C. 1984. The interpretation of ecological data. Wiley, New York.

Rai, L.C., J.P. Gaur, and H.D. Kumar. 1981. Phycology and heavy metal pollution. *Biological Review*. 56: 99-151.

Rhee, G.Y. and P.A. Thompson, 1992. Sorption of hydrophovic organic contaminants and trace metals on phytoplnakton and implications for toxicity assessment. *Journal of Aquatic Ecosystems and Health*, **1**(3):175-191.

Rosaasen, A. R. 1997. Impact of minewater effluent on phytoplankton in a series of Northern Saskatchewan Lakes. Unpublished M. S. Thesis, University of Saskatchewan.

Smith, M. P. and M. Kalin. 1989. A novel approach to decommissioning: biological polishing through ecological engineering for Uranium operation. Conference of Biohydrometallurgy, Jackson Hole, Wyoming, Aug. 23-18.

SPSS, Inc. 1998. SYSTAT® 8.0 - Statistics. Chicago. pp: 1-1086.

St-Cyr, L., A. Cattaneo, R. Chasse, and Fraikin, C.G.J. 1997. Aquatic Effects Technology Evaluation Program - Technical evaluation of monitoring methods using macrophytes, phytoplankton and periphyton to assess the impacts of mine effluents on the aquatic environment. CANMET, Natural Resources Canada, Ottawa.

Stokes, P.M. 1983. Responses of freshwater algae to metals. *Prog. Phyco. Res.* 2: 88-112.

Stratton, G.W., and C.T. Corke. 1979. The effect of mercuric, cadmium, and nickel ion combinations on a blue-green alga. *Chemosphere*, **10**:731-740.

Stumm and Morgan. 1981. Aquatic chemistry - An introduction emphasizing chemical equilibria in natural waters. John Wiley &Sons, New York.

Ter-Braak, C.J.F. 1987. Ordination. In Jongman, R.H., C.J.F. ter Braak, and O.F.R. Tongeren (eds), Data analysis in community and landscape ecology. Pudoc, Wageningen. Pp91-173.

Titus J.E., R.S. Feldman, and D. Grise. 1990. Submerged macrophyte growth at low pH. I. CO_2 enrichment effects with fertile sediment. *Oecologia*. 84: 307-313.

Turner, M.A., E.T. Howell, G.G.C. Robinson, J.F. Brewster, L.J. Sigurdson, and D.L. Findlay. 1995. Growth characteristics of bloom-forming filamentous algae in the littoral zone of an experimentally acidified lake. Can. J. Fish. Aquat. Sci. 52: 2251-2275.

Ultermohl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* 9: 1-38.

Venrick, E.L. 1978. How many cells to count? In: A. Sournia (eds). Phytoplankton Manual. UNESCO Publication, Paris, France. pp: 167-180.

Voke, R. W., K.L. Sear, J.J. O'Toole, and R.B. Wildman. 1980. Growth responses of selected freshwater algae to trace elements and scrubber ash slurry generated by coal-fired power plants. *Water Research*, 14: 141-150.

Vollenweider, R.A., J.F. Talling, and D.F. Westlake (eds). 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handbook 12. Blackwell Scientific Publication, Oxford, UK.

Winner, R.W. and H.A. Owen, 1991. Toxicity of copper to *Chlamydomonas reinhardtii* (Chlorophyceae) and *Ceriodaphnia dubia* (Crustacea) in relation to changes in water chemistry of a freshwater pond. *Aquatic Toxicology*, **21**(3/4):157-170.

Wong, P.T.S., Y.K. Chau, and P.L. Luxon. 1978. Toxicity of a mixture of metals on freshwater algae. *J. Fish. Res. Board Can.*, 35:479-481.

Wright, J. F., M. T. Furse and P. D. Armitage. 1993. RIVPACS - a technique for evaluating the biological quality of rivers in the UK. European Water Pollution Control, 3 (4):15-25.

Zar, J.H. 1984. Biostatistical Analysis, Prentice-Hall International, Inc., New York.

PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 2

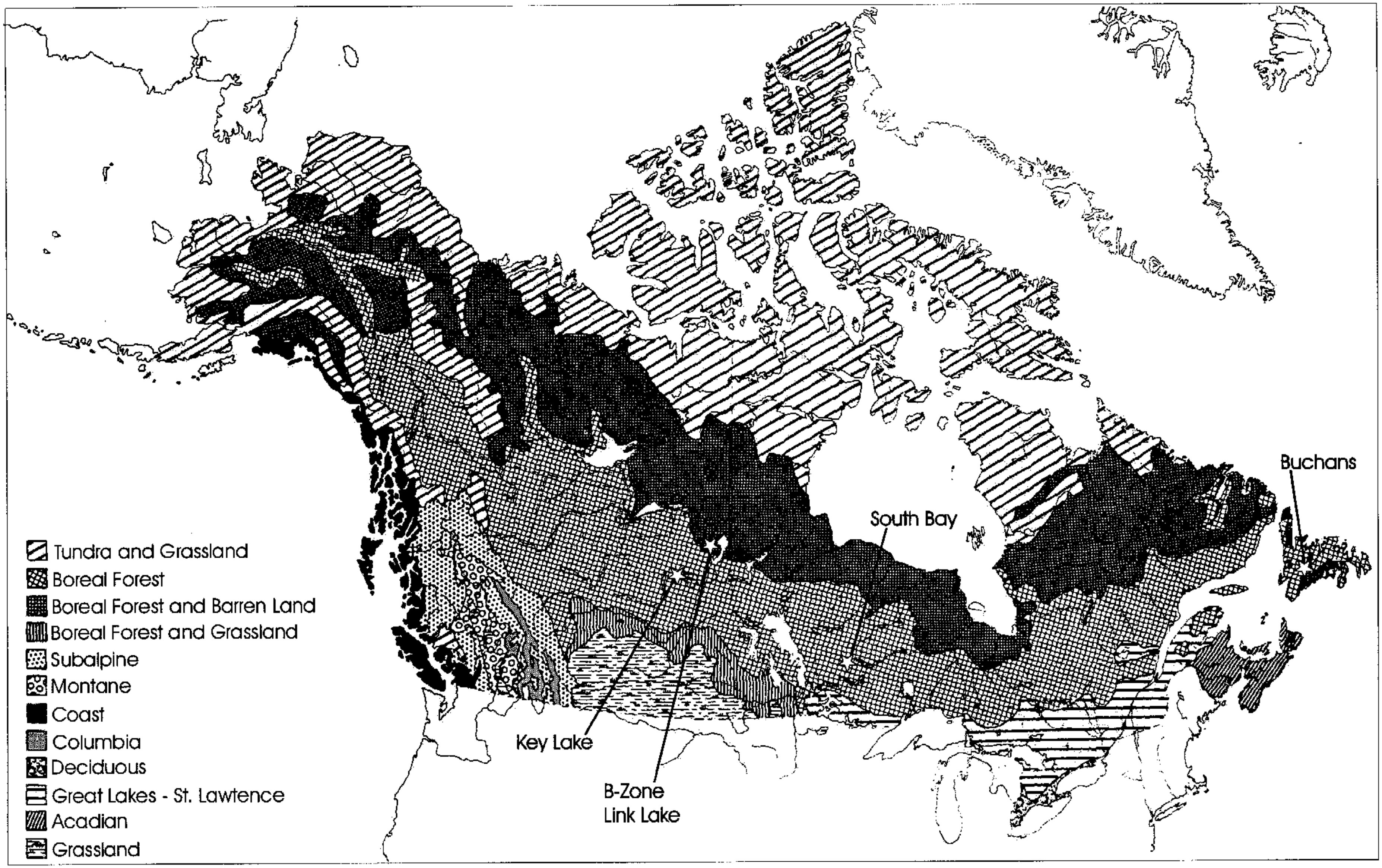
MAPS

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

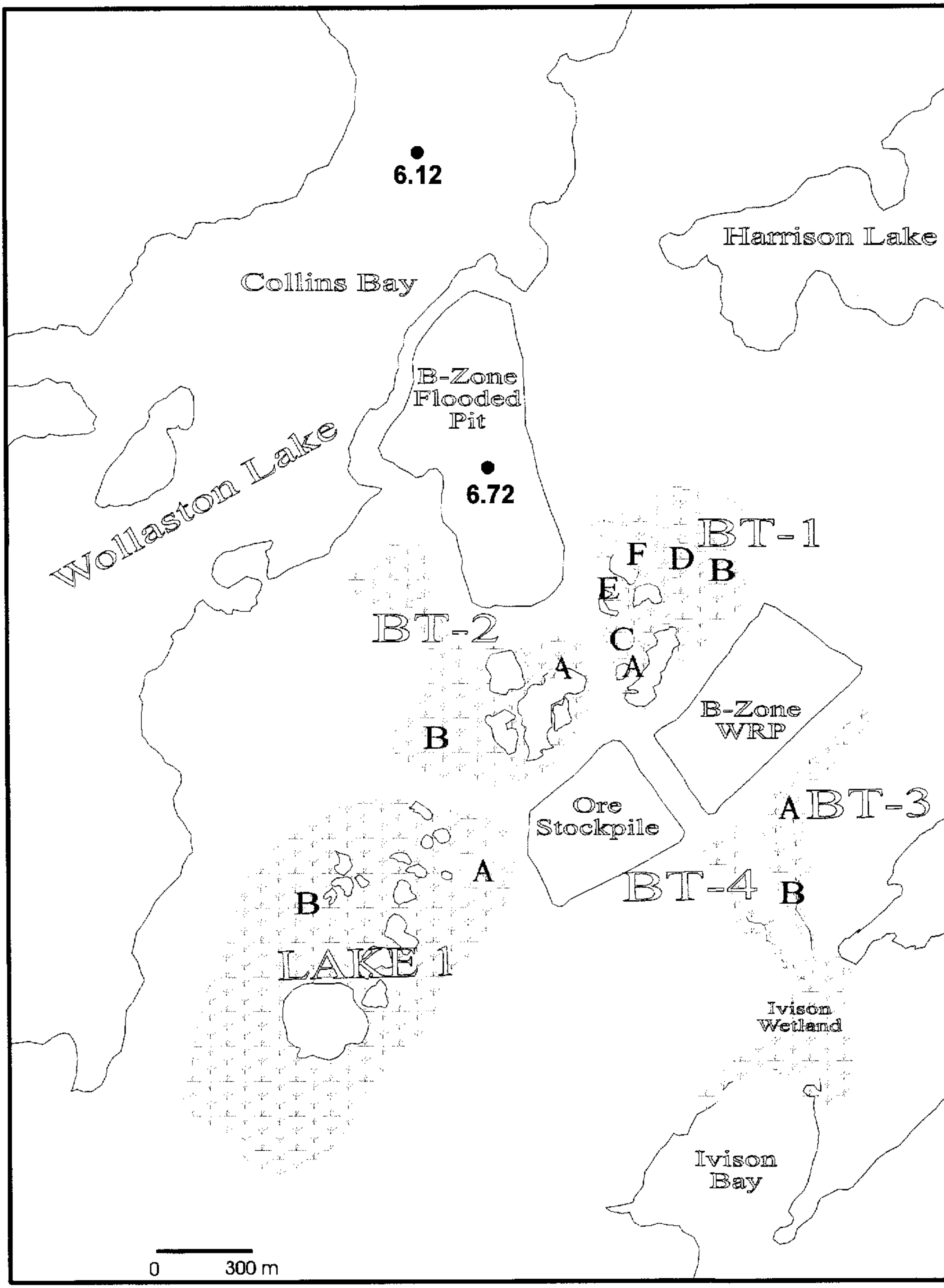
June, 1999

LIST OF MAPS

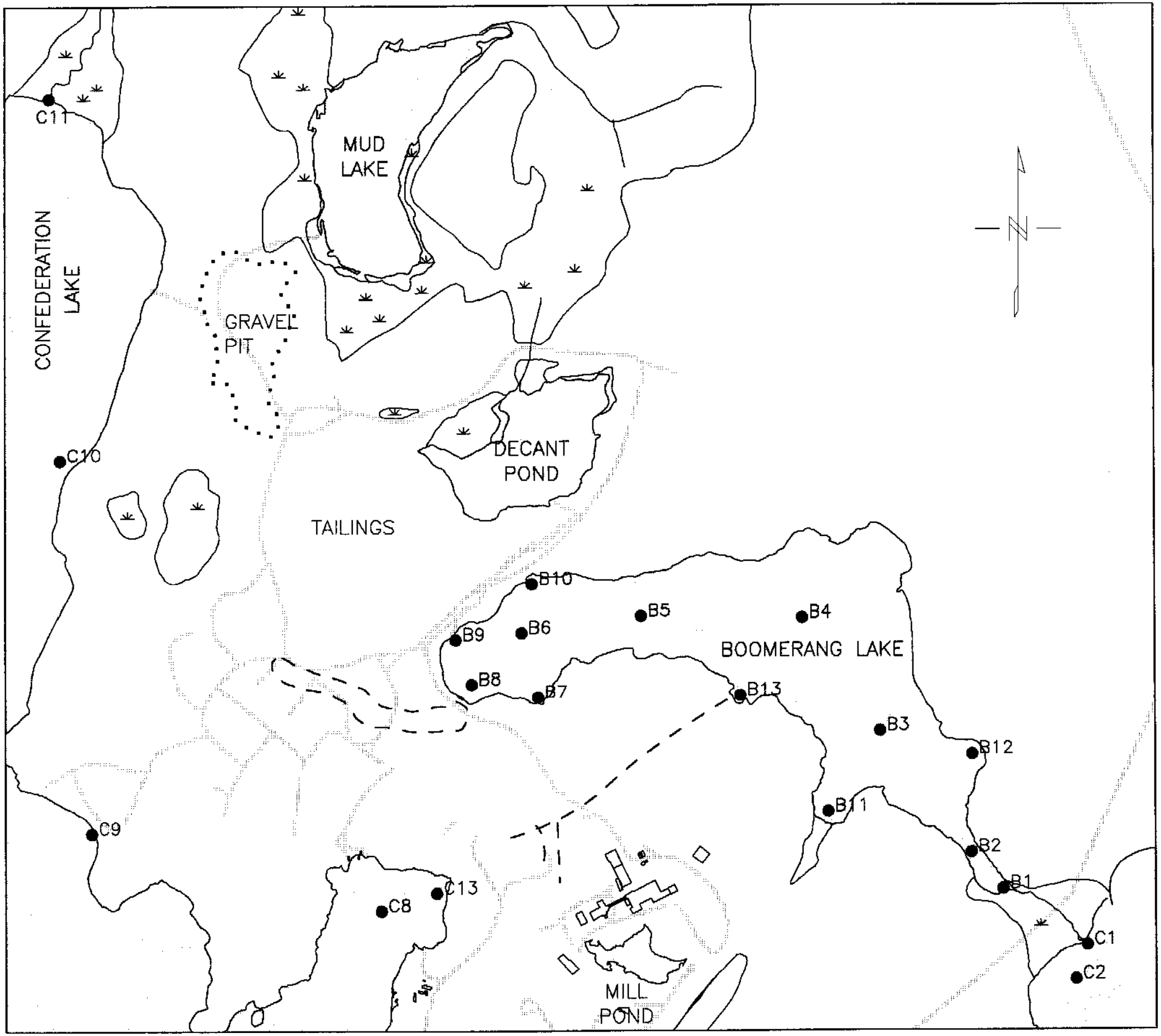


Map 1: Overview of Site Locations

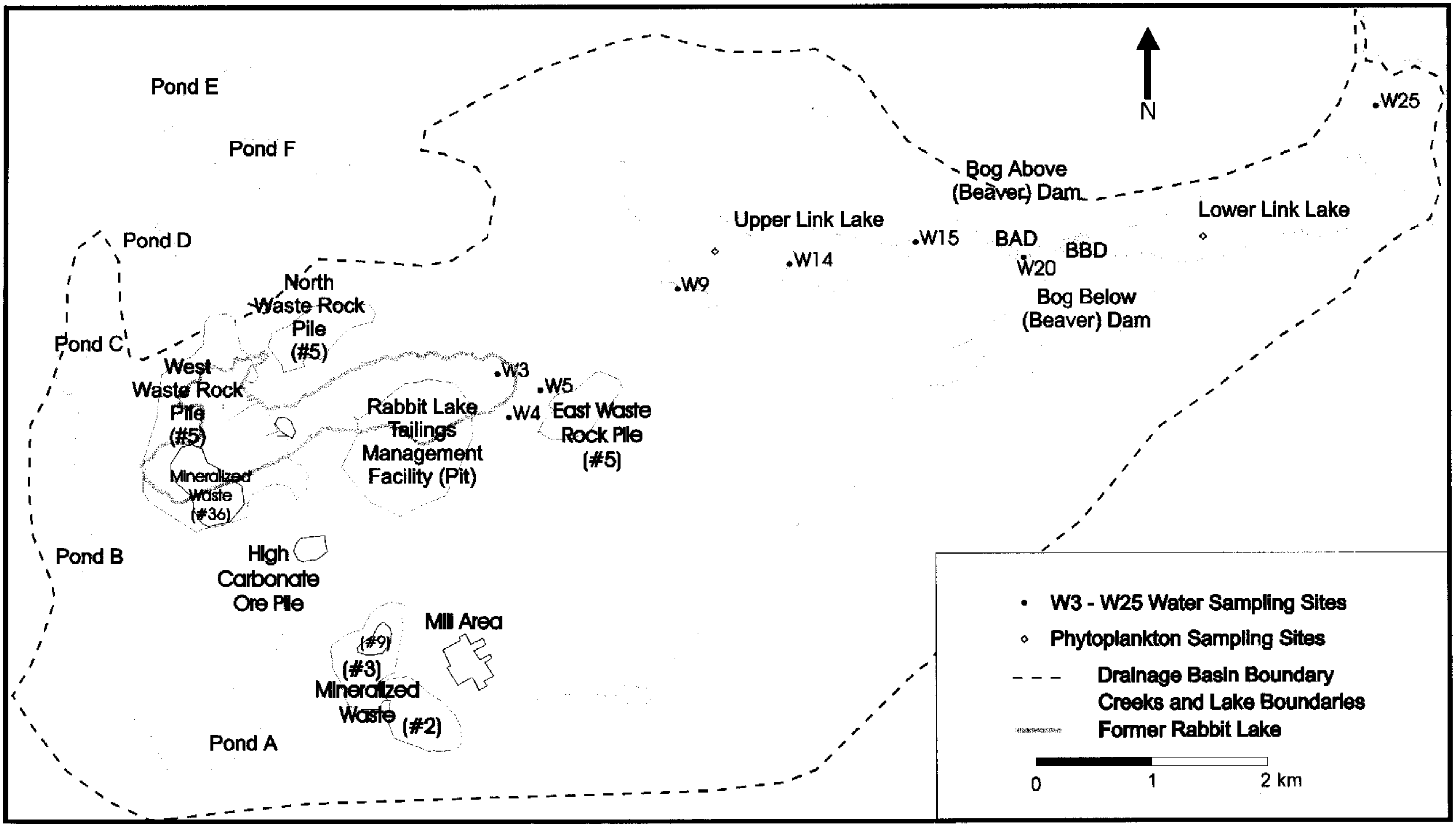
file name : forest map.cdr



Map 2: B-Zone Sampling Locations

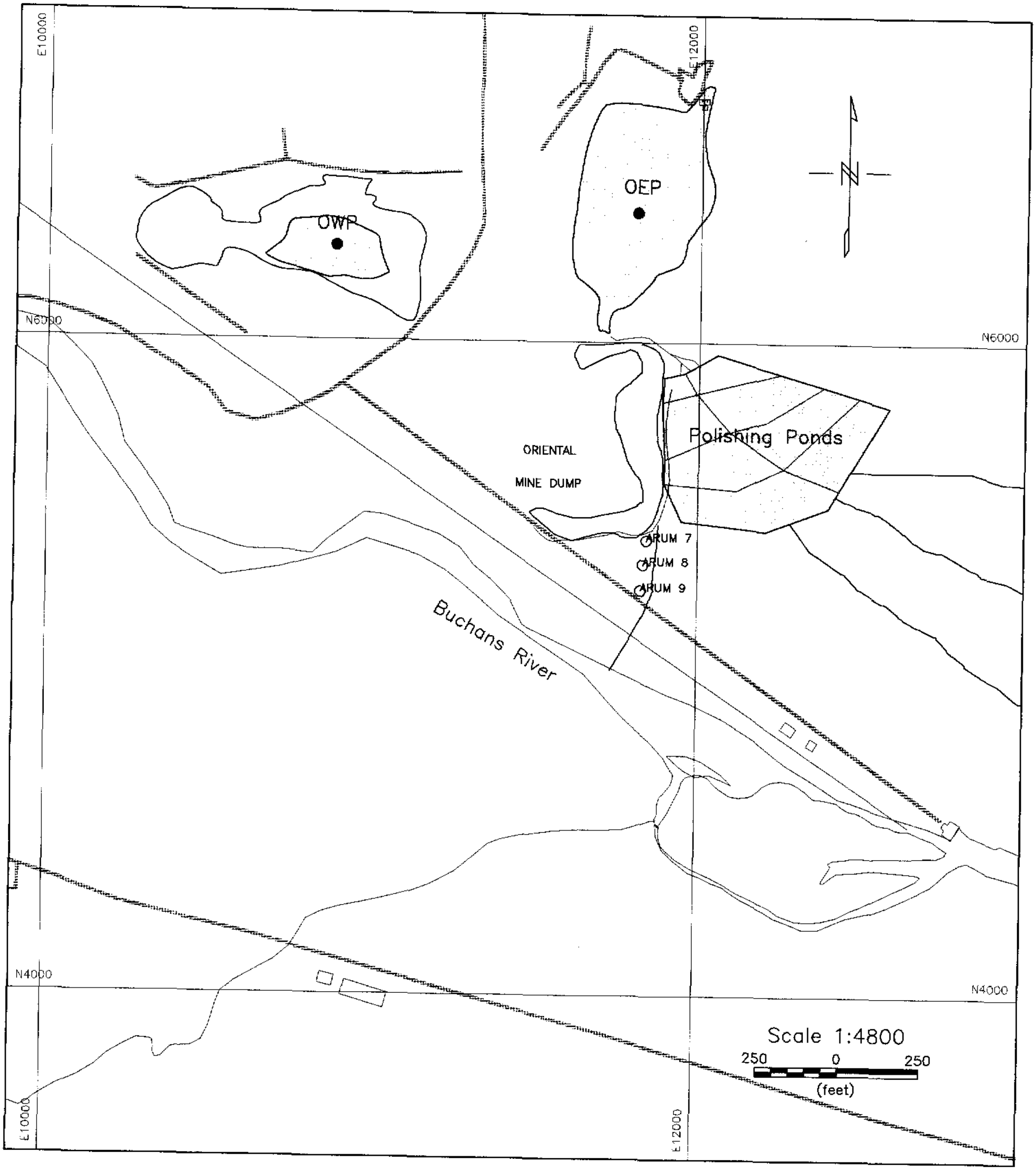


Map 3: South Bay Sampling Locations

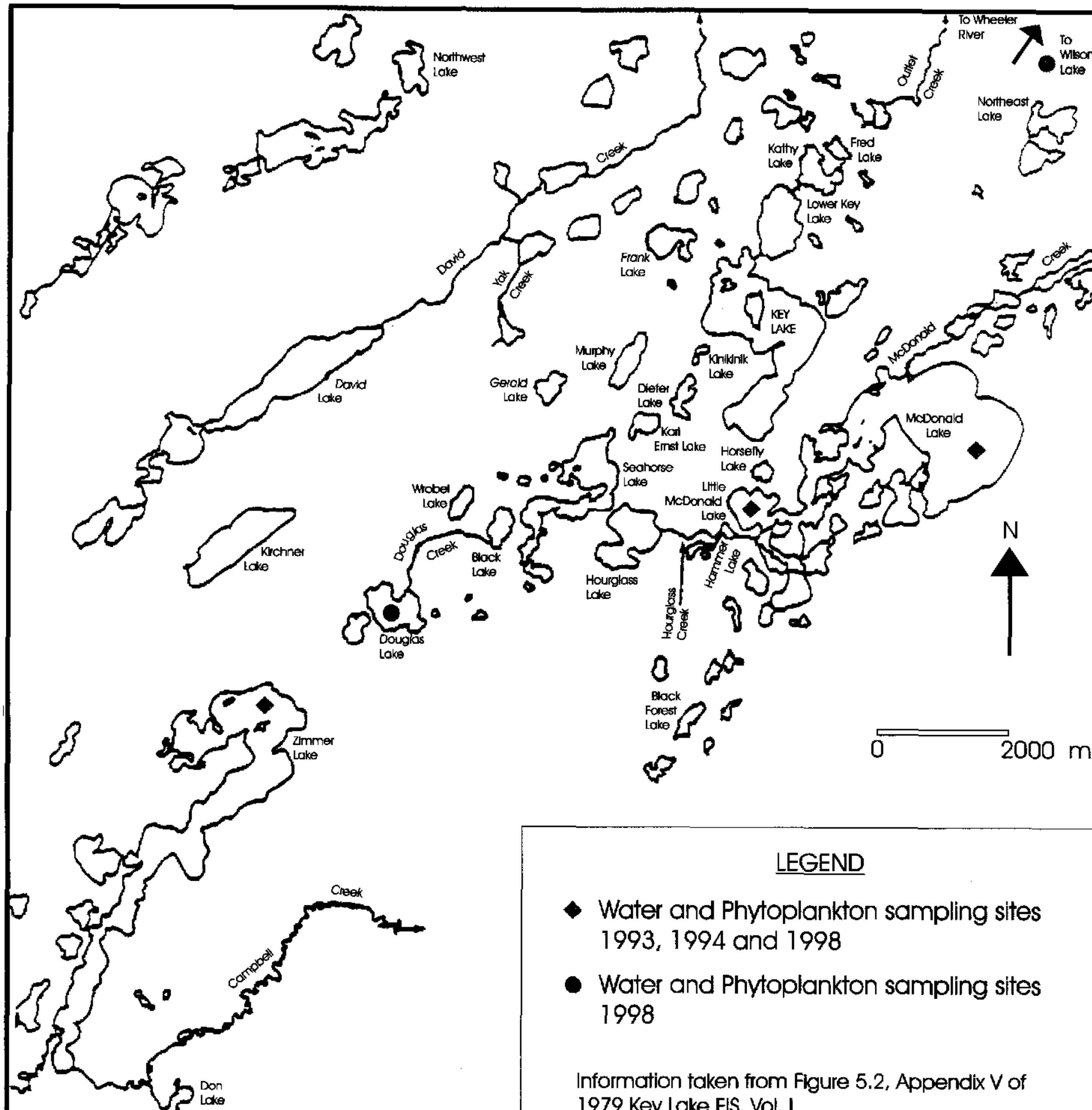


Map 4: Link Lake Sampling Locations

file name LinkLakeSamplingLocations.cdr



Map 5: Buchans Sampling Locations



Map 6: Key Lake Sampling Locations

file name
KL_overview.cdr

PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 3

B-ZONE

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

3.0 Site Description

The B-Zone Pit (Map 2), located on the Harrison Peninsula of Wollaston Lake, Northern Saskatchewan (58°11" N, 103°41" E), was force-flooded in March 1992 with clean and oligotrophic water from the adjacent Collins Bay (part of Wollaston Lake).

The surface area of the pit-lake is approximately 24 ha, and its maximum depth is about 57 m. Uranium was mined between 1984 and 1991. The pH of the water is close to neutral and the pit is high in phosphorus, but relatively poor in nitrogen. The geometry of the pit and its location with respect to Collins Bay, Harrison Lake, and the adjacent Waste Rock pile are shown on the facing page. The B-Zone Pit is largely a closed lake, with little inflow and outflow.

Boojum Research has extensively investigated the limnology of the pit, and observed a strong thermocline in summer, which breaks up in fall (Figures 3.1a-d). The depth profile of water chemistry and phytoplankton have been examined and reported (Boojum 1997).

3. 1 Monitoring Program and Data Scanning

3.1.1 Water Samples

Water samples were collected at the intervals of 5m (i.e., 0m/surface, 5m, 10m, 15m, 20m, 25m, 30m, 35m, 40, 45m) at last three times a year from 1992 to 1998. Sampling was conducted mainly during late spring to early fall, with few samples collected in cold seasons because of the difficulty in access and ice-cover. A range of water quality parameters was measured in the field, including pH, conductivity, Dissolved Oxygen (DO), Redox-potential (Eh), and temperature. Water samples were sent to Saskatchewan Research Council Analysis Service for comprehensive analyses of water chemistry, which included major and trace elements and nutrients. A total of 215 water samples were collected and analysed between 1992 and 1998.

Water quality data were, in the first place, retrieved from the Boojum Paradox database and were saved into Excel 97 files. A data summary is presented in Table 3.1 and 3.2, including the number of observations, averages, standard deviations, and coefficient of variation. The data were being scanned for erroneous records, "outliers" and missing records, as described previously in Section 2.3.1. A limited number of missing data were replaced by:

an average value of upper and lower records; or

the upper layer record if the variable is stabilised at deep layer; or

the average over all depths.

With standardisation to 1 variance and 0 mean, Principal Component Analysis (PCA) was applied to determine "outliers". Two samples were found to stand out markedly from all others in the first two-dimensional PCA plot, and were consequently removed as "outliers". They were collected from 40m depth in March 1993 from the pit and 45m depth in April 1994, respectively. These two samples are associated with exceptionally high levels of Total-P, nickel, SO_4 or arsenic. As a result, a total of 213 water samples were subject to further analysis.

Out of a large number of water physical-chemical variables, 17 major water quality parameters were chosen in this analysis: pH, conductivity, Total Suspended Solid (TSS), Total Organic Carbon (TOC), Redox-potential (Eh), Dissolved Oxygen (DO), Total-P, NH_4, Fe, Ni, As, K, Ca, Mg, Na, SO_4, and HCO_3. Other variables are excluded either because they were consistently at very low levels (i.e., below or close to detect limit), such as many trace metals, B, Hg, Ag, Cd, Cu, Pb, Zn and Cl; or they had too much missing data, such as TKN and NO_3 (only available from late 1994). All chosen variables were subject to a time-trend analysis (Systat 8.0). Then, after standardisation, they were further analysed using Principal Component Analysis (CANOCO 10.1).

3.1.2 Phytoplankton Samples

Boojum Research and Cameco collected phytoplankton samples usually from 6 depths: <0.5m (surface), 2m, 12m, 22m, 32m, and 42m in the pit. During 1992-1994, only surface samples were taken. Samples collected during 1992-94 were subject to absence-presence analysis only, and so binary data are available for these 7 samples. Since then, all taxa were counted and so quantitative data are documented for the other 42 samples. A total of 49 phytoplankton samples was collected from both B-Zone Pit and the Collins Bay up to September 1998. In most years, samples were collected 1-3 times.

3.2 Data Analysis

To explore the available data, we conducted two types of analyses, involving quantitative data (1995-98) and binary data (1992-98). The former can show community structure - water quality relationships quantitatively, while the later can be expected to give a basic, but complete picture how phytoplankton community composition changed in relation to major water quality variables since the pit was forced-flooded.

The change of species/taxa richness was firstly examined using the 1992-98 binary data. Dominance of species/taxa in terms of occurrence frequency and relative abundance was evaluated. Finally, three multivariate approaches were used to investigate the changes in species composition (binary data) and community structure (quantitative data) in relation to major water quality variables.

DCA was applied to the 1995-98 quantitative data set first, after ln (x+1) data transformation. Then, Canonical Correspondence Analysis (CCA) was performed to address the relationships between phytoplankton species/ taxa and major water quality variables, after Ln (x+1) transformation was also conducted. Finally, PCA was used to show the change in species/taxa composition in relation to water quality over 1992-98.

3.3 Results

3.3.1 Water Quality

Time-trend Analysis of Water Quality: The changes in the 17 water quality variables between 1992 and 1998 are shown in Figures 3.2a-f. It is evident that most variables changed markedly over time. TSS, Eh, DO, Total-P and Total-F almost persistently decreased and this trend is significant at the level of 0.05 (Pearson's Correlation). Mg, Ca, K, Na, conductivity, SO_4, and HCO_3 slowly, but steadily increased and this change is also statistically significant (Pearson's Correlation, $P<0.05$). The correlation between As, Ni, TOC and pH and the number of years after flooding is not significant, but their temporal patterns are more complex. Arsenic started to decline after it had reached a peak in 1994. TOC increased at the beginning, peaked in 1995, and slowly decreased thereafter. pH peaked in 1997, but it dropped somewhat in 1998. Nickel increased slightly in the second year of the flooding (1993), and then it slowly decreased. These changes were further analyzed using PCA below.

Multivariate Analysis of Water Quality: The PCA plot of the 213 water samples in the first two dimensions is presented in Figure 3.3. Samples were differentiated using both codes and symbols. Table 3.3 lists codes for all 213 samples and the sampling years are labeled with different symbols (circle, square, diamond and etc.). The 1st PCA axis explains a large proportion of the total variance, 39.6%, and increases to 52.6% together when the 2nd axis is added. Figure 3.4 shows the relationships between the 1st axis and the water quality variables. HCO_3 (-0.92), SO_4 (-0.90), conductivity (-0.82), Ca (-0.81), Fe (0.72), TSS (0.66), and DO (0.56) weigh most heavily on the axis (scores >0.5), followed by TOC (-0.48) and Total-P (0.32). Mg, Na, and K also contributed markedly to the axis, but they co-varied with Ca. The 2nd axis is closely related to As (0.72), Ni (0.59), pH (-0.63), NH_4 (-0.54).

The ordination of the 213 samples showed a clear time-pattern in that the samples of each year were arranged along the 1st axis in a time-series. Pearson's correlation analysis also showed that the axis is significantly and negatively related to the number of days since the first sampling ® = -0.949, n = 213, P<0.001), confirming that the 1st axis represents a time series. Over 1992 to 1998, TSS, Fe, DO, Eh, and Total-P gradually decreased, while most cations (K, Ca, Na, Mg), conductivity, HCO_3, SO_4, and TOC, increased.

The 2nd axis was found to be significantly correlated with water temperature (r=-0.32, n = 213, P<0.01), suggesting that it relates to seasonality, as temperature is a reliable measure of season in the study area. The axis-temperature correlation is not strong, although statistically significant. It was noted that As and Ni weigh heavily on the 2nd axis. Their concentrations decreased from 1993/94 to 97/98, and NH_4 and pH increased at the same time. This appears to compose the second time-trend. In Section 3.5, where the 1995-98 data were analyzed, it was proved that As weighs heavily on the 1st axis, and Ni to a lesser extent. Therefore, the 2nd Axis is more likely to represent the second time-series.

The 3rd axis explains 7.2% of the total variance, but the samples did not show any meaningful pattern along this axis. Whereas the 4th axis is significantly correlated with depth (r=-0.35, n=213, P<0.01), which suggests that it reflects the depth profile of water quality. However, since it only explains 6.7% of the total variance, the variation in water quality over depths is much less important than the time-trends.

It was noted that water quality as measured by the 17 variables, changed very quickly at the beginning (1992-93), but slowed down with time, as well as with the difference over depth, and months in the same year reduced (see Figure 3.3). This indicated that the water quality trends to stabilize gradually in the past couple of years.

3.3.2 Phytoplankton Community in Relation to Water Quality

Phytoplankton Community : a General Description: 21 genera, plus 8 unidentified taxa, were recorded in the B-Zone pit during 1992-98. However, 24 of these taxa occurred in no more than 20% of all 48 samples. Six genera were dominant or frequent at least during certain times of the sampling period:

Dictyosphaerium (88.3% frequency)
Cryptomonas (45.8%)
Oscillatoria (43.8%)
Nitzschia (41.7%)
Chlamydomonas (27.1%)
Dinobryon (27.1%).

Some unidentified taxa were also fairly common, including some small blue-green algae, green algae and diatom. On average, genus/taxa richness in the pit was only 5.5 taxa per sample, which is very much lower than in Collins Bay, where 36 genera were identified in one single sample, collected in June 1992. However, the number of genus/taxa recorded in the pit has rapidly increased (Figure 3.5), from 5 in 1992 to 15 in 1998.

The quantitative data give more details regarding the pit phytoplankton and their changes during 1995-98. A single green algal species, *Dictyospaherium pulchellum* dominated the pit with an average density of 3.59×10^{8} cells per liter in 1995, and retained its dominance in 1996, although more species started occurring. *Oscillatoria limnetica*, a blue-green algae species, became very abundant in 1997, and genus /taxa richness kept increasing. The *Dictyospaherium pulchellum* density dropped significantly, to 5.91×10^{5} cells per liter on average. The community structure continued to change in 1998: *Dictyospaherium pulchellum* almost disappeared, and *Oscillatoria limnetica* also dropped very much. A new green algal species, *Chlamydomonas* became dominant, comprising 50% of the total density, 31.7×10^{5} cells per liter. At the same time, taxon richness further increased, up to

15 taxa per sample, particularly in the surface sample. It is clear that phytoplankton communities in the pit experienced rapid and substantial change during 1995-98, which was characterized by replacement of dominant species and an increase in species richness.

Multivariate Analysis: Out of 42 quantitative samples, 4 were integrated from different depths and removed for comparability. Then, DCA ordination was applied to the remaining 38 samples with all 27 species/taxa retained. The sample and species/taxa codes are listed in Table 3.4.

The 1st DCA axis, with a gradient length of 3.3 SD and an eigenvalue of 0.66, explains 30.8% of the total variance. The 1st two axes together explain 42.6% of the total variance. The 3rd and 4th axes are much less important, adding only 9%. A plot of the first two axes, therefore, adequately presents the solution (Figure 3.6). All 1995 samples clumped around the origin of the plot, indicating that they were almost identical. Indeed, one species, *Dictyospaherium pulchellum*, typically contributed over 90% of the individuals in all those samples. The 1996 samples spread considerably on both axes, appearing to be related to seasonal change, since the April samples were well separated from the August samples. In 1997 and 1998, samples were clustered into their own groups, separated from each other and from the others. The overall pattern indicates that phytoplankton community structure in the pit changed significantly over time.

Figure 3.7 shows the genus/taxa that were best associated with each of the year-based sample clusters. *Dictyospaherium pulchellum* is very closely associated to the 1995 samples, and less so to 1996 samples. However, more genera are related to the later, including *Gymnodium*, *Temnogametum*, *Glenodinium* and two unidentified taxa. More genera are closely related to the 1997 samples, particularly *Nitzschia*, *Dinobryon*, *Cyptomonas*, *Oscillatoria* and *Pseudokephyrion*. A well-defined algal group is almost solely associated with the 1998 samples, including *Chlamydomonas*, *Achnanthes*, *Cryptomonas*, *Oedogonium* and *Gonatozygon*. Meanwhile, several other genera occurring in 1997 remained, *Oscillatoria*, and a small unidentified cyanobacterium.

We further examined the relationships between the community structure/individual species and major water quality variables. As NO_3 was well documented since 1995, we added it to the original 17 variables that were used in the previous section. As mentioned earlier, phytoplankton samples were collected at surface, 2m, 12m, 22m, 32m, and 42m, while water samples were collected at intervals of 5m. Since these two data sets do not exactly match, we used the average of surface 0-1m and 5m records for water variables to match 2m phytoplankton samples. Similarly, 10m, 20, 30, and 40m water samples were matched to 12m, 22m, 32m, and 42m phytoplankton samples. This data handling appears to have been highly successful, as close and significant species-environment relationships were observed using a direct ordination technique, Canonical Correspondence Analysis (CCA).

The 1st CCA axis explains 30.1% of the total variance, almost the same as with DCA (30.8%). The first two axes together account for 46.3% of the total, slightly higher than with DCA (42.6%), implying that these two axes explain a very large proportion of the total variance, and the variables used here can almost fully account for the pattern observed in DCA. This is confirmed by the high species-environment correlation, 0.99 and 0.96 for the 1st and 2nd axis, respectively. This correlation indicates how well the ordination based species data agrees with the one based on water quality data.

The sample ordination in the first two axes is presented in Figure 3.8. The 38 samples were grouped into sampling year-based clusters, except the 1996 samples, which fell into two sub-groups. These year-based clusters distributed along the 1st axis, which obviously a time-related gradient. Figure 3.9 is a tri-plot, which imposes both species and water quality variables to the sample ordination configuration. The relative position of sample, genera and water quality variables indicates their relationships.

As discussed previously, 1995 - 96 samples were associated with relatively high As, Eh, TSS, Fe, Ni, NO_3, and DO concentrations. *Dictyosphaerium pulchellum* reached its highest density under such a harsh condition, and its high biomass is believed to have caused the higher TOC in 1995 - 96. Most genera/taxa were negatively associated with the above variables at reduced levels, however, and associated with higher Ca, Mg, Na, SO_4, and HCO_3 concentrations.

We further examined the binary data of 1992 - 1998, using PCA instead of DCA or CCA, which assume that species abundance follows the Bell-shape distribution along environmental gradients. While this well reflects the reality in most cases, and has been widely accepted, binary data contain no information about abundance, and, therefore, PCA is more appropriate. A total of 48 samples were used, including a sample collected from Collins Bay, which largely represented the phytoplankton community in the lake water which was pumped into the pit. Fifty-four genera/taxa were recorded in these samples. The PCA plot of the 48 samples on the first two axes is shown in Figure 3.10. These two axes explain 48.6% of the total variance, with the 1st axis alone accounting for 32.6%. It is very clear that phytoplankton species composition dramatically changed after the lake water was pumped into the pit, when the majority of genera disappeared. The genus composition in the pit showed no signs of returning to its original form characterized by the Collins Bay samples, even though it changed substantially over time. This is not surprising considering the differences between the two systems in terms of hydrology, limnology, and topology.

Figure 3.11 showed the genera that associated with the pit and Collins Bay, respectively. As indicated by CCA ordination, *Dictyosphaerium* dominated the samples before 1995, but decreased greatly after 1997 while more genera showed up. To reveal more details of the pit phytoplankton changes, we excluded the Collins Bay sample and applied PCA to the 47 pit samples, and imposed environmental correlation to the solution.

The first two PCA axes account for 53% of the total variance, even higher than for the data set of using the 48 binary samples. The 1st axis alone explains 38%, indicating a strong gradient or trend in the data set. Figure 3.12 showed the sample ordination on the first two PCA axes. The samples collected between 1992 to 1995 are clumped densely to the left of the origin, indicating high degrees of similarity in the taxonomy of these samples. In other words, phytoplankton composition did not change obviously during the first 4 years. By 1996, however, some samples stand apart. The 1997 samples, and even more clearly 1998 samples were separated from the large clump. This agrees with the CCA result, in that the change in phytoplankton community speeded up since 1996. Figure 3.13 shows the change in genera/taxa composition. The codes are described in Table 3.5. As the community changed little during 1992-95, the description given earlier for CCA is largely applicable. A bi-plot is shown in Figure 3.14 to help visualize the relationship between genera/taxa and samples.

When environmental relationships were imposed on the PCA plot (Figures 3.14, 3.15), Arsenic, Eh, NO_3, and Total-P were shown to be strongly associated with 1992 - 95 samples, and TSS was particularly high in the 1992-93 samples. Although the other variables are also well correlated with the phytoplankton samples, their concentrations fell within lower and narrower ranges, and were not thought to be ecologically significant.

For the entire monitoring period (1992 - 1998), the most important changes in phytoplankton community composition took place in the last two-three years. The earlier CCA result based 1995-98 data, therefore, covers the majority of the change. The species-environment relationship is shown in Figure 3.15, which is similar to what observed in CCA plot (Figure 3.16), since the taxonomic composition changed little between 1992-1995 when *Dictyosphaerium* dominated.

The ordination techniques and the data sets /sub-data sets used in this analysis is summarized below:

Data Set	Period	Sample No.	Sites	Data Type	Ordination	Figures
Water Quality (WQ)	1992-98	213	Pit	Numeric	PCA	3.3-4
Phytoplankton	1995-98	38	Pit	Numeric	DCA	3.6-7
Phytoplankton + WQ	1995-98	38 each	Pit	Numeric	CCA	3.8-9
Phytoplankton	1992-98	48	Pit & Collins Bay	Binary	PCA	3.10-11
Phytoplankton + WQ	1992-98	47 each	Pit	Binary	PCA	3.12-16

3.4 Discussion

The analysis clearly showed the temporal changes in water quality in B-Zone pit. Natural water bodies typically were formed hundreds of thousands / millions of years ago and have experienced a variety of natural changes, and man-made impacts only more recently. The B-Zone pit provides an excellent opportunity to observe changes in water quality in a newborn aquatic system and the result of this study is of general significance when trying to predict the physical-chemical changes in other force-flooded pits.

When the pit was force-flooded in early 1992, high TSS levels were observed, which reduced gradually with the ongoing settling of particles. The flooding with clear oligotrophic water from Collins Bay of Wollaston Lake also brought high DO into the pit initially, but over time DO decreased in the deep layer. This is the consequence of chemical oxidation in the earlier years and followed by biological decomposition.

The B-Zone mineralization is rich in phosphorus, which weathered and was released into water when the pit was flooded. This resulted in peak concentrations in 1993. Since then, chemical precipitation and biological utilization appear to have consumed much of the total-

P. Compared with most natural lakes, P levels in the pit are still relatively high.

The flooding also resulted in an initial release of Arsenic and Nickel, which peaked in 1993 and 94, respectively. Thereafter, their concentrations decreased to varying degrees. Ni decrease was much slower than As, due to different geochemistry.

NO_3 was excluded from this analysis because of data missing, but its concentration was highest in 1994-1995 and then decreased rapidly. Meanwhile, NH_4 did not show proportional increase, suggesting that a large proportion of N has disappeared from the water column. One possible explanation is that some N has been either restored in the bottom of the pit as dead biomass, or has been lost via denitrification, or both. The increases in several cations (K, Na, Mg, and Ca) over time are very consistent. Increases in Total Organic Carbon, HCO_3, and NH_4 should be attributed to biological productivity. The highest TOC, in 1995, corresponds to the peak in phytoplankton density.

In summary, the statistical analysis indicated that water physical and chemical variables changed significantly between 1992 and 1998. TSS, Fe, DO all decreased, while HCO3, SO4, conductivity, major cations, and TOC increased. As and Ni mainly decreased after 1994/95, when pH and NH_4 increased to some extent. The PCA result also suggested that water quality varies over seasons and with depths, but these changes are less important compared with the time-trends, and appear to be the consequence of both chemical-physical and biological processes.

In order to assess the change in water quality of the pit lake comprehensively, it is necessary to combine both PCA and time-trend analyses. As shown earlier, most variables show clear time-trends, and are weighted heavily in PCA plots. However, their changes typically fall into limited ranges, which are unlikely to be significant in environmental and biological terms. Mg ranged over 1.7 -2.7 mg/L (annual average), Ca 3.6 - 6.3 mg/L, K 1.2-1.6 mg/L, Na 1.8-2.2 mg/L, SO_4 6.2-13 mg/L, HCO_3 12-24.3 mg/L, Cl 0.4-1 mg/L, Ni 0.20-0.29 mg/L, pH 6.5-7.2, conductivity 42-82 µs/cm, and TOC 3.0-4.4 mg/L.

In contrast, several other variables showed substantial changes over time. The annual average of TSS dropped from 53.7 mg/L in 1992 to 1.4 mg/L in 1998, indicating a marked improvement occurred in water transparency, an important factor for phytoplankton communities. Eh also decreased from 437 mV in 1992 to 147 mV in 1998, which could have strongly influenced the chemical processes in the water column. Total-P dropped from a high of 0.21mg/L in 1993 to 0.09 mg/L in 1998 and $N\text{-}NO_3$ followed a similar pattern, but with less significant decreases, from 0.66mg/L in 1994 to 0.13mg/L in 1998. As, the major contaminant in the pit also dropped from a high of 0.34 mg/L in 1994 to 0.08 mg/L in 1998. These changes were regarded as environmentally and ecologically significant.

In concluding, the water quality changes in the B-Zone pit are largely a result of decreases in TSS, Eh, total-P, $N\text{-}NO_3$, and As. These changes are likely to influence the other variables examined, which changed to a lesser extent.

The analysis also clearly showed the changes of phytoplankton community in the B-Zone pit. As a whole, the pit phytoplankton that developed after the Collins Bay water has much lower genera/taxa richness with very different taxonomic composition than its origin. The community itself also has experienced rapid and substantial change since 1996.

Relating phytoplankton community structure to water quality variables in the pit is the focus of this study. As mentioned in the Introduction, however, this task is not easy and requires caution, because of the complex responses of biological systems to their physical environment and the interactions among different components of both systems. Multivariate approaches are favored by ecologists and environmental scientists for their power in defining and quantifying "species - environment" relationships. Since the different techniques used in this study (PCA, DCA, and CCA) produced similar results, the analytic solutions appear to be robust.

Statistical analysis, when used alone to infer "species-environment" relationships and underlying environmental processes, has its limitation. Several ecologists have questioned whether statistical significance necessarily means environmental / ecological importance (McBride et al. 1993, Davis & Simmons 1995). Cao et al. (1999) addressed this question in more detail when they argued that a statistically significant change may well have little environmental significance, and moreover the same magnitude of change in different variables can result in very different environmental effects. The only way to overcome this shortcoming is to take both the statistics and the biology/toxicology of water quality variables into account, which in this study was done by examining the ranges of all statistically important variables with respect to the biology and toxicology of the ranges.

The majority of the variables showed strong time-trends, but only some of them changed beyond the range that is ecologically significant, notably TSS, As, Eh, Total-P and NO_3. TSS appears to have played a major role in shaping the pit phytoplankton community. In the first few years, TSS was very high due to the force flooding, and likely restricted other species from establishing there while allowing *Dictyospaherium* to explore the new habitat and resources. The interaction among TSS, light intensity, and the ecological traits of *Dictyosphaerium* was studied in Kalin and Olaveson (1997). Arsenic is probably another major factor here. Since the high density of *Dictyosphaerium* before 1995 corresponded to relatively high levels of As (0.21 - 0.34 mg/L), these As concentrations obviously did not negatively influence this green algae species, but they may well have eliminated many others. There is no detailed study of the tolerance of algae to As, but *Dictyosphaerium* was already the pioneer species in the newly formed pit-lake. However, this species substantially decreased in 1997, and almost disappeared from 1998 samples, while other genera gained dominance and taxonomic diversity greatly increased. Competition seems to have caused this significant change, as more species joined the community as a consequence of lower TSS and As. It was also noted that the total density of phytoplankton has decreased since 1995-96, mainly due to the drop of *Dictyosphaerium* abundance (Boojum 1998). This change is well matched by the decrease in nutrients, particularly in NO_3, which dropped from 0.65mg/L in 1994, to 0.36mg/L in 1995, and 0.13mg/L in 1998, totally by 80%. Total-P decreased by 43% during 1994-98. Another factor may be the increase of rotifers in the pit. Although we did not present zooplankton data in this analysis, we observed that rotifer density has significantly increased in the past few years, and their grazing could be partially responsible for the fall of phytoplankton density. The interactions between phytoplankton and zooplankton have been well examined, and it is known that zooplankton can remove significant amounts of algae cells from the water column.

Ni concentrations remained at relatively high levels throughout the sampling period, and, therefore, obviously did not inhibit the peak of *Dictyosphaerium* density in 1994 -1995 or the increase of species diversity in the past two years (1997-98). At the present time, Ni does not appear to be a major restriction to pit phytoplankton community.

The tolerance of phytoplankton to metals can be inferred from two ways: toxicity tests and field survey. As mentioned in the Introduction Section, both approaches have some advantages and disadvantages. Water quality criteria are typically based on single metal-single species toxicity test under standard laboratory conditions. In the B-Zone pit, As and Ni co-exists at intermediate levels, but many algae genera were found. B-Zone phytoplankton was further compared with those from the other sites, Boomerang Lake, Buchans Pits, Key Lakes and Rabbit Lakes. This multi-area comparison provided more information regarding the effects of different metals and other water quality variables on phytoplankton communities.

Table 3.1. Major Water Quality Variables in B-Zone Pit for 1992-1998

Sampling		Basic Variables*					Nutrients, mg/L			Dissolved Ion Concentrations, mg/L											
Dates	Depth (m)	Temp (°C)	Cond us/cm	pH	DO (mg/l)	Eh (mV)	Total-P (mg/l)	N-NO_3 (mg/l)	N-NH_4 (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	Total-Fe (mg/l)	SO_4 (mg/l)	Cl (mg/l)	HCO_3 (mg/l)	As (mg/l)	Ni (mg/l)	TOC (mg/l)	TSS (mg/l)
30-Jun-92	5	13.4	38	7.1	11.1	393	0.133		0.010	1.1	3.4	1.6	1.7	2.1	4.7	0.4	12	0.12	0.12	2.9	53
	10	5.7	38	7.1	13.2	413	0.153		0.052	1.1	3.4	1.6	1.8	2.0	4.7	0.4	12	0.11	0.12	3.1	50
	15	5.0	39	6.8	13.2	416	0.143		0.052	1.1	3.3	1.6	1.8	2.1	4.6	0.4	12	0.10	0.14	3.0	40
	20	4.8	39	6.7	13.2	419	0.123		0.091	1.3	3.4	1.6	1.8	2.4	4.7	0.4	12	0.11	0.14	2.9	76
	25	4.7	39	6.7	13.1	422	0.143		0.065	1.2	3.4	1.7	1.8	2.4	4.8	0.4	12	0.12	0.15	2.8	83
	30	4.6	40	6.7	13.0	424	0.164		0.065	1.2	3.5	1.7	1.8	2.6	5.0	0.4	12	0.13	0.15	3.0	82
	35		41	6.7			0.184		0.052	1.2	3.6	1.8	1.8	2.6	5.6	0.4	12	0.14	0.18	3.0	90
	40		42	6.7			0.204		0.052	1.3	3.6	1.8	1.8	2.9	5.8	0.4	12	0.16	0.18	2.9	100
	45		44	6.7			0.194		0.065	1.4	3.6	1.9	1.9	3.4	6.5	0.4	13	0.21	0.21	2.9	140
	50		42	6.7			0.245		0.026	1.5	3.6	2.0	1.8		6.1	0.4	12	0.22	0.22	2.8	160
1-Sep-92	0	17.4	41	7.2	10.9	383	0.041		0.010	1.0	3.0	1.3	1.7	0.9	5.2	0.5	13	0.17	0.12	3.4	48
	5	12.4	39	7.2	11.3	376	0.092		0.010	1.0	3.1	1.3	1.6	0.9	5.2	0.3	13	0.15	0.12	3.5	48
	10	5.6	39	7.0	13.6	400	0.143		0.010	1.0	3.1	1.4	1.7	1.6	5.2	0.3	12	0.13	0.15	3.4	63
	15	4.9	41	6.9	12.8	406	0.164		0.026	1.1	3.3	1.5	1.7	2.5	6.0	0.3	12	0.20	0.22	3.3	36
	20	4.8	42	6.9	12.4	409	0.480		0.039	1.2	3.4	1.6	1.8	2.5	6.5	0.3	12	0.19	0.23	3.3	40
	25	4.8	43	7.0	12.1	413	0.020		0.026	1.3	3.5	1.6	1.8	2.5	7.1	0.3	12	0.26	0.24	3.0	46
	30	*4.9*	*44*	*6.7*	*11.9*	*415*	*0.215*		*0.026*	1.3	*3.6*	*1.7*	*1.8*	*2.3*	*7.5*	*1.3*	12	*0.27*	*0.25*	*2.9*	*54*
	35	4.8	46	6.8	11.9	418	0.041		0.039	1.3	3.6	1.8	1.9	2.5	8.0	0.3	12	0.30	0.30	2.8	54
	40	4.7	47	6.8	11.8	421	0.225		0.039	1.4	3.8	1.9	1.9	2.2	9.1	0.3	12	0.36	0.35	3.0	60
	45	4.7	50	6.7	11.6	421	0.245		0.039	1.4	3.9	1.9	1.8					0.40	0.35	3.0	61
1-Oct-92	0	6.9	42	6.8	12.0	472	0.133		0.026	1.1	3.9	1.8	1.8	1.0	6.9	0.3	12	0.26	0.22	3.3	20
	5	6.8	42	6.8	12.1	469	0.143		0.026	1.1	3.9	1.8	1.8	1.1	6.9	0.3	12	0.26	0.22	3.0	22
	10	6.8	42	6.8	12.0	468	0.153		0.026	1.1	4.0	1.8	2.0	1.2	6.6	0.5	12	0.26	0.22	3.2	22
	15	6.7	43	6.8	12.0	466	0.010		0.039	1.1	4.0	1.8	1.8	1.2	7.0	0.4	11	0.24	0.20	3.3	20
	20	5.2	43	6.8	12.4	478	0.082		0.013	1.1	3.7	1.8	1.8	1.2	7.0	0.4	12	0.27	0.20	3.1	23
	25	4.8	44	6.9	12.2	485	0.112		0.039	1.2	3.8	1.8	1.8	1.2	6.9	0.5	12	0.22	0.21	3.3	25
	30	4.8	43	6.8	11.8	489	0.092		0.026	1.1	3.8	1.8	1.8	1.0	7.0	0.4	12	0.22	0.21	3.2	23
	35	4.7	43	6.8	11.5	490	0.133		0.039	1.1	3.8	1.8	1.8	1.2	7.0	0.4	12	0.26	0.20	3.2	23
	40	4.7	43	6.8	11.3	491	0.133		0.026	1.1	3.8	1.7	1.8	1.3	7.0	0.4	12	0.19	0.19	3.2	24
	45	4.7	44	6.7	11.2	493	0.010		0.026	1.2	4.0	1.8	2.1		6.5	0.4	11	0.22	0.21	3.0	26
7-Mar-93	0	0.1	48.8	6.7	14.5	378	0.154		0.013	1.6	6.0	2.0	2.2	1.1	8.0	2.0	14	0.24	0.28	4.1	6
	5	1.4	41.7	6.8	14.2	375	0.113		0.013	1.4	4.0	2.0	2.0	0.8	7.0	2.0	12	0.21	0.26	3.3	7
	10	1.8	39.6	6.8	14.3	374	0.082		0.026	1.3	5.0	2.0	1.9	0.9	7.0	2.0	12	0.18	0.24	3.0	6
	15	*2.0*	*40*	*6.7*	*14.2*	*375*	*0.133*		*0.026*	1.3	*5.0*	*2.0*	*1.8*	*0.9*	*7.0*	*2.0*	*12*	*0.17*	*0.24*	2.9	7
	20	2.2	42.9	6.6	13.8	379	0.072		0.026	1.3	5.0	2.0	1.8	1.0	8.0	2.0	12	0.17	0.27	3.1	9
	25	2.5	41.2	6.6	13.3	379	0.092		0.013	1.3	5.0	2.0	1.8	1.0	7.0	2.0	12	0.18	0.26	2.8	5
	30	2.7	42.2	6.6	12.8	380	0.092		0.013	1.3	5.0	2.0	1.8	1.0	8.0	2.0	12	0.21	0.24	3.2	8
	35	2.8	42.4	6.6	12.6	381	0.102		0.013	1.3	5.0	2.0	1.8	0.9	8.0	2.0	11	0.23	0.27	2.9	8
	40	2.8	42.8	6.5	12.5	383	0.113		0.013	1.4	5.0	2.0	2.0	1.0	8.0	2.0	12	0.24	0.31	3.1	8
	45	2.8	96.2	6.2	12.3	391	0.614		0.026	2.4	9.0	5.0	2.8	1.1	28.0	2.0	11	0.25	1.20	3.1	8
29-Jun-93	0	11.3	44.7	6.6	9.6	412	0.552		0.010	1.3	5.0	3.0	2.0	0.8	9.0	1.0	14	0.27	0.23	3.4	15
	5	10.3	44.1	6.8	10.1	404	0.388		0.013	1.3	5.0	3.0	1.7	0.8	9.0	1.0	15	0.36	0.25	3.4	16
	10	7.8	45.7	6.7	10.7	407	0.532		0.026	1.4	4.0	3.0	2.0	0.9	9.0	1.0	13	0.23	0.27	3.1	14

Continued

Table 3.1 continues

	Depth	Temp	Cond	pH	DO	Eh	Total-P	$N\text{-}NO_3$	$N\text{-}NH_4$	K	Ca	Mg	Na	Fe	SO_4	Cl	HCO_3	As	Ni	TOC	TSS
29-Jun-93	15	5.1	42.6	6.5	11.3	414	0.644		0.026	1.4	4.0	3.0	1.8	1.0	9	1	13	0.22	0.27	3.0	12
	20	4.6	44.9	6.4	11.4	418	0.429		0.026	1.4	4.0	3.0	1.8	0.9	9	1	13	0.26	0.27	2.8	14
	25	4.7	43	6.4	11.3	418	0.419		0.013	1.3	4.0	4.0	1.8	0.7	9.0	1.0	13	0.30	0.29	2.8	13
	30	4.6	44	6.4	11.2	422	0.266		0.312	1.4	5.0	4.0	1.8	0.4	9.0	1.0	13	0.24	0.28	3.1	33
	35	4.6	42.7	6.4	11.2	423	0.348		0.013	1.4	5.0	4.0	1.8	0.4	9.0	1.0	13	0.25	0.28	3.2	13
22-Aug-93	0	17.8	46.9	7.0	9.5	423	0.102		0.010	1.3	3.7	1.6	1.7	0.2	10.0	3.0	13	0.27	0.15	3.2	4
	5	13.9	45.9	7.3	11.1	422	0.113		0.010	1.6	3.9	1.6	1.7	0.2	10.0	3.0	13	0.31	0.17	2.6	13
	10	8.5	46.9	6.6	12.0	458	0.113		0.013	1.5	4.0	1.7	1.8	0.6	10.0	3.0	13	0.32	0.27	2.4	5
	15	5.3	46.7	6.5	12.2	469	0.164		0.013	1.7	4.1	1.8	1.8	0.8	9.0	3.0	13	0.28	0.29	2.4	6
	20	4.9	47.5	6.4	12.3	473	0.133		0.013	1.6	4.2	1.8	1.8	0.8	9.0	3.0	14	0.33	0.28	2.8	6
	25	4.8	45.9	6.4	12.3	477	0.194		0.013	1.7	4.2	1.8	1.9	0.9	9.0	3.0	13	0.31	0.29	2.1	10
	30	4.7	47.9	6.3	12.4	481	0.164		0.013	1.8	4.4	1.8	1.9	0.9	10.0	3.0	13	0.35	0.31	2.6	11
	35	4.6	50.9	6.3	12.4	484	0.164		0.013	2.0	4.1	1.7	1.8	0.8	10.0	3.0	13	0.38	0.33	2.8	9
9-Oct-93	0	4.2	44.8	6.8	11.3	267	0.154	0.620	0.010	1.3	5.0	2.0	1.6	0.7	10.0	1.0	14	0.32	0.25	3.0	11
	5	4.2	45.2	6.8	11.3	267	0.154	0.620	0.010	1.3	5.0	2.0	1.6	0.6	10.0	1.0	14	0.33	0.26	2.8	13
	10	4.2	45.1	6.8	11.3	267	0.154	0.580	0.010	1.3	5.0	2.0	1.6	0.6	10.0	2.0	14	0.32	0.27	2.8	11
	15	4.2	45.6	6.8	11.4	266	0.164	0.580	0.010	1.3	5.0	2.0	1.6	0.5	10.0	1.0	14	0.31	0.30	2.8	9
	20	4.2	45.2	6.8	11.4	266	0.143	0.580	0.010	1.3	5.0	2.0	1.6	0.6	10.0	1.0	14	0.30	0.29	3.1	10
	25	4.3	44.8	6.8	11.4	266	0.113	0.660	0.010	1.3	5.0	3.0	1.6	0.5	10.0	1.0	14	0.29	0.27	2.7	12
	30	4.3	45.8	6.8	11.4	266	0.113	0.660	0.010	1.3	6.0	2.0	1.6	0.5	10.0	1.0	14	0.32	0.29	3.3	8
	35	4.3	45.3	6.8	11.3	266	0.143	0.710	0.010	1.3	5.0	1.0	1.6	0.6	10.0	1.0	14	0.30	0.25	3.3	11
	40						0.143	0.660	0.010	1.3	6.0	3.0	1.6	0.5	10.0	1.0	14	0.29	0.28	3.1	10
	45						0.154	0.660	0.010	1.3	6.0	2.0	1.6	0.6	10.0	1.0	14	0.32	0.26	2.8	18
17-Apr-94	0	0.2	41.7	5.3	14.4	500	0.133		0.010	1.5	7.0	3.0	2.4	0.4	11	1	16	0.37	0.26	2.7	1
	5	1.0	54.7	6.0	14.5	477	0.143		0.010	1.7	6.0	2.0	2.6	0.4	11	1	16	0.38	0.28	2.0	1
	10	1.7	53.8	6.3	13.8	471	0.143		0.010	1.4	5.0	3.0	2.3	0.4	11	1	17	0.38	0.20	2.0	1
	15	2.2	53.6	6.4	13.3	469	0.133		0.010	1.6	5.0	2.0	2.1	0.4	12	1	16	0.35	0.20	1.9	2
	20	2.4	52.5	6.4	12.8	471	0.123		0.010	1.4	5.0	2.0	2.0	0.4	11	1	16	0.31	0.26	1.6	2
	25	2.5	52	6.4	12.1	471	0.133		0.010	1.4	5.0	3.0	2.1	0.5	10		16	0.31	0.27	1.9	1
	30	2.7	57.2	6.4	11.5	472	0.133		0.010	1.3	5.0	2.0	1.8	0.5	10	1	15	0.31	0.26	2.0	1
	35	2.7	55.9	6.4	11.2	474	0.143		0.010	1.4	6.0	3.0	1.8	0.5	11	1	16	0.36	0.30	2.1	1
	40	2.7	58.4	6.3	10.9	475	0.164		0.010	1.5	7.0	3.0	1.8	0.5	12	1	16	0.40	0.32	2.4	2
	45	2.8	84.4	6.2	10.4	479	0.430		0.010	2.3	8.0	5.0	2.8	0.6	22	1	17	0.72	0.67	2.6	3
28-Jun-94	0	15.2	59.1	7.5	10.1	370	0.143	0.396	0.010	1.2	6.0	2.0	2.0	0.5	11	1	15	0.39	0.24	3.8	8
	5	8.1	58.4	7.4	13.3	377	0.143	0.748	0.010	1.2	5.0	2.0	1.8	0.8	11	1	15	0.39	0.32	3.7	5
	10	5.2	56.7	6.7	13.6	392	0.143	0.880	0.010	1.2	5.0	2.0	1.8	0.6	10	1	15	0.40	0.29	3.4	8
	15	4.6	55.3	6.6	13.3	397	0.154	0.880	0.010	1.2	5.0	2.0	2.0	0.9	10	1	15	0.41	0.32	3.5	7
	20	4.3	53.2	6.5	7.0	400	0.133	0.836	0.010	1.1	5.0	2.0	2.0	0.7	10	1	15	0.40	0.30	3.5	7
	25	4.2	53.9	6.4	12.8	402	0.143	0.924	0.010	1.2	5.0	2.0	2.0	0.6	10	1	15	0.39	0.29	3.5	8
	30	4.2	53.7	6.4	12.5	402	0.225	0.924	0.010	1.4	5.0	2.0	1.8	0.9	10	1	15	0.38	0.27	3.4	9
	35	4.1	53.3	6.4	12.4	404	0.164	0.924	0.010	1.5	5.0	2.0	2.0	1.0	10	1	15	0.39	0.30	3.5	7
	40	4.0	52.8	6.3	12.4	405	0.113	0.924	0.010	1.5	5.0	2.0	2.0	0.6	11	1	15	0.40	0.28	3.5	10
	45	4.0	55.3	6.4	12.3	406	0.154	0.968	0.010	1.4	6.0	2.0	2.0	0.8	11	1	15	0.42	0.28	3.4	7
16-Aug-94	0	13.4	73.7	7.0	10.0	387	0.164	0.044	0.010	1.3	6.0	2.0	1.6	0.3	11	1	15	0.29	0.12	4.8	4
	5	13.4	74.1	7.4	10.7	374	0.164	0.040	0.010	1.4	7.0	1.0	2.3	0.3	11	1	15	0.30	0.12	4.2	7
	10	5.2	69	6.8	13.5	395	0.154	0.704	0.020	1.4	6.0	2.0	2.2	0.4	10	1	14	0.29	0.28	4.4	3

Continued

Table 3.1 continues

	Depth	Temp	Cond	pH	DO	Eh	Total-P	$N\text{-}NO_3$	$N\text{-}NH_4$	K	Ca	Mg	Na	Fe	SO_4	Cl	HCO_3	As	Ni	TOC	TSS
16-Aug-94	15	4.5	69.5	6.5	12.5	405	0.164	0.792	0.010	1.4	6.0	2.0	2.0	0.4	10	1	14	0.27	0.28	2.5	2
	20	4.3	69.3	6.4	11.8	409	0.154	0.792	0.010	1.3	6.0	2.0	2.0	0.5	10	1	15	0.28	0.28	4.0	1
	25	4.4	73.9	6.3	11.4	411	0.143	0.792	0.010	1.3	6.0	1.0	2.2	0.5	10	1	15	0.24	0.32	3.5	1
	30	4.3	70.4	6.3	11.2	413	0.154	0.792	0.010	1.3	6.0	1.0	2.2	0.5	10	1	15	0.27	0.30	3.8	3
	35	4.2	68.8	6.2	11.0	414	0.164	0.836	0.010	1.5	7.0	1.0	2.3	0.5	10	1	15	0.28	0.34	3.9	2
	40	4.1	72.6	6.2	10.9	416	0.174	0.836	0.010	1.5	6.0	1.0	2.3	0.5	10	1	15	0.28	0.36	3.1	2
	45	4.0	73.8	6.1	10.7	418	0.194	0.880	0.020	1.6	6.0	1.0	2.3	0.5	12	1	15	0.32	0.40	3.2	4
9-Oct-94	0	7.0	65.6	6.8	11.2	363	0.184	0.040	0.010	1.3	6.0	3.0	2.0	0.5	11	1	15	0.26	0.17	4.5	6
	5	7.0	65.1	6.9	11.6	362	0.123	0.040	0.010	1.3	6.0	3.0	2.1	0.4	11	1	14	0.24	0.15	4.3	1
	10	7.0	64.7	6.9	11.7	363	0.133	0.040	0.010	1.3	6.0	4.0	2.1	0.4	11	1	14	0.25	0.16	4.3	1
	15	6.9	65.1	6.9	11.8	364	0.133	0.040	0.010	1.2	3.0	3.0	1.6	0.4	11	1	14	0.28	0.17	4.5	5
	20	4.5	65.1	6.4	11.2	383	0.143	0.704	0.010	1.5	3.0	2.0	2.2	0.7	11	1	14	0.26	0.31	3.4	2
	25	4.3	64.7	6.4	10.6	387	0.143	0.792	0.020	1.2	3.0	3.0	1.6	0.5	11	1	14	0.25	0.26	3.3	2
	30	4.3	65	6.3	10.3	390	0.123	0.792	0.030	1.2	3.0	2.0	1.6	0.5	11	1	14	0.26	0.27	3.3	3
	35	4.2	65.1	6.3	10.1	391	0.194	0.836	0.020	1.2	3.0	2.0	2.0	0.5	12	1	14	0.28	0.26	3.3	1
	40	4.1	67.8	6.2	9.9	393	0.164	0.880	0.010	1.3	3.0	3.0	2.0	0.5	12	1	14	0.31	0.34	3.4	11
12-Apr-95	0	0.4	64.8	6.8	13.6	400	0.153	0.440	0.010	1.6	6.0	3.0	2.3	0.3	11	1	20	0.28	0.29	4.4	1
	5	1.0	62.4	6.8	12.8	397	0.143	0.440	0.030	1.5	5.0	2.0	2.2	0.3	11	1	19	0.26	0.28	4.3	10
	10	1.3	61.6	6.8	12.3	398	0.133	0.400	0.010	1.5	5.0	2.0	2.1	0.3	10	1	19	0.28	0.27	4.3	2
	15	2.1	61.1	6.7	11.1	399	0.133	0.350	0.030	1.4	6.0	2.0	2.1	0.4	10	1	19	0.25	0.25	4.2	2
	20	2.7	61.1	6.6	10.0	403	0.133	0.440	0.030	1.3	6.0	3.0	2.1	0.5	10	1	20	0.22	0.26	4.3	2
	25	3.1	61.8	6.6	9.8	404	0.133	0.570	0.030	1.3	4.0	3.0	2.6	0.5	10	1	19	0.23	0.24	4.4	3
	30	3.2	62.3	6.5	9.7	404	0.123	0.400	0.010	1.2	6.0	3.0	2.0	0.5	10	1	20	0.24	0.25	4.0	2
	35	3.2	62.8	6.5	9.2	406	0.123	0.530	0.030	1.5	6.0	2.0	2.2	0.5	10	1	19	0.24	0.26	4.1	2
	40	3.2	62.8	6.4	8.6	408	0.163	0.700	0.010	1.6	6.0	2.0	2.2	0.6	11	1	20	0.30	0.30	4.2	2
	45	3.3	74.4	6.3	6.6	412			0.040	1.8	5.0	3.0	2.4	0.6	15	1	22			4.2	
14-Jun-95	0	16.3	67.5	7.5	11.5	281	0.133	0.130	0.050	1.3	5.0	3.0	2.0	0.5	10	1	19	0.22	0.22	4.5	6
	5	7.1	64.9	7.2	16.4	307		0.530		1.2	5.0	3.0	2.0	0.5	10	1	19	0.29	0.25	4.2	5
	10	5.1	62.9	6.7	16.2	325	0.460	0.350	0.050	1.4	4.0	2.0	2.0	0.5	11	1	19	0.33	0.27	4.3	2
	15	4.6	62.3	6.6	15.4	331	0.093	0.400	0.080	1.4	5.0	2.0	2.0	0.5	10	1	19	0.20	0.27	4.1	5
	20	4.4	62.2	6.5	14.9	337	0.070	0.350	0.030	1.4	5.0	2.0	2.0	0.5	10	1	19	0.19	0.29	4.0	4
	25	4.3	60.8	6.5	14.8	340	0.093	0.400	0.120	1.4	5.0	3.0	2.0	0.5	10	1	19	0.22	0.29	4.3	4
	30	4.2	62.6	6.5	14.6	342	0.133	0.400	0.100	1.4	5.0	2.0	1.7	0.5	10	1	19	0.26	0.28	4.0	5
	35	4.1	63.9	6.4	14.5	345	0.153	0.350	0.030	1.3	5.0	2.0	2.1	0.5	11	1	19	0.25	0.31	4.1	4
	40	4.0	67.8	6.3	14.1	349	0.163	0.440	0.070	1.5	6.0	3.0	1.8	0.6	11	1	20	0.27	0.31	4.1	5
17-Aug-95	0	15.3	61.2	8.1	10.8	349	0.127	0.040	0.100	1.4	5.0	3.0	2.1	0.4	10	1	19	0.27	0.17	5.5	7
	5	13.2	60.4	7.4	9.8	374	0.153	0.040	0.050	1.4	5.0	3.0	2.1	0.5	10	1	18	0.26	0.18	7.5	5
	10	6.0	60.6	6.8	10.3	400	0.257	0.350	0.050	1.4	5.0	3.0	2.1	0.5	10	1	19	0.28	0.24	4.1	5
	15	4.7	59.4	6.6	10.3	407	0.123	0.310	0.040	1.4	5.0	3.0	2.1	0.5	10	1	19	0.28	0.25	5.1	3
	20	4.6	59.9	6.6	10.2	408	0.133	0.350	0.050	1.4	5.0	3.0	2.1	0.5	10	1	19	0.22	0.26	7.3	4
	25	4.4	60.6	6.5	10.1	409	0.173	0.440	0.180	1.4	6.0	3.0	2.2	0.5	10	1	19	0.23	0.26	5.0	1
	30	4.4	60.8	6.5	10.0	410	0.173	0.480	0.090	1.5	6.0	3.0	2.1	0.5	10	1	19	0.24	0.28	4.7	2
	35	4.3	60.8	6.5	9.7	411	0.203	0.400	0.130	1.5	6.0	3.0	2.2	0.6	11	2	19	0.29	0.29	5.0	2
	40	4.2	62.5	6.5	9.5	413	0.213	0.620	0.220	1.4	5.0	3.0	2.2	0.6	10	1	19	0.31	0.32	3.5	3
	45	4.1	64.4	6.5	8.9	412	0.257	0.400	0.040	1.5	6.0	3.0	2.8	0.9	11	1	19	0.37	0.36	4.2	11

Continued

Table 3.1 continues

	Depth	Temp	Cond	pH	DO	Eh	Total-P	$N\text{-}NO_3$	$N\text{-}NH_4$	K	Ca	Mg	Na	Fe	SO_4	Cl	HCO_3	As	Ni	TOC	TSS
14-Oct-95	0	7.0	61	6.9	11.9	387	0.123	0.130	0.050	1.4	5.0	2.0	2.0	0.5	10	1	21	0.10	0.24	5.4	7
	5	7.0	60.8	6.9	11.8	384	0.143	0.090	0.030	1.4	5.0	3.0	2.0	0.5	11	1	21	0.19	0.24	6.8	6
	10	7.0	61.3	6.9	11.8	382	0.103	0.090	0.050	1.4	5.0	3.0	1.8	0.5	10	1	21	0.17	0.22	5.8	6
	15	7.0	60.9	6.9	11.7	381	0.093	0.180	0.030	1.4	5.0	3.0	1.8	0.5	10	1	21	0.18	0.22	8.5	11
	20	5.5	60.5	6.7	9.9	393	0.143	0.090	0.030	1.4	5.0	2.0	2.1	0.6	10	1	21	0.24	0.24	7.6	12
	25	4.6	62.4	6.4	9.2	401	0.060	0.310	0.080	1.4	5.0	3.0	1.8	0.5	11	1	21	0.14	0.24	5.7	9
	30	4.5	61.9	6.3	9.0	402	0.103	0.480	0.030	1.4	5.0	3.0	1.9	0.5	11	1	22	0.25	0.29	4.4	8
	35	4.4	62.8	6.3	8.7	403	0.143	0.480	0.030	1.4	6.0	2.0	2.1	0.5	11	1	21	0.27	0.33	3.6	7
	40	4.3	66.2	6.3	8.1	404	0.163	0.440	0.030	1.4	5.0	3.0	1.9	0.6	13	1	22	0.34	0.31	4.6	10
9-May-96	0	1.2	47.8	6.8	11.0	533	0.133	0.660	0.030	1.2	5.5	2.5	1.9	0.4	12	0.5	21	0.23	0.28	4.4	2
	5	3.4	78.6	6.8	10.4	545	0.133	0.750	0.010	1.3	5.5	2.4	2.2	0.4	12	0.5	21	0.23	0.28	4.4	2
	10	3.3	78.4	6.8	10.3	548	0.123	0.750	0.030	1.2	5.7	2.4	2.3	0.4	12	0.5	22	0.22	0.27	4.4	3
	15	3.3	78.4	6.7	10.0	548	0.133	0.480	0.030	1.2	5.6	2.4	2.3	0.4	12	0.5	21	0.21	0.28	4.4	3
	20	3.3	78.5	6.7	9.8	548	0.133	0.480	0.030	0.7	5.6	2.4	2.2	0.5	12	0.4	21	0.19	0.27	4.6	3
	25	3.3	78.3	6.7	9.3	548	0.133	0.530	0.030	1.0	5.6	2.4	2.2	0.5	12	0.4	22	0.19	0.27	4.3	4
	30	3.3	77.1	6.7	8.5	548	0.103	0.570	0.040	1.7	5.7	2.4	2.1	0.5	12	0.4	22	0.20	0.28	4.4	4
	35	3.3	78.4	6.6	6.8	548	0.123	0.350	0.040	1.7	5.6	2.4	2.2	0.5	12	0.4	23	0.20	0.28	4.3	3
	40	3.3	80.9	6.5	5.7	546	0.133	0.180	0.040	1.4	5.8	2.5	2.2	0.5	13	0.5	22	0.24	0.31	4.4	4
26-Aug-96	0	16.3	93.5	7.5	10.2	358	0.080	0.040	0.050	1.6	5.8	2.3	2.2	0.3	12	0.5	21	0.19	0.22	4.8	2
	5	14.9	91.9	7.4	10.5	350	0.070	0.040	0.030	2.3	5.9	2.4	2.0	0.3	13	0.5	21	0.20	0.22	5.1	2
	10	6.7	83.5	6.8	11.3	372	0.070	0.040	0.020	1.8	5.7	2.4	2.1	0.3	13	0.5	21	0.20	0.27	4.8	3
	15	5.0	82.2	6.7	11.0	376	0.070	0.040	0.010	2.3	5.9	2.4	2.1	0.3	13	0.6	21	0.20	0.28	4.4	2
	20	4.5	81.1	6.6	10.7	378	0.080	0.040	0.050	2.2	6.1	2.5	2.2	0.3	13	0.5	21	0.21	0.29	4.1	2
	25	4.3	83.9	6.5	10.4	379	0.070	0.040	0.100	2.0	5.2	2.1	1.9	0.3	11	0.6	21	0.16	0.27	4.9	2
	30	4.2	85.3	6.5	10.0	379	0.070	0.040	0.040	1.9	5.3	2.1	1.9		11	0.6	21	0.22	0.27	4.8	1
	35	4.1	86.5	6.4	9.4	381	0.103	0.090	0.030	1.9	5.6	2.3	1.9	0.4	12	0.5	22	0.28	0.33	4.8	
	40	4.0	88.3	6.4	9.0	382	0.123	0.130	0.040	2.1	5.8	2.4	2.1	0.5	13	0.6	22	0.31	0.32	4.6	3
28-Oct-96	0	3.4	85.6	6.7	13.2	327	0.113	0.130	0.010	2.0	5.8	2.6	2.2	0.3	13	0.5	22	0.22	0.27	4.4	3
	5	3.5	85.5	6.7	13.2	328	0.103	0.180	0.040	1.9	5.9	2.6	2.2	0.3	13	0.5	22	0.20	0.27	4.1	2
	10	3.6	85.8	6.7	13.2	327	0.103	0.130	0.010	1.3	5.9	2.6	2.1	0.3	13	0.5	22	0.20	0.25	4.1	3
	15	3.6	86.2	6.7	14.0	326	0.103	0.090	0.050	1.8	5.9	2.6	2.2	0.3	13	0.5	22	0.20	0.26	4.1	12
	20	3.7	85.9	6.7	13.2	326	0.103	0.220	0.030	1.4	5.9	2.6	2.3	0.3	13	0.5	22	0.21	0.27	4.1	2
	25	3.7	85	6.7	14.0	324	0.103	0.130	0.010	1.7	5.8	2.6	2.3	0.4	13	0.5	21	0.20	0.27	4.1	3
	30	3.7	85.9	6.7	13.3	323	0.103	0.180	0.010	2.3	5.8	2.6	2.3	0.3	13	0.6	21	0.21	0.26	4.0	4
	35	3.7	87.1	6.7	13.8	323	0.123	0.130	0.010	1.7	5.9	2.6	2.4	0.4	13	0.5	21	0.20	0.26	4.1	
	40	3.7	85.3	6.7	13.7	335	0.213	0.040	0.050	1.2	6.0	2.7	2.3	0.7	13	0.6	21	0.18	0.25	4.3	
29-Jun-97	0	19.4	68.2	7.3	9.2	102	0.100	0.040	0.100	1.3	5.5	2.5	2.0	0.2	13		22	0.13	0.21	3.8	2
	5	13.9	67.6	7.5	10.2	104	0.100	0.040	0.100	1.5	5.5	2.5	2.0	0.2	12		22	0.14	0.22	4.0	2
	10	6.0	65.7	7.1	10.2	123	0.030	0.040	0.100	1.2	5.5	2.5	2.0	0.2	12		22	0.13	0.24	4.0	2
	15	4.8	65.2	6.9	9.9	129	0.110	0.040	0.120	1.4	5.6	2.4	2.0	0.2	13		22	0.14	0.25	4.0	1
	20	4.4	65	6.8	9.8	131	0.140	0.040	0.100	1.5	5.6	2.4	2.1	0.3	12		22	0.14	0.26	4.0	2
	25	4.3	64.2	6.8	9.7	134	0.160	0.040	0.090	1.4	5.5	2.4	2.1	0.3	12		22	0.15	0.26	4.0	1
	30	4.2	66	6.7	9.4	136	0.110	0.040	0.080	1.5	5.6	2.4	2.1	0.3	12		23	0.15	0.25	4.0	4
	35	4.1	68	6.7	9.3	137	0.130	0.040	0.090	1.5	5.7	2.5	2.1	0.3	12		23	0.16	0.26	4.0	1
	40	4.0	69.8	6.7	9.0	138	0.140	0.040	0.100	1.7	5.8	2.5	2.1	0.3	12		23	0.18	0.28	4.0	2

Continued

Table 3.1 continues

	Depth	Temp	Cond	pH	DO	Eh	Total-P	$N\text{-}NO_3$	$N\text{-}NH_4$	K	Ca	Mg	Na	Fe	SO_4	Cl	HCO_3	As	Ni	TOC	TSS
12-Aug-97	0	16.0	71.1	7.1	8.8	108		0.044	0.052	1.6	5.8	2.4	2.1	0.2	12		23	0.15	0.21	3.0	2
	5	15.9	70.9	7.4	9.0	109		0.044	0.052	1.8	5.8	2.4	2.1	0.2	12		23	0.15	0.22	3.2	2
	10	8.0	66.2	7.6	9.9	127		0.044	0.039	2.0	5.7	2.4	2.0	0.2	12		22	0.13	0.26	2.8	3
	15	4.7	66.1	7.6	9.4	134		0.044	0.039	1.7	5.6	2.4	2.1	0.2	12		22	0.13	0.25	2.8	2
	20	4.4	64.1	7.6	9.1	141		0.044	0.026	1.9	5.7	2.4	2.1	0.2	12		22	0.13	0.26	2.8	2
	25	4.3	66.4	7.6	8.9	144		0.044	0.039	1.5	5.8	2.4	2.1	0.3	12		23	0.13	0.26	2.8	2
	30	4.2	67.8	7.6	8.7	147		0.044	0.026	1.5	5.9	2.5	2.1	0.3	12		23	0.13	0.25	2.9	1
	35	4.2	67.8	7.6	8.7	150		0.040	0.026	1.7	5.9	2.5	2.1	0.3	12		23	0.14	0.26	2.9	1
	40	4.0	67	7.6	8.2	153		0.176	0.052	1.6	6.0	2.5	2.1	0.3	12		23	0.15	0.25	3.0	2
6-Oct-97	0	6.9	68.3	7.1	10.5	74	0.090	0.220	0.078	1.8	6.1	2.6	2.3	0.3	13		23	0.12	0.23	3.5	5
	5	6.9	68.4	7.3	11.0	79	0.080	0.176	0.078	1.8	6.0	2.5	2.2	0.3	13		23	0.12	0.23	3.8	5
	10	6.8	66.5	7.3	11.6	82	0.080	0.176	0.100	1.8	5.9	2.5	2.3	0.3	13		22	0.12	0.23	3.9	7
	15	6.8	69.1	7.4	11.8	85	0.070	0.176	0.090	1.9	5.8	2.5	2.2	0.3	13		22	0.11	0.23	4.0	7
	20	6.5	67	7.4	12.0	88	0.070	0.220	0.208	1.9	5.9	2.5	2.2	0.3	13		22	0.12	0.24	3.4	6
	25	4.5	70.9	7.4	9.6	100	0.060	0.308	0.130	1.7	6.1	2.6	2.3	0.2	13		23	0.11	0.27	4.1	4
	30	4.4	68.8	7.4	8.0	105	0.060	0.264	0.156	1.8	6.1	2.5	2.3	0.2	13		23	0.1	0.27	3.4	3
	35	4.3	71.7	7.4	7.7	108	0.060	0.308	0.156	1.8	6.1	2.6	2.3	0.2	13		23	0.11	0.27	4.1	3
	40	4.2	73.1	7.4	7.1	111	0.080	0.352	0.182	1.4	6.2	2.7	2.3	0.3	14		23	0.13	0.28	4.0	3
18-Apr-98	0	0.9	61.9	7.0	9.6	84	0.287	0.260	0.040	1.8	6.4	2.7	2.2	0.2	13		26	0.11	0.25	4.2	1
	5	3.0	65.6	7.1	9.5	89	0.113	0.260	0.040	1.3	6.3	2.7	2.1	0.2	13		24	0.10	0.24	4.1	1
	10	3.0	65.5	7.1	9.5	93	0.113	0.180	0.030	1.4	6.3	2.7	2.1	0.2	13		24	0.10	0.25	3.9	1
	15	3.1	65.7	7.0	8.8	98	0.093	0.220	0.030	1.7	6.3	2.7	2.2	0.3	13		24	0.08	0.25	4.0	1
	20	3.1	66.6	6.9	8.2	103	0.080	0.220	0.040	1.4	6.4	2.8	2.2	0.4	13		24	0.06	0.24	4.1	1
	25	3.1	67.2	6.9	7.8	107	0.093	0.260	0.040	1.5	6.4	2.8	2.3	0.5	13		26	0.07	0.24	4.0	1
	30	3.1	68.1	6.9	7.6	109	0.070	0.260	0.050	1.4	6.4	2.8	2.2	0.5	13		26	0.06	0.25	3.9	2
	35	3.1	69.2	6.8	7.5	111	0.080	0.260	0.050	1.3	6.5	2.8	2.3	0.5	13		26	0.07	0.24	4.2	2
	40	3.2	67.9	6.8	7.2	114	0.070	0.350	0.050	1.7	6.4	2.8	2.3	0.4	13		26	0.07	0.23	4.0	1
1-Jun-98	*0*	*7.2*	*65.9*	*7.2*	*8.8*	*181*	*0.203*	*0.040*	*0.070*	1.4	6.2	*2.7*	2.1	*0.3*	13		24	*0.09*	0.23	*3.8*	2
	5	7.2	67.5	7.1	8.6	177	0.103	0.040	0.080	1.9	6.2	2.6	2.1	0.3	13		24	0.09	0.23	3.8	2
	10	6.8	66.3	7.1	8.5	175	0.080	0.040	0.080	1.6	6.2	2.7	2.1	0.3	13		24	0.08	0.24	3.8	1
	15	4.8	66.4	7.0	8.2	179	0.093	0.130	0.070	1.7	6.0	2.6	2.1	0.3	13		24	0.09	0.25	3.5	2
	20	4.4	63.8	6.8	8.1	181	0.040	0.090	0.040	1.9	6.1	2.6	2.1	0.3	13		24	0.08	0.25	3.7	2
	25	4.3	66	6.8	8.0	181	0.060	0.180	0.050	1.8	6.0	2.5	2.1	0.3	13		24	0.09	0.25	3.6	2
	30	*4.2*	*61.5*	*6.8*	*8.0*	*181*	*0.060*	*0.090*	*0.050*	*1.8*	*6.2*	*2.6*	*2.1*	*0.3*	13		*24*	*0.09*	*0.25*	3.6	2
	35	4.2	66.4	6.7	7.8	181	0.070	0.130	0.040	1.5	6.1	2.7	2.1	0.4	13		24	0.09	0.25	3.7	2
	40	4.2	65.1	6.7	7.9	181	0.183	0.130	0.070	1.8	6.2	2.7	2.1	0.4	13		24	0.09	0.25	3.6	1
2-Sep-98	0	13.9	71.1	5.8	9.3	152	0.060	0.040	0.050	1.7	6.7	2.8	2.3	0.1	13		24	0.07	0.18	3.7	1
	5	13.8	71.7	6.1	9.1	144	0.143	0.040	0.040	1.8	6.6	2.8	2.3	0.1	13		24	0.07	0.18	3.8	2
	10	8.7	68.8	6.3	9.7	142	0.080	0.040	0.040	1.6	6.3	2.7	2.2	0.2	13		24	0.06	0.22	3.5	2
	15	*5.5*	*68*	*6.4*	9.5	*152*	*0.060*	*0.040*	*0.040*	1.4	*6.3*	2.7	2.2	0.3	13		24	0.05	0.24	3.4	1
	20	4.6	65.7	6.4	9.2	155	0.103	0.040	0.010	1.6	6.5	2.7	2.3	0.3	13		24	0.06	0.25	3.4	1
	25	4.5	68		9.1	158	0.060	0.040	0.030	1.5	6.3	2.7	2.2	0.4	13		24	0.05	0.24	3.5	1
	30	4.4	68		9.2	159	0.050	0.040	0.050	1.8	6.5	2.7	2.3	0.4	13		24	0.06	0.25	3.4	1
	35	4.3	70		9.1	160	0.060	0.040	0.070	1.7	6.4	2.8	2.3	0.4	13		24	0.07	0.14	3.3	1
	40	4.3	69			161	0.050	0.160	0.050	1.6	6.6	2.7	2.3	0.4	13		24	0.07	0.26	3.4	1

*. These variables were measured in the same month as the others, but not the same day.

Table 3.2. Summary of Major Water Quality Variables in B-Zone Pit for 1992-1998

Parameters	Temp (°C)	Cond us/cm	pH	DO (mg/l)	Eh (mV)	PO4 (mg/l)	N-NO3 (mg/l)	N-NH4 (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	Fe (mg/l)	SO4 (mg/l)	Cl (mg/l)	HCO3 (mg/l)	As (mg/l)	Ni (mg/l)	TOC (mg/l)	TSS (mg/l)
NO	218	222	218	217	218	213	157	223	224	224	224	224	220	223	168	223	223	223	224	220
Maximum	19.41	96.2	8.07	16.39	548	0.644	0.968	0.312	2.4	9	5	2.8	3.4	28	3	26	0.72	1.2	8.5	160
Minimum	0.14	38	5.29	5.73	74	0.01	0.04	0.01	0.7	3	1	1.6	0.11	4.6	0.3	11	0.051	0.12	1.6	1
Median	4.36	62.7	6.73	11.12	382.5	0.133	0.26	0.03	1.4	5.5	2.4	2.05	0.475	11	1	19	0.223	0.26	3.5	4
Mean	5.32	60.78	6.74	10.98	335.98	0.145	0.33	0.04	1.46	5.24	2.37	2.02	0.64	10.67	0.99	17.95	0.22	0.26	3.70	11.44
StDev#	3.43	13.63	0.37	1.97	130.33	0.09	0.29	0.04	0.27	1.04	0.61	0.23	0.56	2.64	0.61	4.49	0.10	0.08	0.94	21.43
C.V.*	0.64	0.22	0.06	0.18	0.39	0.65	0.86	1.01	0.19	0.20	0.26	0.12	0.87	0.25	0.62	0.25	0.44	0.33	0.25	1.87

Standard Deviation

* Coefficient of Variation (StDev/Mean)

Table 3.3. Sample Codes for PCA Plots.

Sampling Dates	Depth (m)	Sample Codes	Sampling Dates	Depth (m)	Sample Codes	Sampling Dates	Depth (m)	Sample Codes
30-Jun-92			**22-Aug-93**	0	B30800	**9-Oct-94**	0	B41000
	5	B2605		5	B30805		5	B41005
	10	B2610		10	B30810		10	B41010
	15	B2615		15	B30815		15	B41015
	20	B2620		20	B30820		20	B41020
	25	B2625		25	B30825		25	B41025
	30	B2630		30	B30830		30	B41030
	35	B2635		35	B30835		35	B41035
	40	B2640					40	B41040
	45	B2645						
	50	B2650						
1-Sep-92	0	B20900	**9-Oct-93**	0	B31000	**12-Apr-95**	0	B50400
	5	B20905		5	B31005		5	B50405
	10	B20910		10	B31010		10	B50410
	15	B20915		15	B31015		15	B50415
	20	B20920		20	B31020		20	B50420
	25	B20925		25	B31025		25	B50425
	30	B20930		30	B31030		30	B50430
	35	B20935		35	B31035		35	B50435
	40	B20940		40	B31040		40	B50440
	45	B20945		45	B31045		45	B50445
1-Oct-92	0	B21000	**17-Apr-94**	0	B40400	**14-Jun-95**	0	B50600
	5	B21005		5	B40405		5	B50605
	10	B21010		10	B40410		10	B50610
	15	B21015		15	B40415		15	B50615
	20	B21020		20	B40420		20	B50620
	25	B21025		25	B40425		25	B50625
	30	B21030		30	B40430		30	B50630
	35	B21035		35	B40435		35	B50635
	40	B21040		40	B40440		40	B50640
	45	B21045		45				
7-Mar-93	0	B30300	**28-Jun-94**	0	B40600	**17-Aug-95**	0	B50800
	5	B30305		5	B40605		5	B50805
	10	B30310		10	B40610		10	B50810
	15	B30315		15	B40615		15	B50815
	20	B30320		20	B40620		20	B50820
	25	B30325		25	B40625		25	B50825
	30	B30330		30	B40630		30	B50830
	35	B30335		35	B40635		35	B50835
	40	B30340		40	B40640		40	B50840
				45	B40645		45	B50845
29-Jun-93	0	B30600	**16-Aug-94**	0	B40800	**14-Oct-95**	0	B51000
	5	B30605		5	B40805		5	B51005
	10	B30610		10	B40810		10	B51010
	15	B30615		15	B40815		15	B51015
	20	B30620		20	B40820		20	B51020
	25	B30625		25	B40825		25	B51025
	30	B30630		30	B40830		30	B51030
	35	B30635		35	B40835		35	B51035
				40	B40840		40	B51040
				45	B40845			

Continued

Table 3.3 continues

9-May-96	0	B60500	**6-Oct-97**	0	B71000
	5	B60505		5	B71005
	10	B60510		10	B71010
	15	B60515		15	B71015
	20	B60520		20	B71020
	25	B60525		25	B71025
	30	B60530		30	B71030
	35	B60535		35	B71035
	40	B60540		40	B71040
26-Aug-96	0	B60800	**18-Apr-98**	0	B80400
	5	B60805		5	B80405
	10	B60810		10	B80410
	15	B60815		15	B80415
	20	B60820		20	B80420
	25	B60825		25	B80425
	30	B60830		30	B80430
	35	B60835		35	B80435
	40	B60840		40	B80440
	0	B61000	**1-Jun-98**	0	B80600
28-Oct-96	5	B61005		5	B80605
	10	B61010		10	B80610
	15	B61015		15	B80615
	20	B61020		20	B80620
	25	B61025		25	B80625
	30	B61030		30	B80630
	35	B61035		35	B80635
	40	B61040		40	B80640
29-Jun-97	0	B70600	**2-Sep-98**	0	B80900
	5	B70605		5	B80905
	10	B70610		10	B80910
	15	B70615		15	B80915
	20	B70620		20	B80920
	25	B70625		25	B80925
	30	B70630		30	B80930
	35	B70635		35	B80935
	40	B70640		40	B80940
12-Aug-97	0	B70800			
	5	B70805			
	10	B70810			
	15	B70815			
	20	B70820			
	25	B70825			
	30	B70830			
	35	B70835			
	40	B70840			

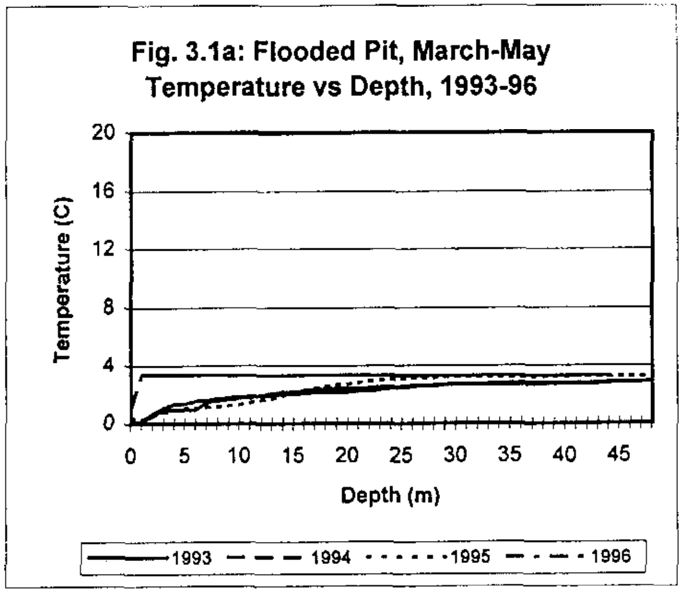
Table 3.4. Phytoplankton and Sample Codes for B-Zone Numeric Data (1995-98)

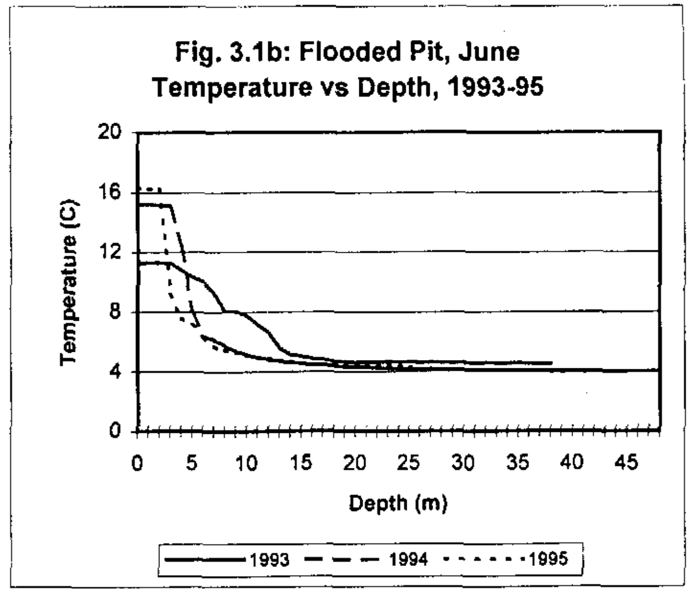
Phytoplankton				Samples			
No.	Genera	Species	Codes	No.	Dates	Depths	Codes
1	*Achnanthes*	sp.	Achnan	1	12-Apr-95	surface	B54S
2	*Ankistrodesmus*	*falcatus*	Ankist	2	26-Jun-95	surface	B56S
3	*Chlamydomonas*	spp.	Chlamy	3	10-Aug-95	surface	B58S
4	*Cryptomonas*	*erosa*	Cryero	4	16-Sep-95	surface	B59S
5	*Cryptomonas*	sp.	Crypto	5	26-Jun-95	2 m	B5602
6	*Dictyospaherium*	*pulchellum*	Dictyo	6	10-Aug-95	2 m	B5802
7	*Dinobryon*	*divergens*	Dindiv	7	16-Sep-95	2 m	B5902
8	*Dinobryon*	*sertularia*	Dinser	8	26-Jun-95	12 m	B5612
9	*Dinobryon*	spp.	Dinobr	9	10-Aug-95	12 m	B5812
10	*Euglena*	sp.	Euglen	10	12-Apr-95	22 m	B5422
11	*Glenodinium*	spp.	Glenod	11	26-Jun-95	22 m	B5622
12	*Gonatozygon*	sp.	Gonato	12	10-Aug-95	22 m	B5822
13	*Gymnodinium*	spp.	Gymnod	13	10-Aug-95	32 m	B5832
14	*Oedogonium*	sp.	Ochrom	14	12-Apr-95	42 m	B5442
15	*Nitzschia*	sp.	Nitzst	15	26-Jun-95	42 m	B5642
16	*Oscillatoria*	*limnelica*	Oscill	16	31-Aug-96	surface	B68S
17	*Peridinium*	*inconspicum*	Peridi	17	25-Apr-96	0.5 M	B64.5
18	*Pseudokephyrion*	spp.	Pseudo	18	25-Apr-96	2 M	B6402
19	*Sphaerellopsis*	sp.	Sphaer	19	31-Aug-96	2 M	B6802
20	*Tabellaria*	*fenestrata*	Tabell	20	25-Apr-96	12 M	B6412
21	*Temnogametum*	species	Temnog	21	31-Aug-96	12 M	B6812
22	Unidentified	sp.	Ucrypt	22	31-Aug-96	22 M	B6822
23	unidentified	spp.	Uchry_1	23	25-Apr-96	32 M	B6432
24	Unidentified sm	species	Uchl_s	24	31-Aug-96	32 M	B6832
25	Unidentified small	sp.	Ucyano_s	25	25-Apr-96	42 M	B6442
26	Unidentified small	spp.	Uchl_spp	26	31-Aug-96	47 M	B6847
27	Z-Unidentified	spp.	UDiatom	27	26-Aug-97	2M	B7802
				28	26-Aug-97	22M	B7822
				29	26-Aug-97	surface	B78S
				30	5-Sep-98	2m	B8902
				31	26-Aug-97	42 M	B7842
				32	26-Aug-97	12 M	B7812
				33	26-Aug-97	32 M	B7832
				34	2-Sep-98	42m	B8942
				35	2-Sep-98	32m	B8932
				36	2-Sep-98	22m	B8922
				37	2-Sep-98	12m	B8912
				38	2-Sep-98	surface	B89S

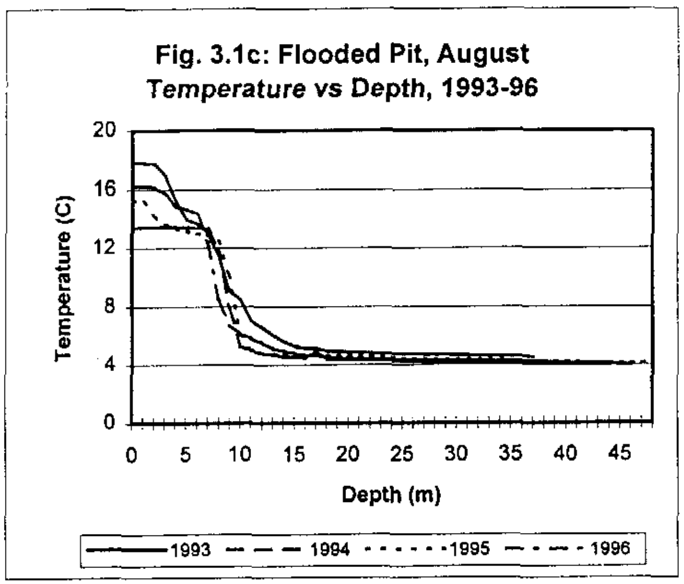
Table 3.5. Taxa Names and Codes for B-Zone Pit and the Collins Bay

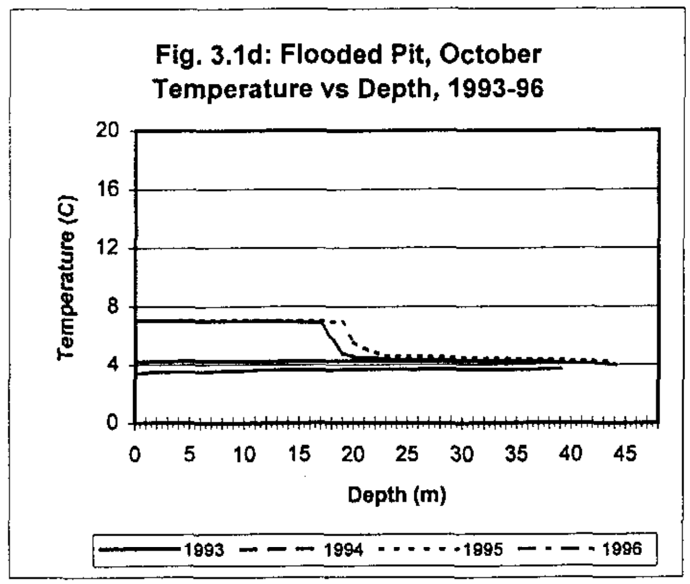
No.	Genus	species	Code	No.	Genus	species	Code
1	Achnanthes	sp.	Achnan	28	Nitzschia	sp.	Nitszc
2	Anabaena	sp.	Anabae	29	Ochromonas	sp.	Ochrom
3	Anabaenopsis	sp.	Anasis	30	Oedogonium	sp.	Oedogo
4	Ankistrodesmus	falcatus	Ankist	31	Oocystis	sp.	Oocyst
5	Arthrodesmus	incus	Arthro	32	Oscillatoria	sp.	Oscill
6	Asterionella	ralfsii	Asteri	33	Peridinium	inconspicuum	Peridi
7	Bitrichia	chodatii	Bitric	34	Pinnularia	sp.	Pinnul
8	Chlamydomonas	sp.	Chlamy	35	Pseudokephyrion	sp.	Pseudo
9	Chlorella	sp.	Chlore	36	Rhizosolenia	sp.	Rhizos
10	Chromulina	sp.	Chromu	37	Scendesmus	sp.	Scende
11	Chroococcus	minutus	Chrooc	38	Sphaerellopsis	sp.	Sphaer
12	Cosmarium	sp.	Cosmar	39	Staurastrum	paradoxum	Staura
13	Cryptomonas	sp.	Crypto	40	Stauroneis	sp.	Staueis
14	Cymbella	sp.	Cymbel	41	Stephanodiscus	astraea	Stepha
15	Dictyosphaerium	sp.	Dictyo	42	Synedra	sp.	Synedr
16	Dinobryon	sp.	Dinobr	43	Tabellaria	sp.	Tabell
17	Euglena	sp.	Euglen	44	Temnogametum	sp.	Temnog
18	Fragilaria	crotonensis	Fragil	45	Tetraedron	sp.	Tetrae
19	Glenodinium	sp.	Glenod	46	Ulothrix	sp.	Ulothr
20	Gomphonema	acuminatum	Gompho	47	Unid-Crypt	sp.	Ucrypt
21	Gonatozygon	sp.	Gonato	48	unidentified	spp.	Uchry_1
22	Gymnodinium	sp.	Gymnod	49	Unidentified flagg	green algae	Uchl_flg
23	Melosira	sp.	Melosi	50	Unidentified sm	green algae	Uchl_s
24	Merismopedia	tenuissima	Merism	51	unidentified sm ch	sp.	Uchry_sp
25	Monoraphidium	sp.	Monora	52	Unidentified small	spp.	Uchl_spp
26	Navicula	sp.	Navicu	53	Unidentified small	sp.	Ucyano_s
27	Nephrocytium	obessum	Nephro	54	Z-Unidentified	spp.	UDiatom

Figures 3.1a-3.1d. Occurrences of Thermocline in the B-Zone Pit

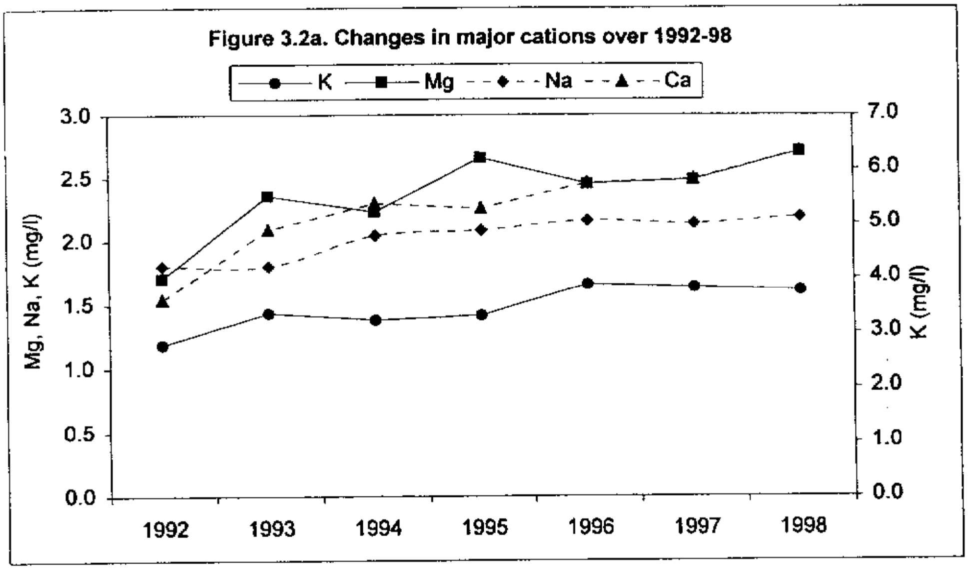


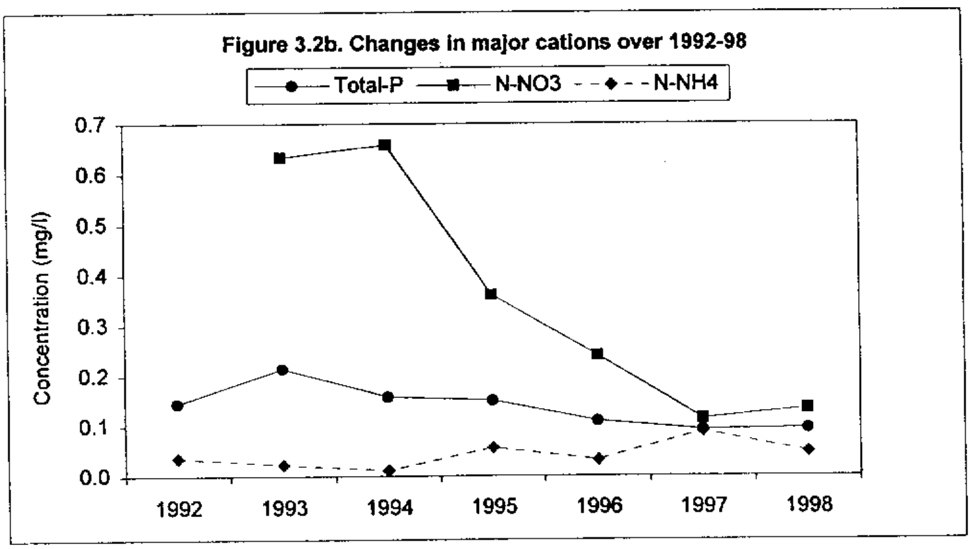


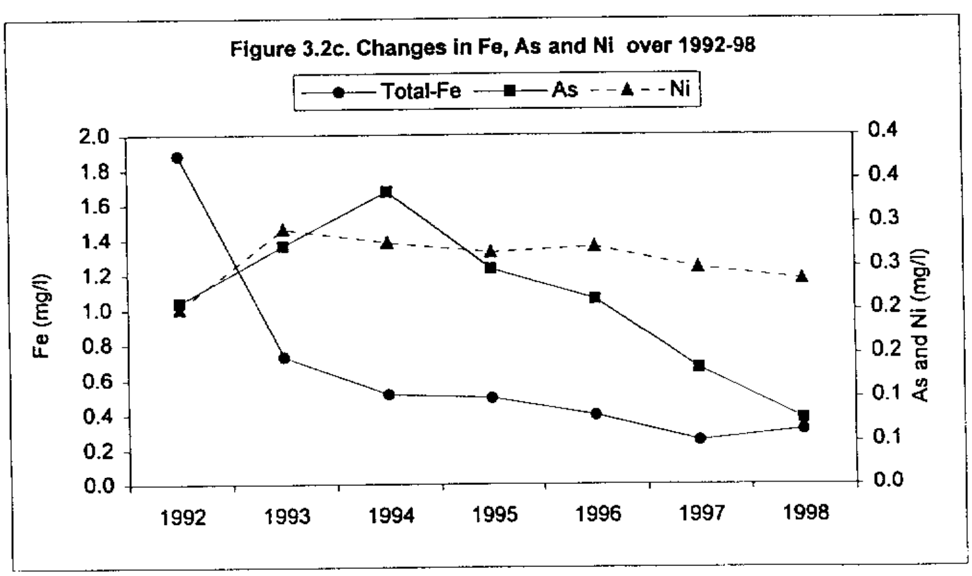




Figs. 3.2a-c. Time Trends of 19 Major Water Quality Variables in the B-Zone Pit (1992-98)







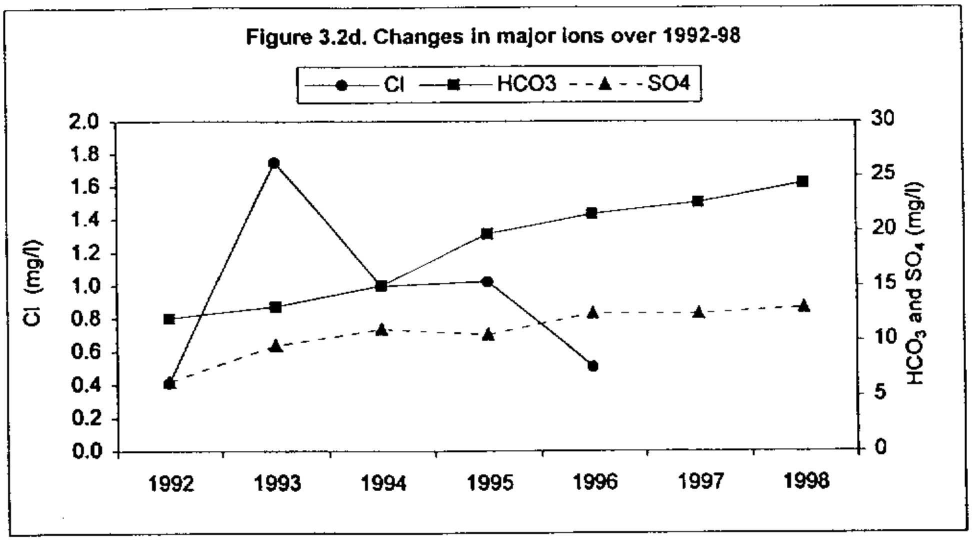


Figure 3.2d. Changes in major ions over 1992-98
Cl
HCO3
SO4
Cl (mg/l)
HCO_3 and SO_4 (mg/l)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
30
25
20
15
10
5
0
1992
1993
1994
1995
1996
1997
1998

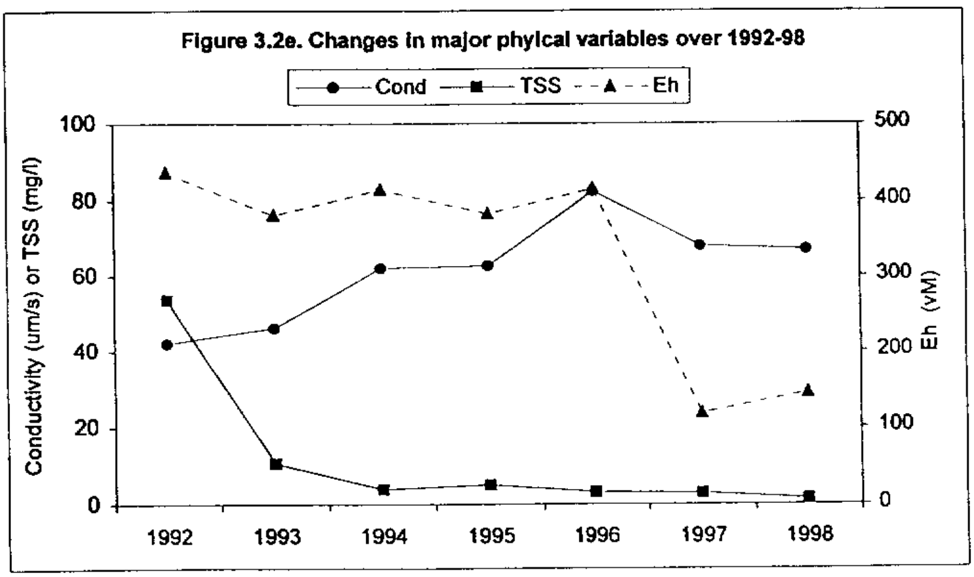


Figure 3.2e. Changes in major phyical variables over 1992-98
Cond
TSS
Eh
Conductivity (um/s) or TSS (mg/l)
Eh (vM)
100
80
60
40
20
0
500
400
300
200
100
0
1992
1993
1994
1995
1996
1997
1998

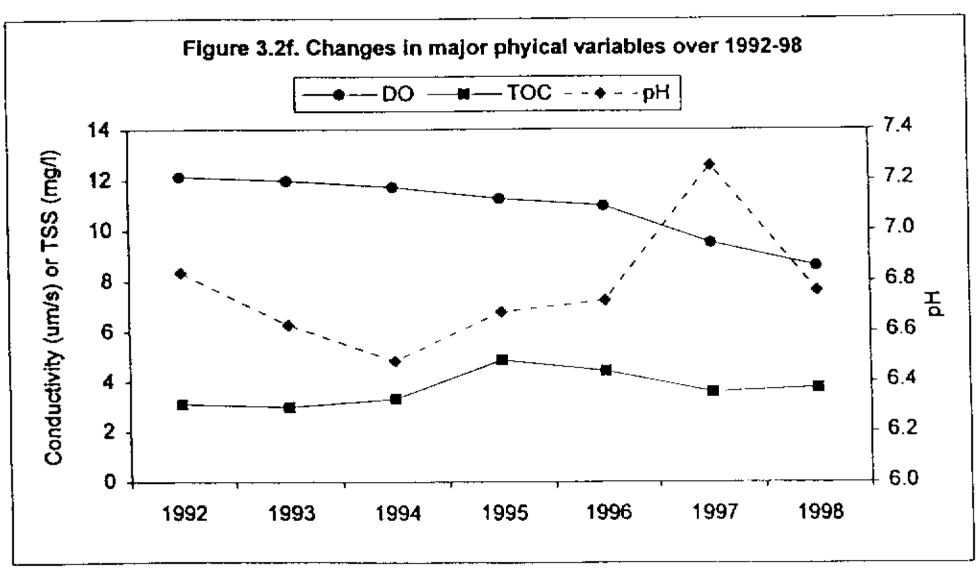


Figure 3.2f. Changes in major phyical variables over 1992-98
DO
TOC
pH
Conductivity (um/s) or TSS (mg/l)
pH
14
12
10
8
6
4
2
0
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
1992
1993
1994
1995
1996
1997
1998

Figure 3.3. PCA Plot of 213 Samples

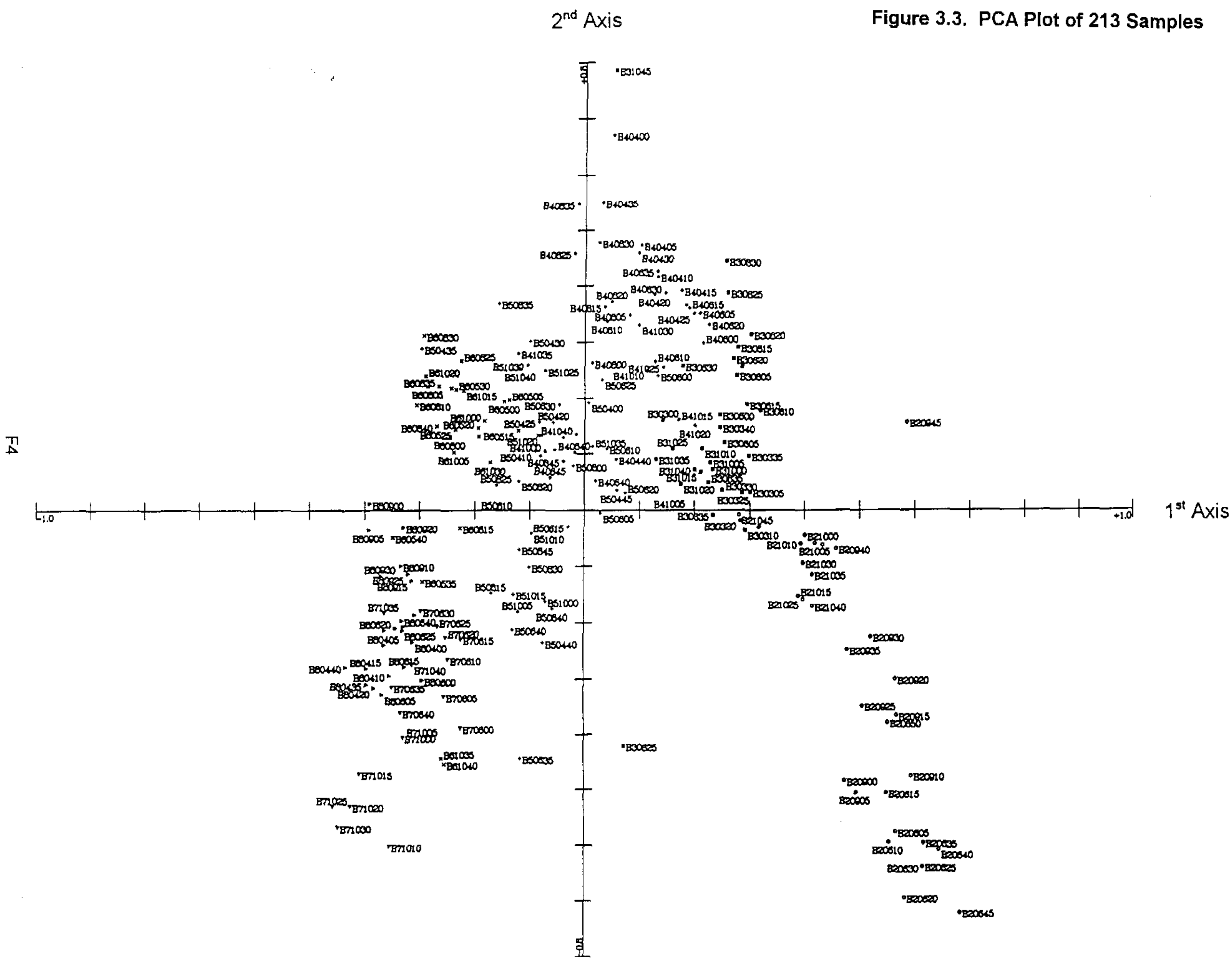


Figure 3.4. PCA Plot of 213 Samples and 17 Variables

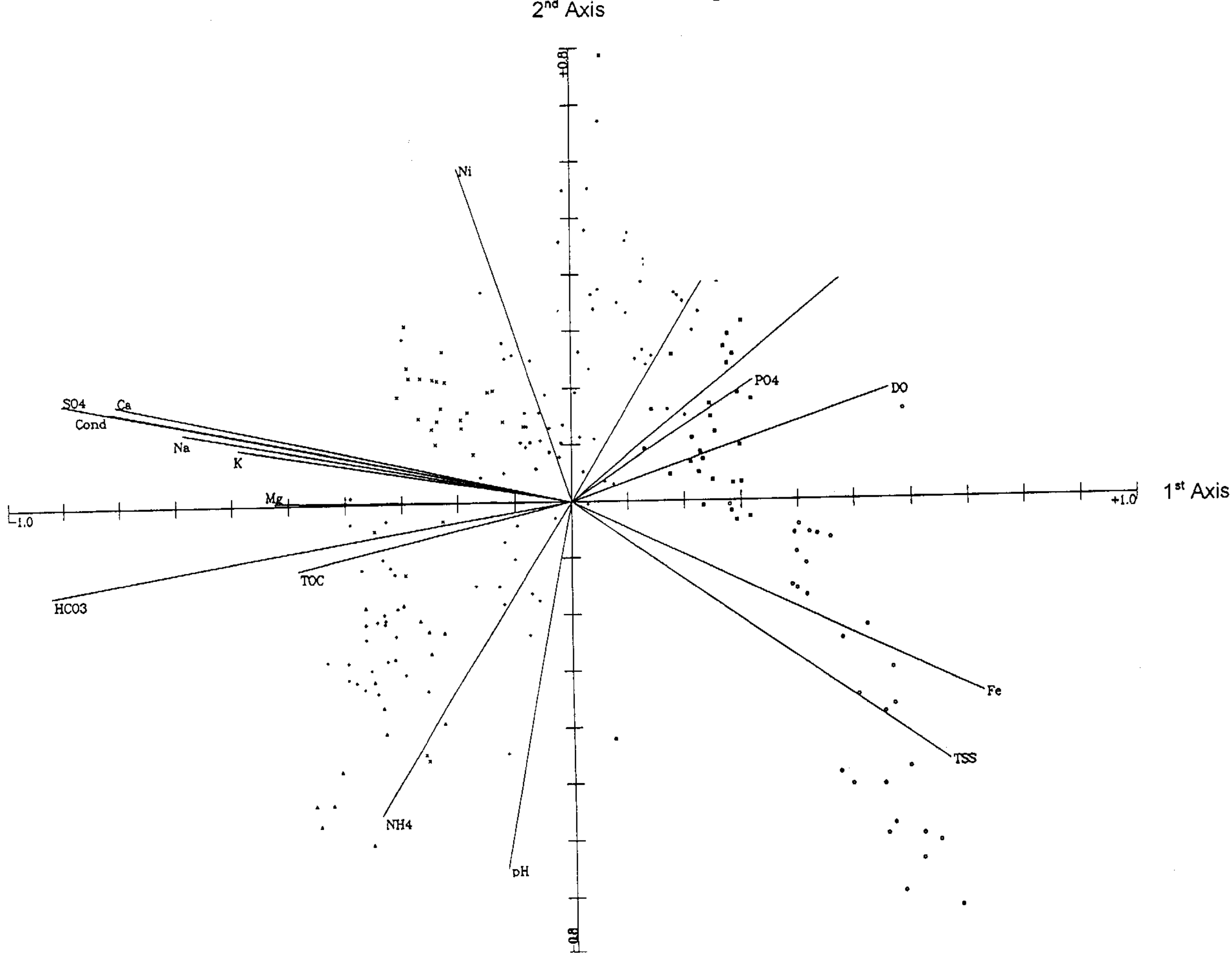


Figure 3.5. Genus/Taxon Richness in B-Zone Pit surface Water (1992-1998) compared with Collins Bay (CB)

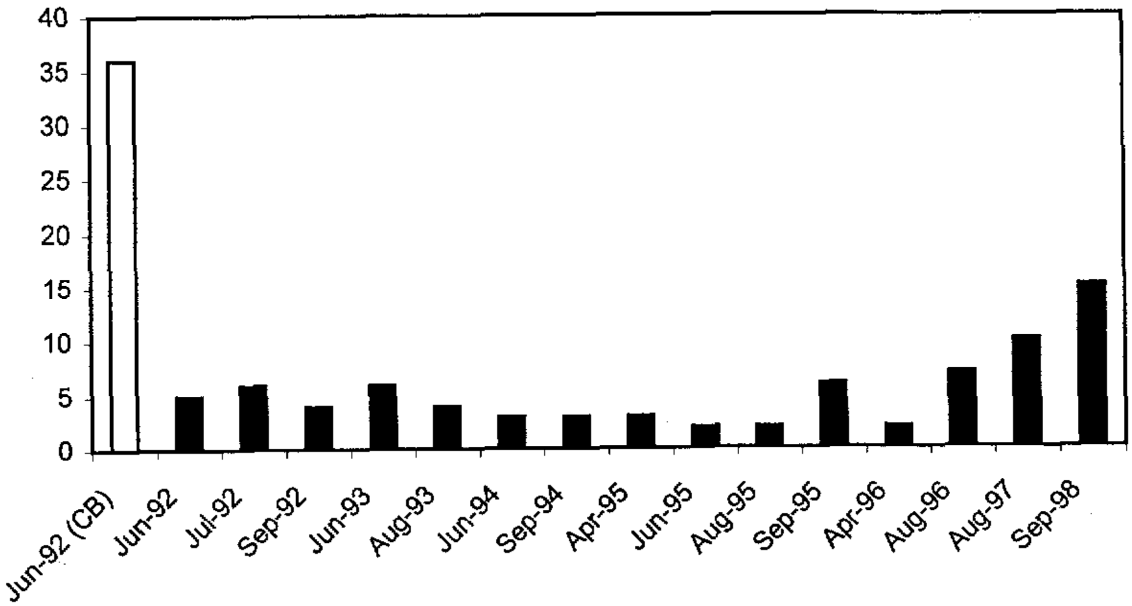


Figure 3.6. DCA Plot of 38 Phytoplankton Samples (1995-98)
(see Table 4 for sample codes)

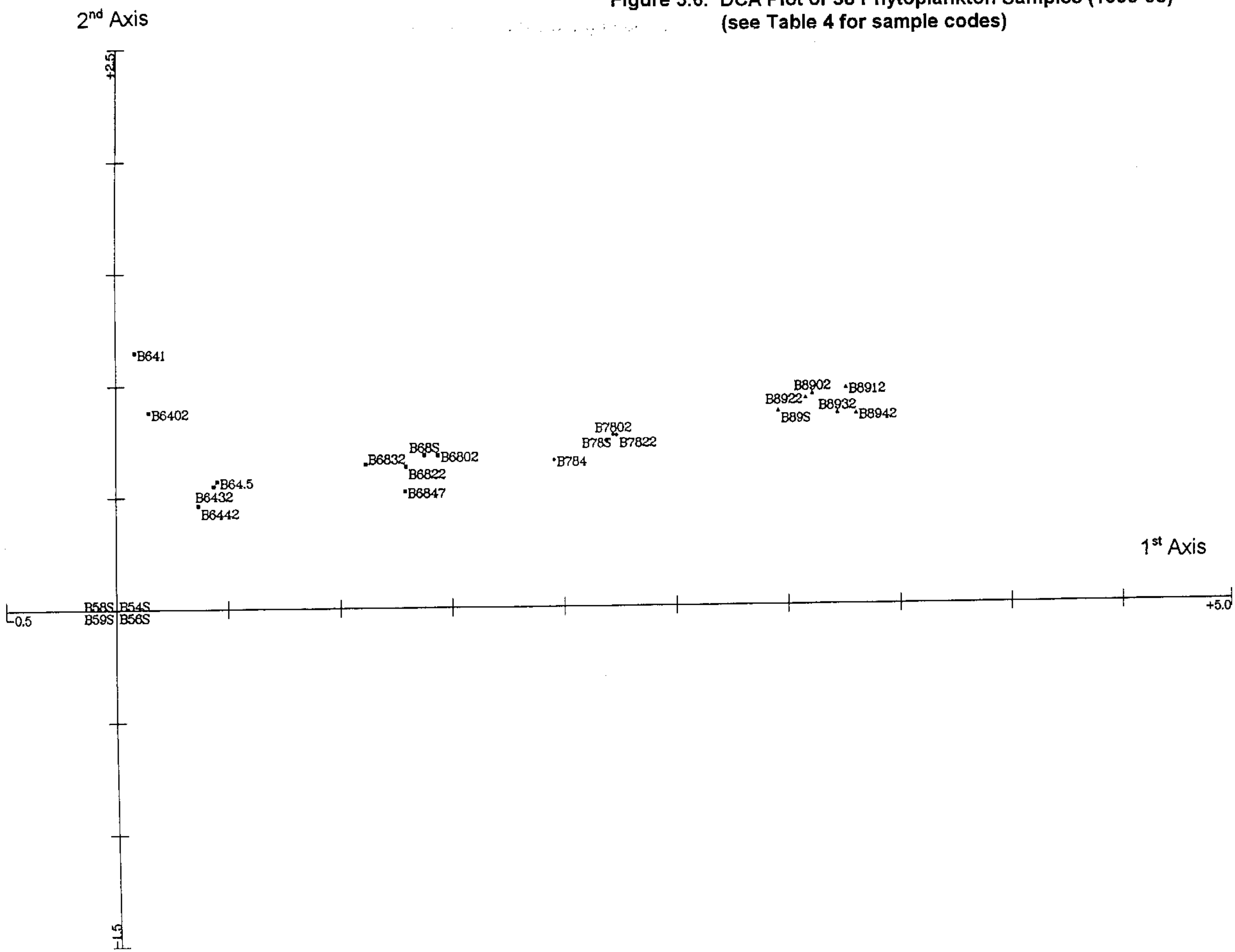


Figure 3.7. DCA Plot of Phytoplankton Species/Taxa (38 Samples)
(refer to Table 6 for taxa-sample relationships)

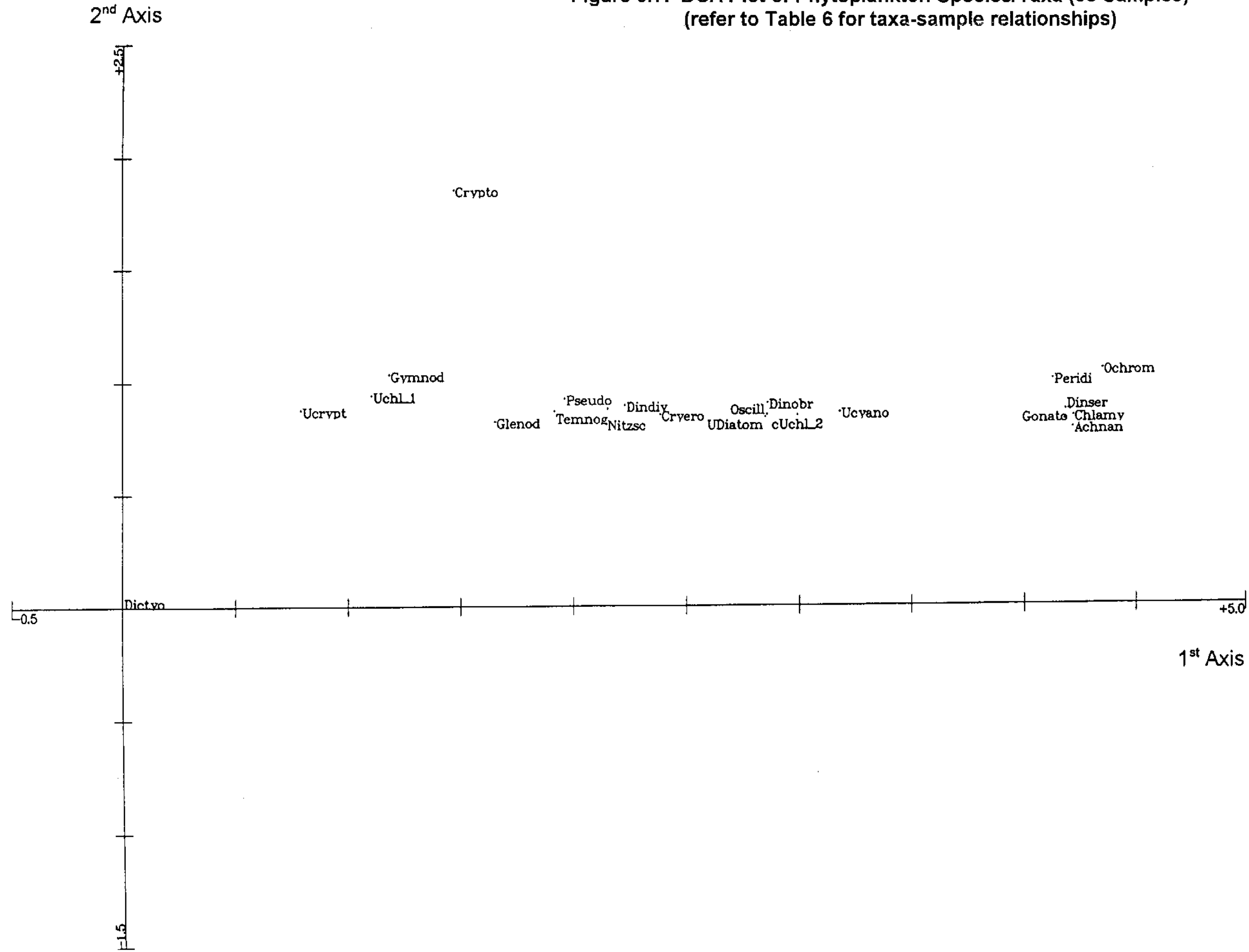
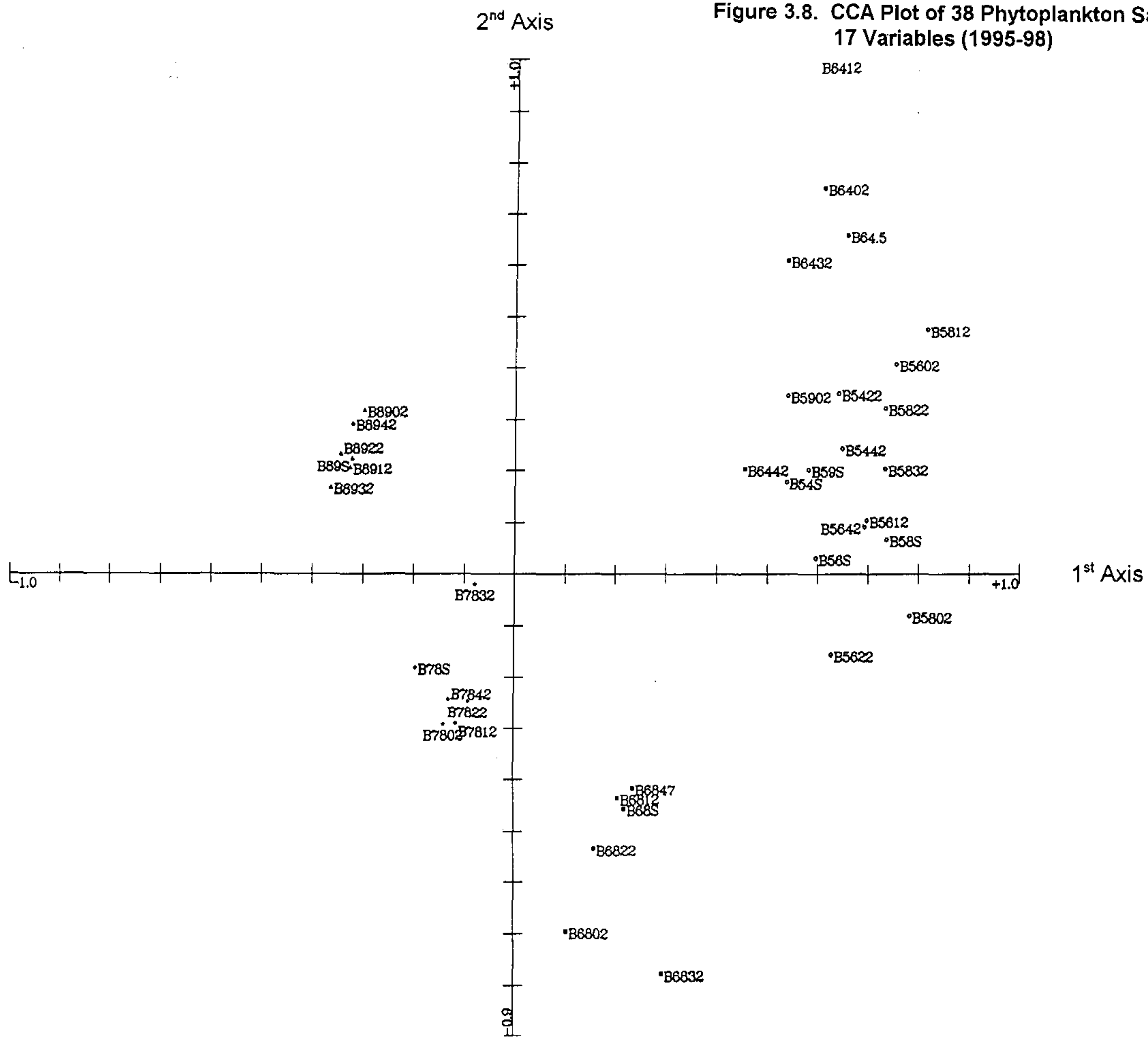
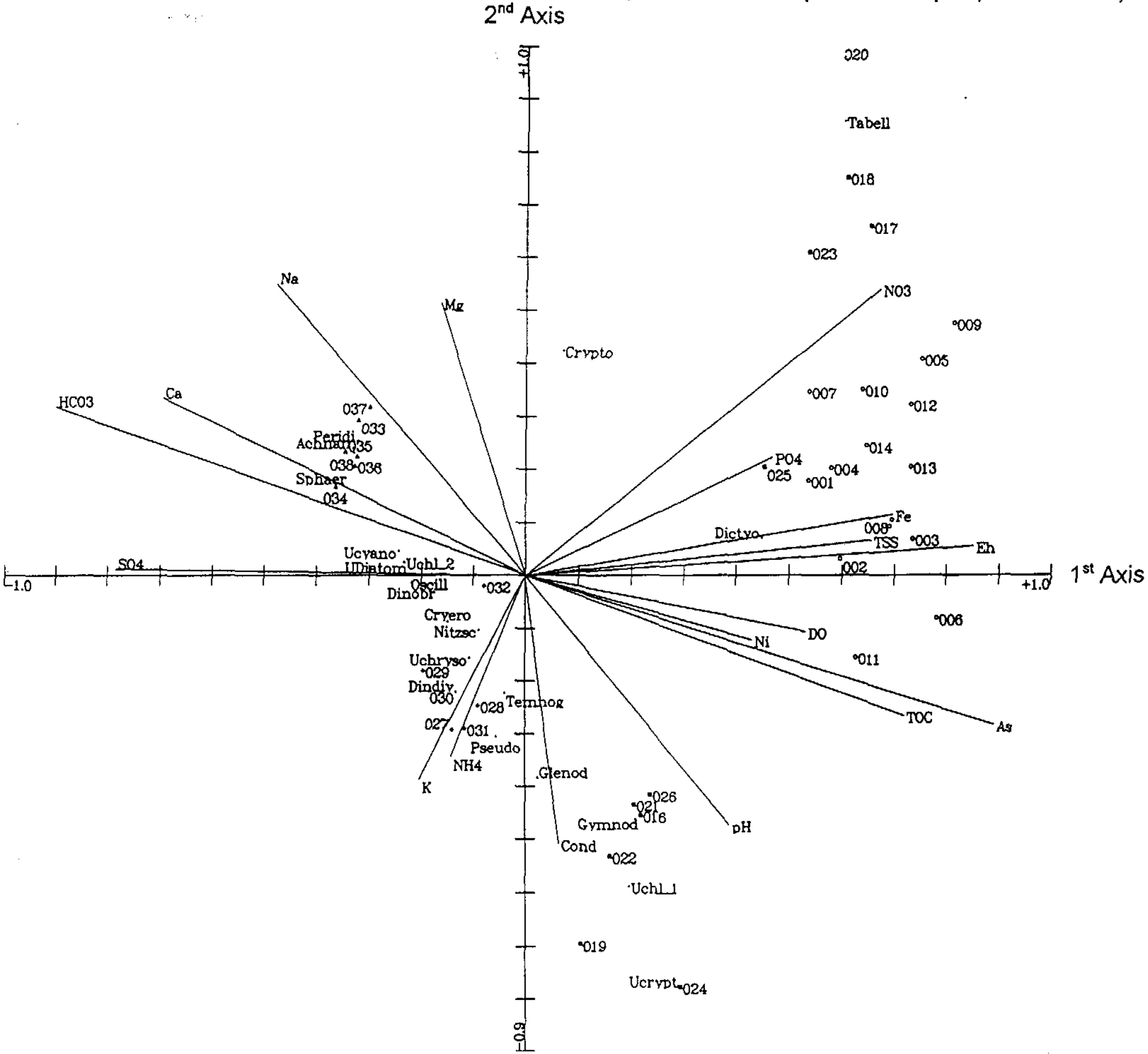


Figure 3.8. CCA Plot of 38 Phytoplankton Samples and 17 Variables (1995-98)



F9

Figure 3.9. CCA Triplot: 38 Samples; 17 Variables; 27 Taxa (1995-98)



F10

Figure 3.10. PCA Plot of 47 Phytoplankton B-Zone Pit Samples (1992-95) and 1 from Collins Bay (1992) (binary data)

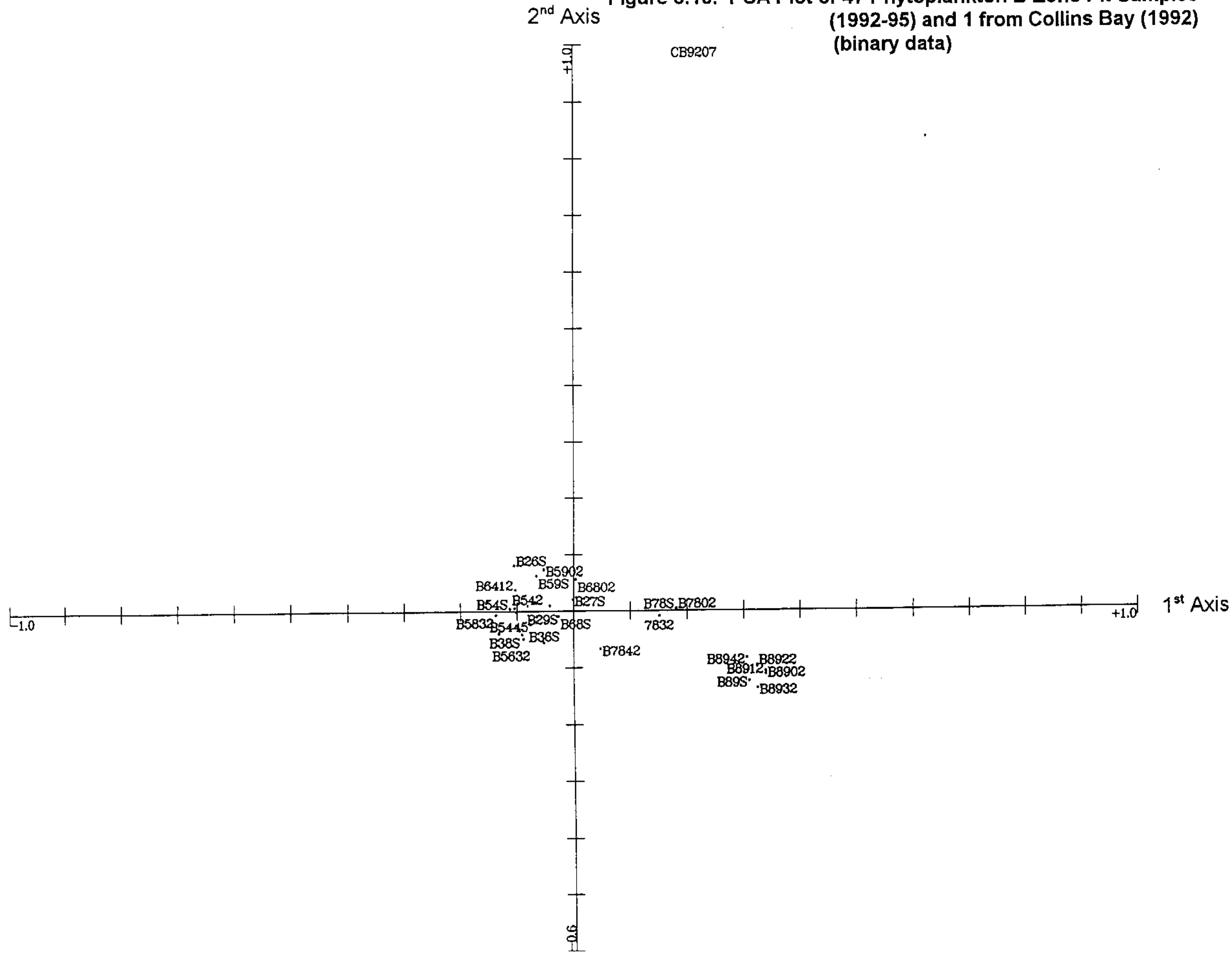
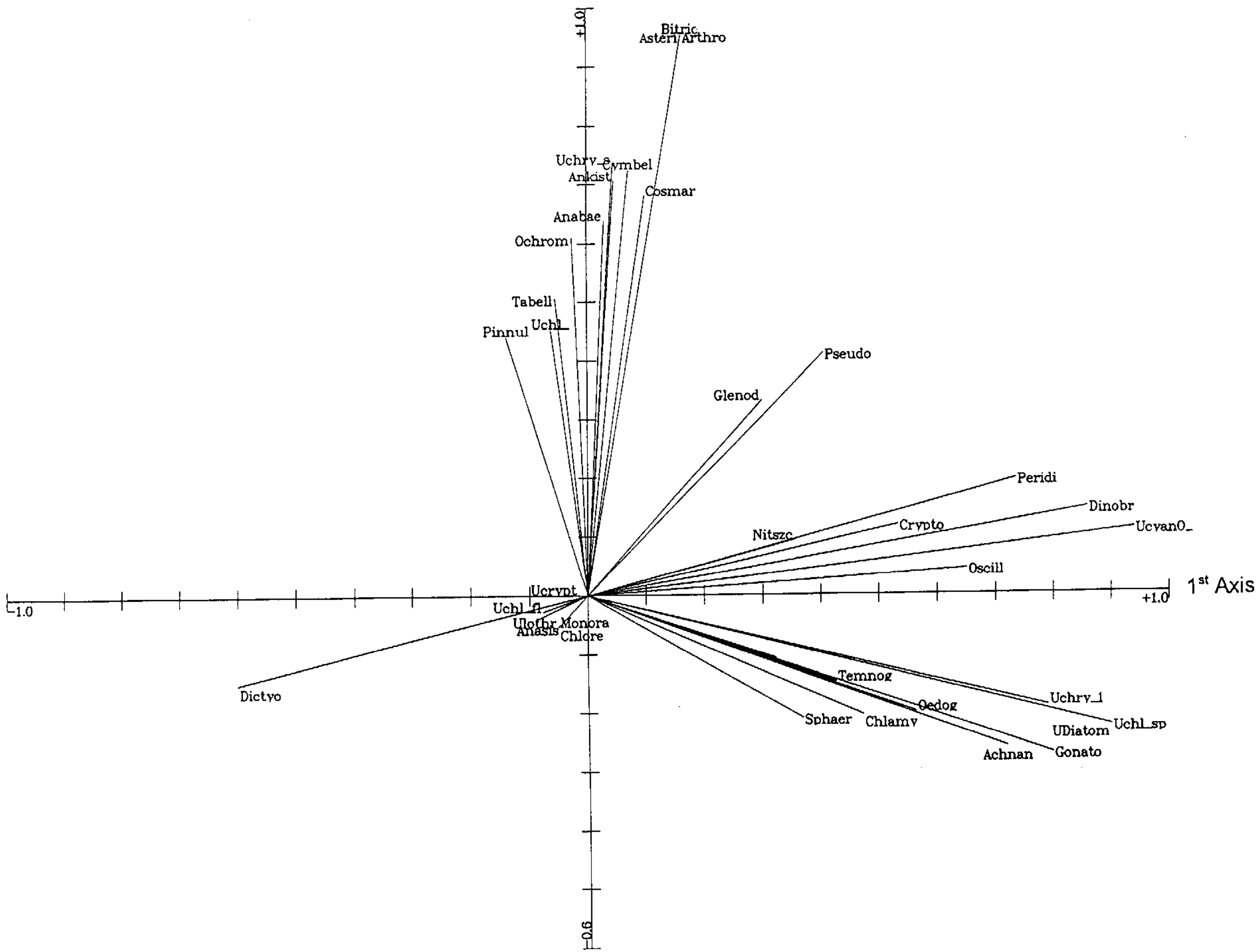
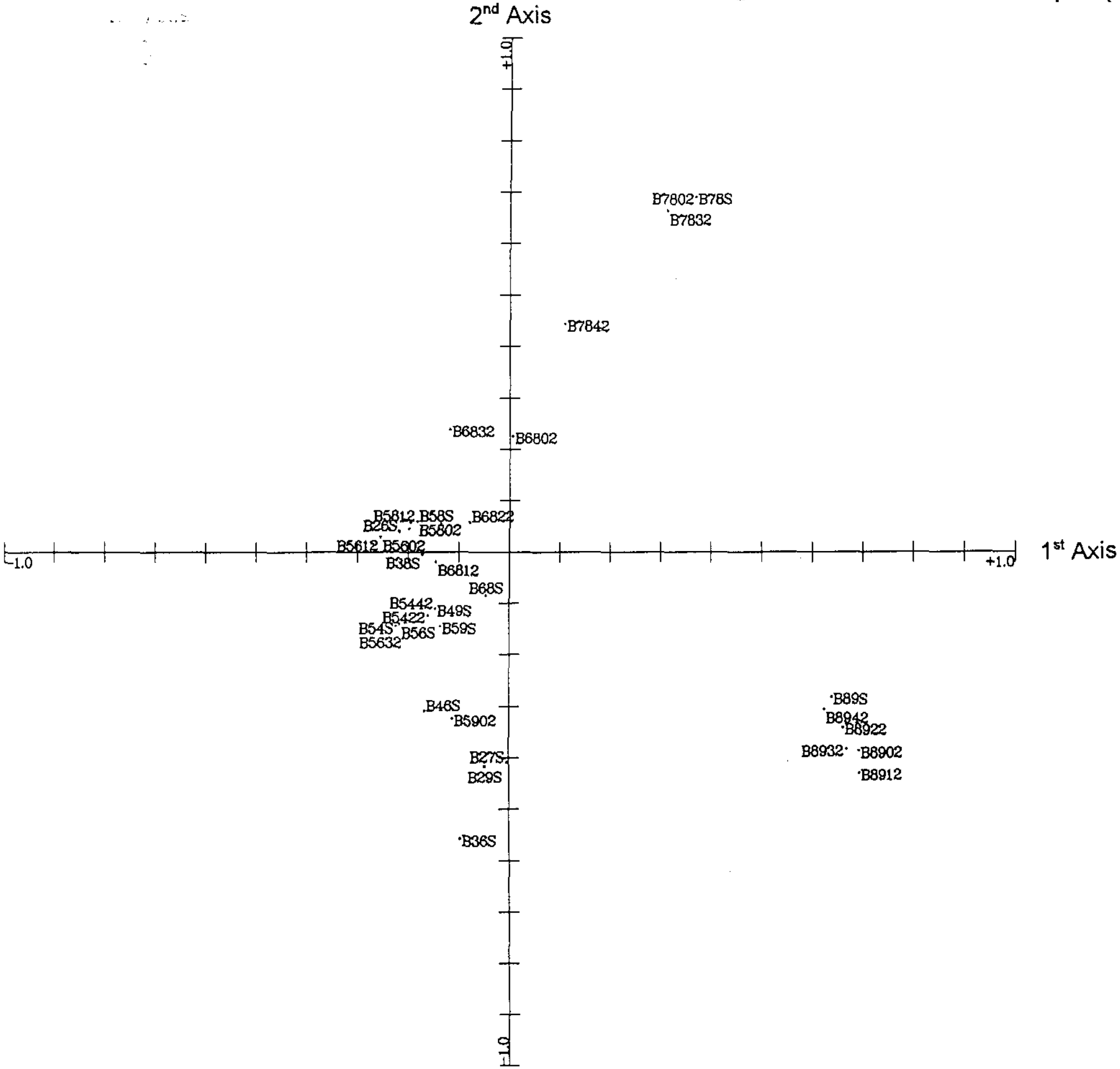


Figure 3.11. PCA Plot of 54 Taxa (binary data) (see Table 5 for taxa codes)



F12

Figure 3.12. PCA Plot of 47 Samples (1992-98 binary data)



F13

Figure 3.13. PCA Plot of Phytoplankton Taxa (47 samples binary data)
(see Table 5 for taxa codes)

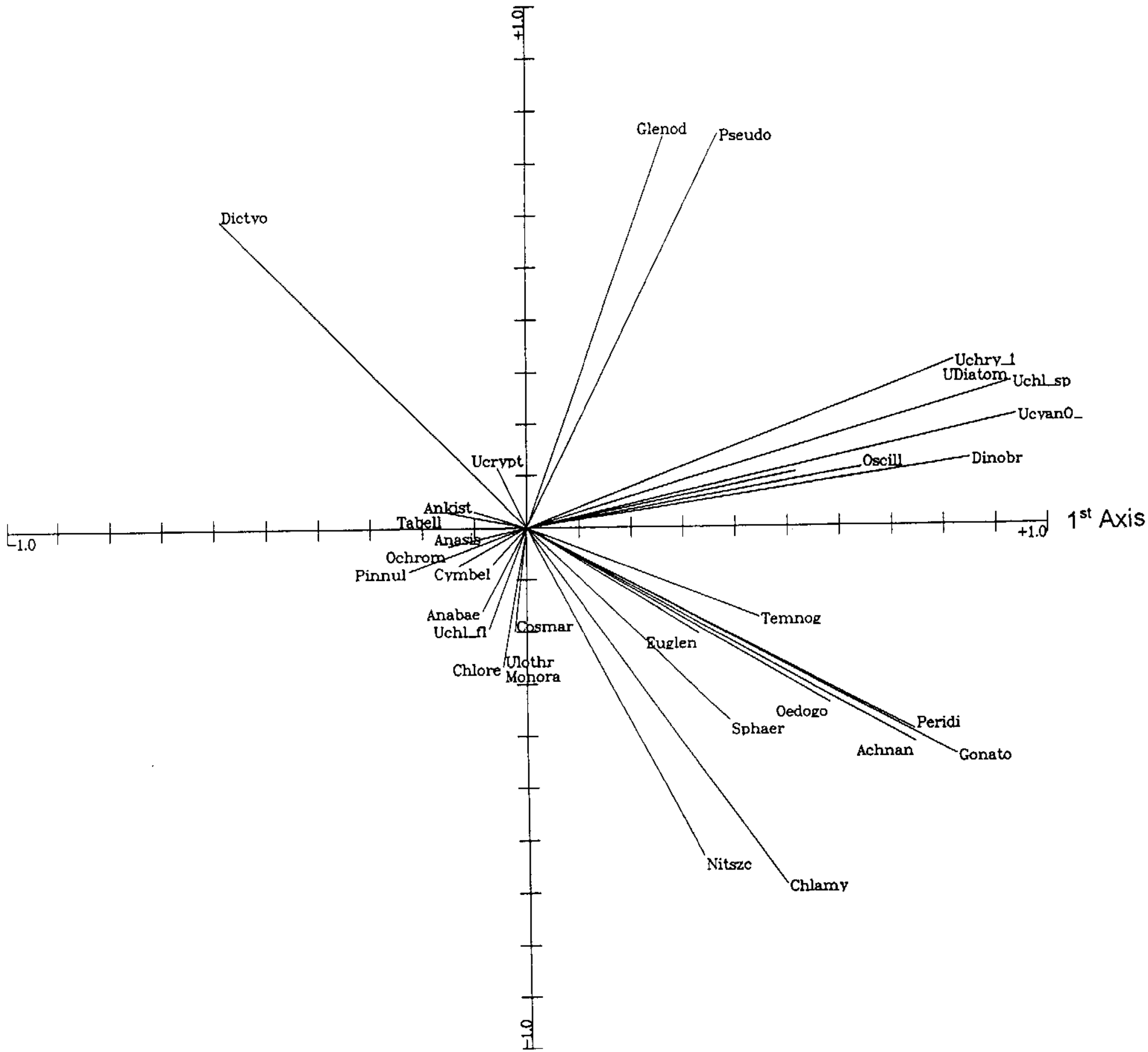
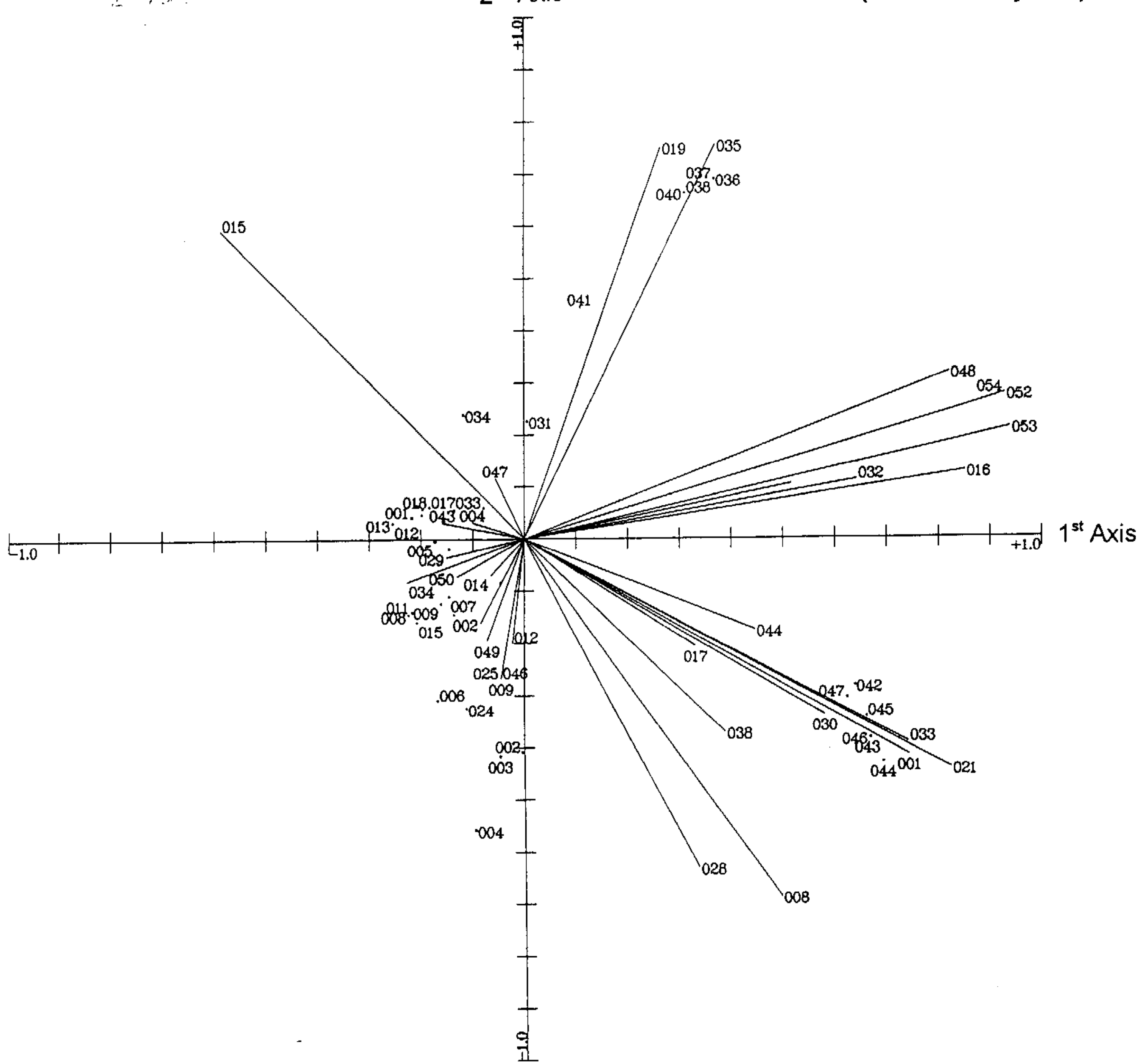


Figure 3.14. PCA Biplot of Phytoplankton Taxa and Samples (1992-98 binary data)



F15

Figure 3.15. PCA Plot of Phytoplankton Samples and Water Quality Variables (1992-98 binary data)

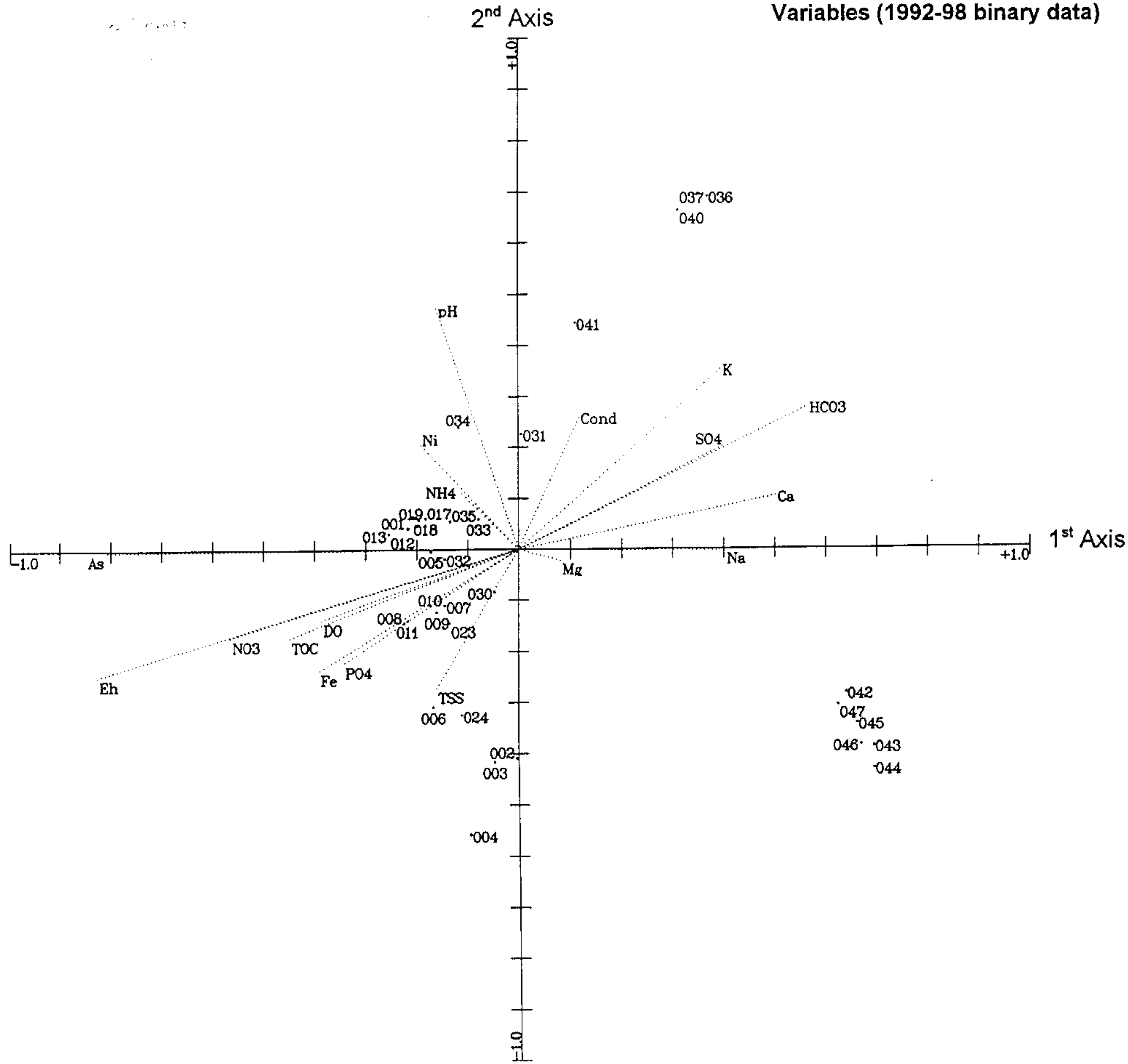
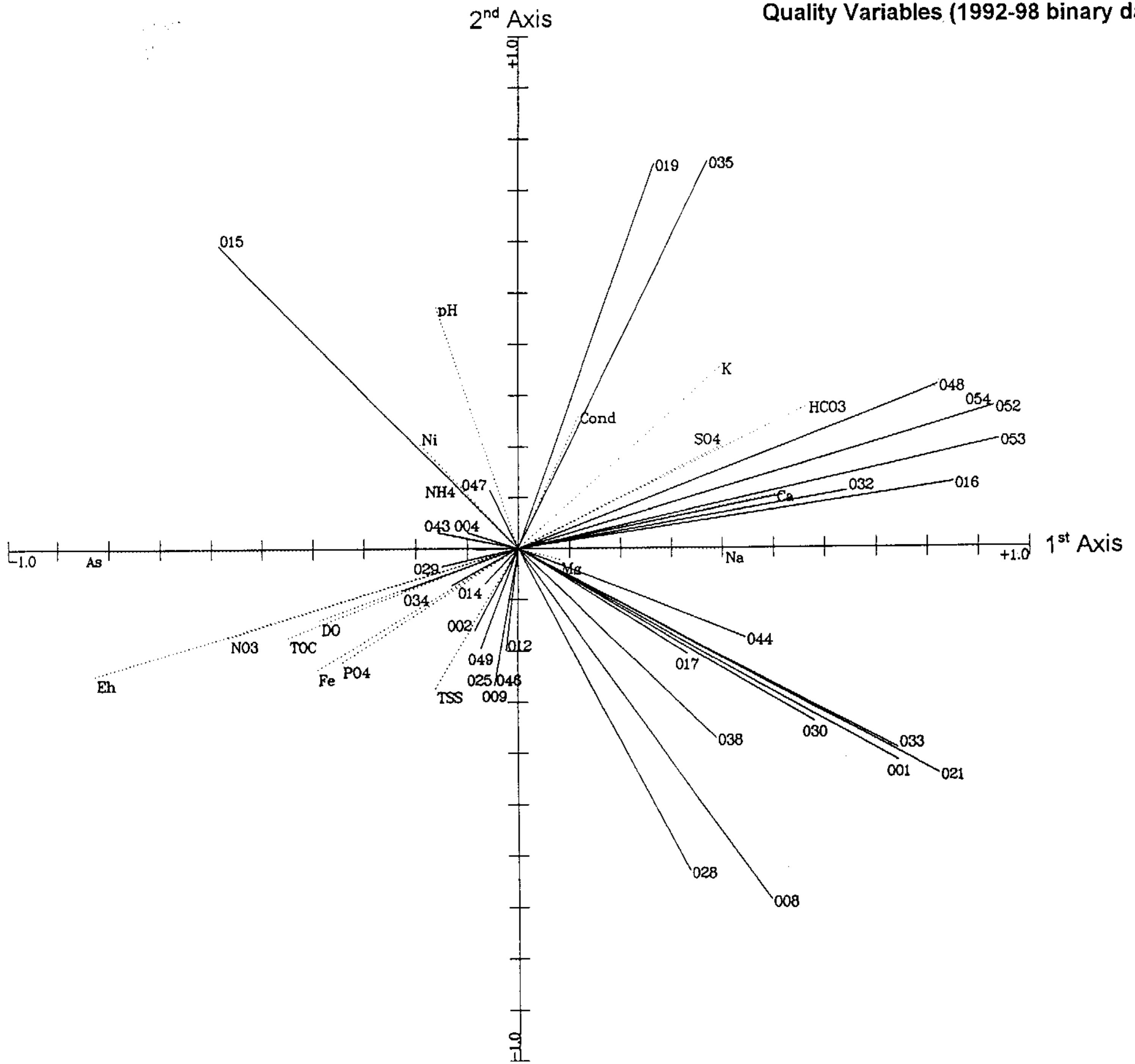


Figure 3.16. PCA Triplot of Phytoplankton Samples, Taxa and Water Quality Variables (1992-98 binary data)



PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 4

SOUTH BAY

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

4.0 Site Description

A former Zn and Cu mine in Northern Ontario, the South Bay mine is approximately 85 km north-east of Ear Falls (51° 08" N, 92° 40" E). The mine site is surrounded by Confederation Lake, part of the English River Basin.

The entire waste management area covers about 73 ha. It consists of several water bodies (Boomerang Lake, Mud Lake, Decant Pond and Mill Pond) and dry areas (tailings, pit, sand, gravel, mill and warehouse). Mining took place between 1971 and 1981, during which time approximately 1 million tonnes of tailings, containing 41% pyrite and 4% pyrrhotite, were generated. Water leaching through the mine sites and tailings gathers high levels of dissolved concentrations of metals (Zn, Cu, Mn, Fe), sulphate and acid. The majority of the wastewater discharges into the water systems within the area, mostly into Boomerang Lake, which has a maximum depth of about 5 m. Because of the continuous seepage and contaminated groundwater discharge, its water quality has deteriorated substantially in the past few years.

Since the mid-1980s, the process of decommissioning this mine has involved integrated ecological engineering approaches. Management measures have included:

1. phosphorus rock dispersion in Boomerang Lake to remove Fe and stimulate periphyton growth;
2. the introduction of acid-tolerant aquatic moss which, after five years, covers the lake bottom;
3. the addition of cut brush, which is providing surface area for periphytic algae.

4.1 Monitoring Program

4.1.1 Water Quality

The water quality and phytoplankton monitoring of Boomerang Lake and Confederation Lake was initiated by Boojum in 1986. Some water chemical data had also been collected by the mining company, BP Selco Inc., in earlier years. The original sampling design included 12 locations (B1-B12) for each of Boomerang Lake (Map 3) and Confederation Lake (C1-C12). It was soon noted, however, that this plan was neither financially feasible nor necessary, especially since Boomerang lake is so well-mixed. As a result, only three locations have been regularly sampled at Confederation Lake (C1, C8 and C11: see Map 3). C1 represents one of the inflows in Lost Bay from Boomerang Lake while C11 was assigned to monitor the outflow of the tailings drainage basin. C8 is well away from the shoreline in South Bay (about 500 m), generally representing the reference conditions of the study area. More sampling locations have been retained in Boomerang Lake, but B1 to B6 were sampled more frequently than the others.

Water samples were usually collected at quarterly intervals except in winter. Sampling was also less extensive in 1990, 1993 and1994 for financial reasons. Nevertheless, this sampling intensity well matches phytoplankton sampling. In both Boomerang and Confederation Lakes, water samples were collected from different depths on several occasions, but little difference was found with depth.

A total of 230 water samples were retrieved from the Boojum Paradox Database for Boomerang Lake. A total of 64 variables/elements were determined, but 35 of them were measured too infrequently to be included. A further 14 elements were removed as they were at very low concentrations or at below the detect limit. After the data refining, 15 elements remained and are summarised in Table 4.1.

Seventy-three samples were removed because they i) missed too many data (10), ii) were from unclear sampling locations (9), iii) were collected for specific tests (4), or iv) are redundant (27). Consequently, 157 samples were used for Boomerang Lake. Table 4.1 summarised the 15 variables/ elements retained for the 157 water quality samples. These samples were later pooled in order to create lake-composite samples when the water quality was found to vary little among locations (B1 and B6) compared to the long-term trends. In Confederation Lake, only the three most extensively sampled locations: C1, C8 and C11, were retained with a total of 111 samples available for these locations over 1986 -1998. Table 4.3 summarizes 15 major water quality variables pooled for the three locations in Confederation Lake.

4.1.2 Phytoplankton

Phytoplankton samples have been collected from Boomerang Lake since 1986, typically at several locations. The sample processing and taxa identification is as described in Section 2.2. The identification level adopted in this analysis is genus, with a number of exceptions where the tolerance and distribution of various species from the same genus are known to be different (*Euglena*, *Cryptomonas*, and *Lepocinclis*).

Quantitative samples were collected in some years (1986-1989, 1996, 1997), but for all other years samples were processed only for the presence-absence of taxa. In order to arrive at a complete understanding of the phytoplankton community changes in the lake, binary data were used. It should be noted that many studies have shown that binary data retains most information contained in the quantitative data (Gauch 1982). Binary data for the period of 1986 -1997 for a total of 57 samples were available for analysis (Table 4.4). From the table, it is clear that in many sampling events, only one or two samples were collected, while a few more samples from different locations were taken at other times. As all these samples represent the same lake and species number and species composition can be strongly influenced by sample size, taxon occurrence was averaged over all lake locations that were sampled at the same date. If the frequency is larger than 0.5, it was regarded as presence (1.0), and if <0.5, it was regarded as absence (0.0). This procedure eliminates subtle within-lake variation in phytoplankton community to evaluate temporal patterns. This data handling yielded 26 composite samples, which comprised a sampling date-based, binary data set.

4.2 Data Analysis

For Boomerang Lake, several sampling locations exist. If the within-lake variation in water quality is very small compared with the long-term changes, it would be preferable to pool the original location-based samples to obtain an composite sample, which exclude within-lake variation and reduce the "noise" to major gradient (s) or pattern (s).

Firstly, Principal Component Analysis (PCA) was applied to the 157 location-based samples to assess the spatial variation of water quality within the lake in relation to the long-term changes. As water chemistry was reasonably consistent within the Boomerang Lake sampling locations, the records from all locations of each sampling event were averaged. The data handling reduced the number of samples to 52 for the period from 1986 to 1997. All 52 composite samples were then subjected to a trend analysis, assessing May-June changes.

There are a variety of techniques available for time trend analysis, but they typically require regular sampling. A simple linear correlation, Pearson's Correlation, was used to quantify the time-trend of the water quality variables selected. PCA was also applied to the pooled Boomerang Lake sample to show the time trend of water quality. The three locations in the Confederation Lake are different in water quality, as C1 receives discharge from Boomerang Lake, and C11 is the collective drainage in which the tailings are contained and might occasionally be affected by discharge. However, it was not clear how water quality varies within Boomerang Lake. For Confederation Lake, 111 samples with 15 variables were analyzed using PCA. The data were then standardized to 0 mean and 1 variance before PCA was performed.

In order to relate phytoplankton composition changes to water quality, a water quality data set was drawn from the original 52 composite samples that matched the phytoplankton in sampling date. This reduced the number of phytoplankton samples to 20. The water quality data set included 14 variables, Al, Fe, Mn, Pb, Cd, Cu, Ni, Total-P, Zn, Ca, SO_4, Mg, pH and conductivity. In this set of data, standard PCA was performed. The genera - water quality relationships were examined using more complex multivariate approach, CCA, which is a constrained direct ordination technique and better indicates the responses of phytoplankton to particular water quality variables.

4.3 Results

4.3.1 Water Quality Changes in Boomerang Lake

Within-lake variation of water quality: PCA ordination of 157 samples on the first two axes is show in Figure 4.1. The 1st axis explains 45.4% of the total variance, and together with the 2nd axis, accounts for 77%. All variables except Fe weigh heavily on the 1st axis, and fewer weigh on the 2nd axis.

Sample code in the Figure 4.1 are configured as follows: from the right to the left, the first two digits represent the sampling month (01-12), the next two digits are the year (86-98), and the last digit or two is combined with 'B' to indicate the location (B1-B13, and BB). For example, B49203 means a sample collected from B4 in March 1992. In the plot, locations were differentiated using symbols.

It is evident that no location-based clusters are visible in Figure 4.1, and instead, all the locations are well mixed. It therefore indicates that water quality variation within the lake is not significant, when compared with the major gradient-time series. Those are dominating in the 1st axis, explaining 45.4% of the variance in the data set. The majority of post-1992 samples are located up-right corner with 1996-98 samples at the end, while pre-1992 samples are distributed on the bottom-left corner. This distribution was further analysed by time-trend analysis.

Time-Trend Analysis: All variables except Ni, and Pb, are significantly correlated with time, which is given as weekly intervals since the first collection date (Tables 4.3) (Pearson correlation, $P<0.05$). Figures 4.2 a-m illustrate the details of the temporal changes in water quality parameters. The fitted curves indicate the overall trends in the parameters. Correlation coefficients are given in Table 4.2.

Al, Zn, and Cu showed very similar pattern (Figures 4.2a, g, and h), i.e., the concentrations were relatively stable before 1990, but have rapidly increased since that time. The behaviours of Pb, Cd and Ni are similar to each other in that the concentrations were relatively high in the beginning (approximately 1986-88), dropped in the following two or three years, but since1992, the concentrations have rapidly increased (Figures 4.2e, f, and j). Fe, Ca, Mg, and SO_4 steadily increased since approximately 1992 (Figures 2b, d, I, k). Magnesium changed in differently to the other elements in that the increase in concentration occurring in the early years is more obvious than later on (Figure 4.2c). As most ions increased, conductivity showed a rapid, almost linear increase (Figure 4.2o). In contrast, the pH rapidly decreased from above 4.0 to around 3.0 (Figure 4.2m). Acidity was only measured in the later years. The data for the past 5 years however showed a rapid increase (Figure 4.2n). Total-P dropped over the period, but it has remained at fairly steady, low levels in the past five years (Figure 4.2l). A similar trend to Phosphate was observed in Pb (Figure 4.2e).

From the above trend analysis, it is expected that most of the variables were co-ordinated to varying degrees. Table 4.2a and 4.2b present the correlation coefficients and the corresponding probabilities for the correlation. All metals except Pb and Ni, conductivity, and SO_4 are positively and significantly correlated with each other, and negatively and significantly correlated with pH. Total-P is negatively correlated with most metals. Nickel did not show any correlation with any other variables.

Multivariate analysis: The data set was further analysed using PCA. Two major water quality variables, pH and conductivity were also included. As shown in Table 4.2, these two variables have strong correlation with time. A regression equation was used to estimate the pH and conductivity levels that were missing in the original data set. As a result, 14 variables were used in the following PCA analysis. All other data processing was identical as described earlier.

PCA ordination of the 52 samples on the first two axes is shown in Figure 4.3. The 1st axis explains 52.7% of the total variance, which is higher than in the location-based data set (45.4%) while the 2nd axis accounts for 13.6%, compared with 34% in Figure 4.1. These results can be attributed to the data pooling, which reduces the'noise' level.

The sample codes used here are self-explanatory. It was noted that almost all post-1992 samples were located to the right of the origin with 1997 samples on the farthest right. All pre-1993 samples except one (September, 1991) are to the left of the origin. This pattern clearly indicates, again, a time-trend. Figure 4.4 showed the relationship between the two axes and the 14 water quality variables. Zn, Al, SO_4, conductivity (F-Cond), and field pH (F-pH) weigh most heavily on the 1st axis, followed by Ca, Mg, Cu, Cd, Mn (not shown) and Fe. Pb, P and Ni are related to the 2nd axis, but only a few samples are well dispersed along this axis and, therefore, these elements are of less importance.

It is evident that all metals except Ni and Pb increase over time and the changes in Zn, Al, Ca and Mg are particularly significant from a statistical standpoint. This outcome supports the results of the time-trend analysis, described earlier. Furthermore, water quality appears to deteriorate rapidly after 1992. We used Average Euclidean Distance and Ward Linkage in a cluster analysis (Systat 8.0). After the data were standardised to 0 mean and

1 variance, the 52 samples clustered into two groups (Table 4.6), with most post-1992 samples separated from the pre-1992 samples (Figure 4.5). This demonstrates that water quality was considerable different before and after that year. This significant separation of the data set is explained by the construction of a diversion ditch, which increased the contaminant loading to Boomerang Lake.

4.3.2 Water Quality Changes in Confederation Lake

Table 4.3 summarises 14 major water quality variables, Al, Ca, Cd, Cu, Fe, Mn, Ni, Pb, Zn, SO_4, Total-P, pH, acidity and conductivity. Of 111 samples, 39 were collected from C1, and 36 from each of C11 and C8. As with Boomerang Lake samples, data were standardised to 0 mean and 1 variance, then non-centred PCA was performed using CANOCO.

The PCA ordination of the 111 samples showed that two samples, collected from C8 and C1 in August 1989 (C88908, C18908), were located far from all the other. These were removed, as "outliers", so that potential patterns within the majority of samples could be better described and quantified. PCA was run again using the 109 retained samples. The ordination on the first two axes is shown in Figure 4.6. The 1st axis explains over half of the total variance, 56.9%, and together with the 2nd axis, it comes up to 66.8%, indicating the PCA solution represents the data exceptionally well.

Most variables weigh heavily on the 1st axis, particularly Zn, Mn, Mg, SO_4 (S), Al, acidity, conductivity (F-Cond), Ca, and pH (F-pH) (Figure 4.7). In other words, the water quality changes are associated mainly with these variables. Ni and Pb contributed to the 2nd axis, which accounts only for a small proportion of the total variance. All C8 samples, labeled by a star, are assigned negative scores on the 1st axis (i.e., they lay to the left side of the origin), indicating that the water quality variables examined in the analysis changed little at C8 during the period (1986-97). Many C1 samples are dispersed on the right section of the 1st axis, which is associated with higher Zn, Al, Acidity, Cu and some other ions. This suggests that the water quality at C1 was relatively poor compared to the rest of the lake. This location receives the discharge from Boomerang Lake. Some C1 samples were well mixed with C11 and C8 samples, suggesting that the water quality at C1 varied considerably, but comparable to C11 and C8. The overlap between C11 and C1 is larger than between C8 and C1, indicating that the water quality at these two locations was similar in many sampling occasions.

4.3.3 Phytoplankton Changes in Relation to Water Quality

An examination of the frequency of genus/taxa occurring in the 26 composite samples determines the dominant genera/taxa in the lake. Then, changes in genera/taxa richness over time were plotted. A total of 60 genera/taxa were recorded in the 26 composite samples. The true number was thought to be higher, because some rare genera were eliminated by averaging..

Table 4.5 listed the frequencies of occurrence (% of 26 samples) of all 60 genera/ taxa. *Ochromonas, Chlamydomonas, Staurastrum, Pinnularia, Ulothrix, Eunotia* and a type of unidentified green algae were recorded in more than 50% of the samples, and they were the sole or co-dominant of the lake phytoplankton community at least at a certain times. *Cryptomonas, Euglena, Sphaerellopsis, Navicula, Nitzschia, Peridinium, Chromulina, Euglena, Synedra,* and *Lepocinclis* were also common at different time. Most other genera/taxa occurred only sporadically.

Figure 4.8 shows the changes in the genera/taxa numbers over the period of 1986-97. No clear time-trend is apparent, probably because the seasonal variation was included here. From 1986 to 89, the richness decreased markedly, but relatively high numbers were observed in 1991 and 1992. However, it was noted that the richness was very much reduced in 1997. The data for 1998, which became available after multivariate analysis was completed and was not integrated into this analysis, shows low taxa richness as well, 4 genera/taxa per sample on average.

Multivariate Analysis: The PCA plot of 20 composite samples is shown in Figure 4.8. The first two axes account for 33% of the total variance, with the 1st axis alone explaining 20%. The samples are distributed along the 1st axis more or less in the order of sampling year, i.e., the earlier samples on the right and the later samples on the left. The recent samples (1996-1997) are located at the upper-left corner. Clearly, then the 1st axis represents a time-trend. Figure 4.9 shows the ordination of genera/taxa, reflecting the association between samples and phytoplankton occurrences. More taxa are associated with 1986-87 samples, which agrees with the higher taxon richness at that time, since then the taxon composition changed significantly. Many genera that were common in late 1980s disappeared in the past few years, but some "new" genera appeared.

Figure 4.10, a tri-plot of all the 20 samples, 14 water quality variables and 60 genera/ taxa, better illustrates their relationships. A number of genera, such as *Lepocinclis*, *Staurastrum*, and some unidentified taxa are strongly associated with increased Zn, Al, Cu, conductivity, and decreased pH. *Ochromonas* and *Ulothrix* remained common throughout the period.

The response of phytoplankton to particular water quality variables were further examined using CCA (Figure 4.11, 4.12). The ordination of samples follows a pattern similar to what was observed in PCA (Figure 4.8), i.e., samples dispersed along the 1st axis to form a time series. Pre-1990 samples are on the left side while post-1990 samples are distributed on the right. Furthermore, the post-1990 samples distributed more widely, indicating rapid changes in community composition.

The multivariate analyses performed on different data sets in this section are summarised below:

Data Set	Sample No & Type	System	Data Type	Method	Figure
Water Quality (WQ)	157 location-based	Boomerang Lake	Numeric (N)	PCA	4.1
Water Quality	52 composite	Boomerang Lake	Numeric	PCA	4.3-4.4
Water Quality	52 composite	Boomerang Lake	Numeric	Cluster	4.5
Water Quality	109 location-based	Confederation Lake	Numeric	PCA	4.6-4.7
Phytoplankton + WQ	20 composite	Boomerang Lake	Binary + N	CCA	4.8-4.12

4.4 Discussion

Both the time-trend and multivariate analyses show that Boomerang Lake experienced rapid and significant changes in water quality. This trend appears to be particularly marked in the past few years. In contrast, the reference site - Confederation Lake, remained stable with good water quality, with C8 being most stable and the largest variation seen in C1, the outflow of Boomerang Lake.

Although most variables changed markedly in Boomerang Lake, these changes are not equally important for the lake environment and ecosystem. It is necessary to consider the biology/toxicology of the variables and their ranges. Zinc is no doubt one key variable because it showed high concentration and a strong trend towards increase, from <10mg/L in 1986-90 to approximately 30mg/L in 1997-98. pH value, which has dropped from >4.0 to close to 3.0, is probably equally important since it strongly influences the toxicity of metal contaminants and many physical-chemical processes in water, such as CO_2 dynamics and NH_4 - NH_3 balance. It can influence the all geobiochemical processes in the lake. Al, which increased from <1 mg/L to >5.0 mg/L is another major contaminant in the lake. The toxicity of Al is strongly dependent on pH, and this metal probably, together with Zn and low pH, has imposed a major restriction on the biological system.

Two other metals, Cu and Cd, are almost equally significant in statistical terms, exhibiting strong time-trends, but their concentrations were much lower, <0.5mg/L Cu, and <0.05mg/L Cd. Ca, Mg, and Mn also increased markedly, but these metals are at much lower levels, and their increases should not be regarded as major water quality problems. Ni and Pb did not show an equally significant increase, and their concentrations also close to those of Cd and Cu.

The rapid deterioration of water quality after 1992, which is clear from both PCA ordination and cluster analysis, can be attributed to the management activities. During Nov. 1992 to Jan. 1993, a major ditch was constructed to collect the seepage from the mine site and lead it to Boomerang Lake. This action was taken to protect C8 in Confederation Lake. The seepage water contained high levels of Fe, Zn and other metals, the diversion of which significantly influenced Boomerang Lake.

Since water quality has decreased continuously since 1992, it appears clear that the contaminant loading has exceeded the capacity of the Boomerang Lake biological polishing system. Boojum Research has undertaken a series of management interventions to increase the functioning of the systems, including the dispersion of phosphorus rock (1994-95) to enhance Fe precipitation and to stimulate phytoplankton growth; transplantation of moss into the lake to promote biological polishing; and the addition of NO_3 (1997) for increasing productivity. The effectiveness of these measures is still under assessment, but it is evident that the deterioration of water quality in the lake has not yet been stopped. If no effective operation is taken, the water quality of the lake can be expected to deteriorate further.

As the part of this project, however, Boojum Research has designed a whole lake-scaled treatment to increase the pH and reduce the metal loading, which is expected to change the water chemistry substantially and boost the biological system of the lake.

The changes in phytoplankton community composition over the period 1986-97 are marked, but as noted earlier, some taxa were consistently recorded in the acidified lake. From the more restricted quantitative data in terms of time, those taxa were also present in large number. Because the concentration of the major contaminants, Zn and Al, remained at high level or even further increased after 1992, all phytoplankton taxa that were frequently recorded in the lake, e.g., occurring in >40% samples (see Table 4.5), can be regarded as highly tolerant "species", although their tolerances are not the same.

It was noted that some of those genera, *Chlamydomonas, Cryptomonas, Sphaerellopsis, Peridinium, Pinnularia,* and *Nitzschia*, have been found to be common in B-Zone pit (Appendix 3). However, these genera are also common in most natural waters. This means that they are tolerant to a wide range of environment, not specified to particular contamination. However, several genera, such as *Ochromonas, Staurastrum, Euglena,* and *Lepocinclis*, are specific for Boomerang Lake, suggesting that they be particularly tolerant to the very low pH and high Zn.

Both the PCA and the CCA analyses indicated that the changes in the lake water quality explain the change in phytoplankton composition. It appears that pH and Zn concentration particularly relate to the phytoplankton changes. For the pH manipulation mentioned earlier, Boojum has designed a sampling program to monitor the change in phytoplankton community before and after the operation.

4.5 Field Experimentation of Controlling Factors

It is generally believed that low pH depress phytoplankton communities and primary productivity, and the above analysis also showed that it is the most important water quality variable explaining the change in the phytoplankton community in the lake. Its effects on aquatic ecosystems have also extensively examined in the context of acid rain. In Boomerang Lake, the low pH also resulted in the elevation of metals. The combined effects of low pH and increased metal levels on phytoplankton are thus even more complex.

From the experimental liming programs in many lakes, it became clear that sustainable solutions to counteract acidification need to consider not only the chemical water characteristics, but also the aquatic communities in the water body. In the case of aquatic systems impacted by mining effluents, the neutralisation of water does not only require the consumption of hydrogen ions, but hydroxyls are consumed by metals in water column, which produces sludge. Metal hydroxide sludge has a high affinity for particles and hence would remove almost all phytoplankton cells at the same time and, therefore, the primary production. In addition, neutralisation by lime, limestone, or any other forms of hydroxyls used (sodium hydroxide) is a very fast reaction, and thus this increases pH too rapidly. Ecological engineering for mining-impacted habitats requires restoring the ecosystem using the minimum intervention, rather than altering the system so quickly that no new system can not develop to replace the present functioning, low-pH tolerant system. Neutralisations by hydroxyl additions are therefore not applicable. An alternative method to increase pH and reduce acidity in the water bodies receiving mining effluents is being field tested.

4.5.1 Selecting a site for Field Test

Boomerang Lake was chosen for the field test of pH increase for two reasons: it is a very acidified lake, and its water quality and acidity sources have been well documented. This would allow to design most effective way to increase water pH and examine the consequence of the test.

Boomerang Lake has two major inputs of contaminants. One continuous and long-term source is the flooded abandoned underground workings, which discharges as small seeps from the Backfill Raises, and other former mine workings. The other one is the overburden materials, which are saturated with concentrate from the concentrator and the concentrate storage pads on the Mill side. These contaminant loads are severe since Cu and Zn concentrate during milling and storing have started to oxidise and produce seepage with high level of Zn, e.g., 1 g/L. The seepages oxidise as they merge from the overburden on the mine site and the underground workings, so that redox changes take place in the drainage towards Boomerang Lake, but not in the lake. Redox changes are destructive to phytoplankton and periphyton. Two enclosures have been constructed in October 1998 as part of the project, around the discharge point of the seepage (Backfill Bay, and Mill Pond Runoff Bay) to separate the main lake from the seepage inflow. Polyvinyl Chloride (PVC) sheets were used to provide a lining for the enclosures, which were suspended from log booms anchoring into the sediment by weight. Water is expected to leave the enclosures through the openings between the PVC sheets, which were overlapped, but the seams of the linings were not sealed. The main purpose of the enclosure is to prevent mixing Boomerang Lake water from entering the enclosures.

4.5.2 Removal of Acidity and Hydrogen Ions through Cementation

It is known that cementation processes consume hydroxyls and produce hydrogen gas. However, for wastewater treatment, these processes are generally considered to be too costly. This is clearly the case if the treatment would be required as a long-term option. Here the objective is to reduce the contamination load to the lake for a limited period for increasing the water pH and boosting the phytoplankton production. Extensive experiments have been performed in the laboratory and field tests are currently being conducted. Results of the cementation experiments in the laboratory are represented here. Since many of the parameters, such as flow condition, can not be tested realistically in the laboratory. The cementation test of the water of the enclosures is being carried out in March 1999, which will provide the relevant field-scale up data. Those results along with the effects of phytoplankton and periphyton will be submitted as an addendum report.

Figure 4.13a-b shows the change of pH and conductivity over time in series of cementation experiments using 1 litre Boomerang Lake water at metal surface/water volume ratios of approximately 6.0. There are two factors considered here, stagnant/stirring, and temperature. It is evident that pH 5.0 can be reached quickly, particularly in the stirring condition. To obtain more process data, Boojum is conducting tests in addition to the enclosure tests, using a series of 1 m^3 containers to assess the control parameters for the cementation process. The cementation process will be applied to the two enclosures in April, and will provide information at the time of ice break-up, when a spring algal bloom usually comes.

4.5.3 Expected Changes in Water Quality and Phytoplankton

A literature review has been carried out regarding the possible effects of raising pH by 2 units (from current 3.0 to 5.0). The implication of low pH and water chemistry related to algal growth was to be defined. One consequence of low pH is the decrease of Dissolved Inorganic Carbon (DIC) in water, which algal growth needs. In general, the DIC concentration remains at about 10 μM until pH reaches 5.0. Other variables, such as temperature and buffering capacity of lake water can influence DIC as well. Stumm and Morgan (1981) give a general view of the carbonate system. Titus et al. (1990) used the equation of Stumm and Morgan to calculate the "theoretical" equilibrium DIC concentration for 20 US acid lakes. They reported DIC concentrations ranging from 10 -17μM at pH 6.0 in the lakes. The DIC observed was lower than expected in 6% of the lake, but as much as 5 times higher than expected in 16% lakes. In other 76% lakes, the observed DIC was also well above the theoretical equilibrium level. They attributed the differences to the DIC release from sediments.

Decreasing pH levels in Boomerang Lake were halted in 1995 by adding phosphate rock waste materials to the lake sediment. Since then, moss has established well enough to cover much of the lake bottom, but the amount of DIC that this organic matter produces is difficult to estimate. By increasing pH to 5.0, periphyton should be enhanced and further contribute to the DIC in the water column.

The increased pH would reduce the toxicity of most metals to aquatic life and enhance their precipitation. As more phytoplankton species/taxa were collected when the pH was close to 5.0 in middle 1980s, considerable recovery of the phytoplankton community can be expected. Molot et al. (1990) noted the great change in species composition after neutralisation in some Sudbury lakes.

On the other hand, the effects of neutralisation on algal primary productivity appear to be limited (Molot et al. 1990). Turner et al. (1995) correlated photosynthesis rates of benthic algae with pH in Experimental Lake Area (ELA), and noted that net photosynthesis rate increased to some extent as pH increased up to 6.0, but the largest gain occurred between pH 6.0 - 7.0. A further consequence of the pH manipulation is to increase the buffering capacity of the lake. As pH increases, more carbon species will be found in the lake water, which will help in maintaining pH and acidity.

In summary, the cementation experiment in Boomerang Lake is expected to both improve water quality and reduce the toxicity of Zn to aquatic life, and to increase diversity in phytoplankton composition. While the literature review does not suggest a great increase in phytoplankton primary production, an increase in periphyton might be expected. The experiments should also provide important information on the interactions among pH, algal species composition, biomass, and other water quality variables.

Table 4.1. Summary of 15 major water quality variables in Boomerang Lake (1986-97).

	Al (mg/l)	Ca (mg/l)	Cd (mg/l)	Cu (mg/l)	Fe (mg/l)	Mg (mg/l)	Mn (mg/l)	Ni (mg/l)	Total-P (mg/l)	Pb (mg/l)	SO_4 (mg/l)	Zn (mg/l)	pH	Cond. (um/s)	Acidity (mg/l)
No.	156	148	157	157	157	147	157	157	136	155	157	156	122	113	62
Max	6.00	157.00	1.00	1.50	53.30	30.50	19.04	1.00	3.20	1.00	248.0	49.71	5.42	942	219.0
Min	0.010	8.000	0.004	0.005	0.010	1.200	0.500	0.005	0.005	0.003	10.2	0.750	3.040	140	22.0
Average	1.570	60.478	0.106	0.226	2.175	11.106	5.158	0.109	0.267	0.097	79.9	10.527	3.928	453	91.9
StDev	1.357	19.343	0.271	0.281	5.496	3.786	2.147	0.271	0.485	0.257	28.9	6.494	0.530	172	38.8
Median	0.900	57.750	0.011	0.130	1.200	10.200	5.500	0.020	0.060	0.020	75.0	8.325	3.960	390	87.2

T1

Table 4.2. Pearson's Correlation Coefficients and the probabilities for 14 major water quality variables and time (weeks from the first sampling) in Boomerang Lake (1986-1998), based on the over-lake averages (N = 49).

	Correlation Coefficients														
	Weeks	Cond.	Al	Fe	Mn	Ca	SO_4	Pb	Cd	Cu	Ni	Total-P	Zn	Mg	pH
Weeks	1.000														
Cond	0.950	1.000													
Al	0.885	0.873	1.000												
Fe	0.513	0.573	0.542	1.000											
Mn	0.630	0.588	0.665	0.376	1.000										
Ca	0.784	0.780	0.802	0.422	0.698	1.000									
SO_4	0.854	0.859	0.880	0.586	0.718	0.902	1.000								
Pb	-0.227	-0.227	-0.189	-0.352	-0.044	-0.061	-0.070	1.000							
Cd	0.642	0.642	0.686	0.488	0.369	0.705	0.743	-0.024	1.000						
Cu	0.713	0.728	0.739	0.551	0.291	0.696	0.731	-0.292	0.819	1.000					
Ni	-0.043	-0.043	0.088	0.106	0.002	0.064	0.114	0.147	0.035	0.057	1.000				
Total-P	-0.383	-0.436	-0.342	-0.316	-0.171	-0.225	-0.268	0.475	-0.031	-0.261	0.074	1.000			
Zn	0.877	0.890	0.868	0.540	0.517	0.868	0.873	-0.212	0.825	0.853	-0.029	-0.316	1.000		
Mg	0.772	0.789	0.844	0.460	0.784	0.922	0.917	0.026	0.668	0.623	0.138	-0.237	0.839	1.000	
pH	-0.841	-0.824	-0.770	-0.459	-0.629	-0.585	-0.682	0.305	-0.336	-0.508	0.003	0.448	-0.638	-0.632	1.000

	Probabilities														
Weeks	0.000														
Cond	0.000	0.000													
Al	0.000	0.000	0.000												
Fe	0.000	0.000	0.000	0.000											
Mn	0.000	0.000	0.000	0.008	0.000										
Ca	0.000	0.000	0.000	0.003	0.000	0.000									
SO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
Pb	0.117	0.117	0.193	0.013	0.764	0.677	0.635	0.000							
Cd	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.868	0.000						
Cu	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.042	0.000	0.000					
Ni	0.768	0.771	0.549	0.468	0.991	0.660	0.435	0.315	0.814	0.700	0.000				
Total-P	0.007	0.002	0.016	0.027	0.239	0.120	0.062	0.001	0.834	0.070	0.613	0.000			
Zn	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.144	0.000	0.000	0.844	0.027	0.000		
Mg	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.861	0.000	0.000	0.344	0.100	0.000	0.000	
pH	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.033	0.018	0.000	0.985	0.001	0.000	0.000	0.000

Table 4.3. Summary of 15 major water quality variables at C1, C8 and C11 in Confederation Lake (1986-97).

	Al (mg/l)	Ca (mg/l)	Cd (mg/l)	Cu (mg/l)	Fe (mg/l)	Mg (mg/l)	Mn (mg/l)	Ni (mg/l)	P (mg/l)	Pb (mg/l)	SO4 (mg/l)	Zn (mg/l)	pH	Cond (um/s)	Acidity (mg/l)
No.	111	95	111	111	111	95	111	111	90	108	105	111	92	76	58
Max	3.770	97.800	0.300	0.210	8.000	15.700	7.530	0.100	1.800	0.410	112.000	20.100	8.050	613.000	117.500
Min	0.005	1.284	0.000	0.001	0.010	0.090	0.005	0.001	0.005	0.001	0.010	0.005	3.260	25.000	0.7000
Average	0.243	18.404	0.015	0.024	0.398	2.744	0.789	0.017	0.103	0.023	14.704	1.421	6.446	123.580	15.044
StDev	0.595	17.832	0.041	0.039	1.034	3.078	1.667	0.020	0.228	0.041	23.935	3.516	0.772	112.134	22.965
Median	0.050	11.000	0.005	0.010	0.070	1.600	0.080	0.010	0.060	0.020	4.410	0.200	6.570	80.000	5.950

Table 4.4. Summary of binary phytoplankton data for Boomerang Lake (1986-97)

No.	Sampling Date	Location	Taxa	No.	Sampling Date	Location	Taxa
1	17-Aug-86	B1	16	32	21-Aug-88	B11	16
2	17-Aug-86	B3	18	33	14-May-89	B1	8
3	17-Aug-86	B5	23	34	14-May-89	B4	14
4	15-Oct-86	B3	17	35	14-May-89	B5	8
5	15-Oct-86	B5	18	36	24-Aug-89	B1	6
				37	24-Aug-89	B5	8
6	30-Mar-87	B1	11				
7	30-Mar-87	B4	12	38	13-Oct-90	B4	7
8	25-Apr-87	B3	12				
9	25-Apr-87	B6	7	39	24-Jun-91	B4	18
10	31-May-87	B3	14				
11	31-May-87	B5	16	40	13-Jul-92	B2	11
12	16-Jul-87	B6	21	41	13-Jul-92	B4	15
13	13-Aug-87	B1	7	42	17-Oct-92	B8	6
14	17-Aug-87	B4	22				
15	13-Aug-87	B6	17	43	29-Aug-94	B4	9
16	5-Oct-87	B1	12				
17	5-Oct-87	B3	13	44	11-May-95	B4_S	5
18	5-Oct-87	B6	9	45	11-May-95	B4_B	5
19	5-Oct-87	B11	10	46	11-May-95	B5	6
				47	11-May-95	B5_S	5
20	7-Apr-88	B3	5	48	11-May-95	B5_B	6
21	7-Apr-88	B7	17	49	15-Aug-95	B8	11
22	7-Apr-88	B10	16	50	18-Aug-95	B8	10
23	23-May-88	B1	12				
24	23-May-88	B3	16	51	09-Feb-96	B1	9
25	23-May-88	B5	13	52	23-Feb-96	B4	8
26	14-Jun-88	B3	7	53	29-Mar-96	B4	8
27	14-Jun-88	B5	6	54	09-May-96	B9	4
28	21-Aug-88	B2	6.5	55	28-Jun-96	B5	6
29	21-Aug-88	B3	20				
30	21-Aug-88	B5	23	56	20-Jun-97	B3	3
31	21-Aug-88	B8	12	57	20-Jun-97	B4	5

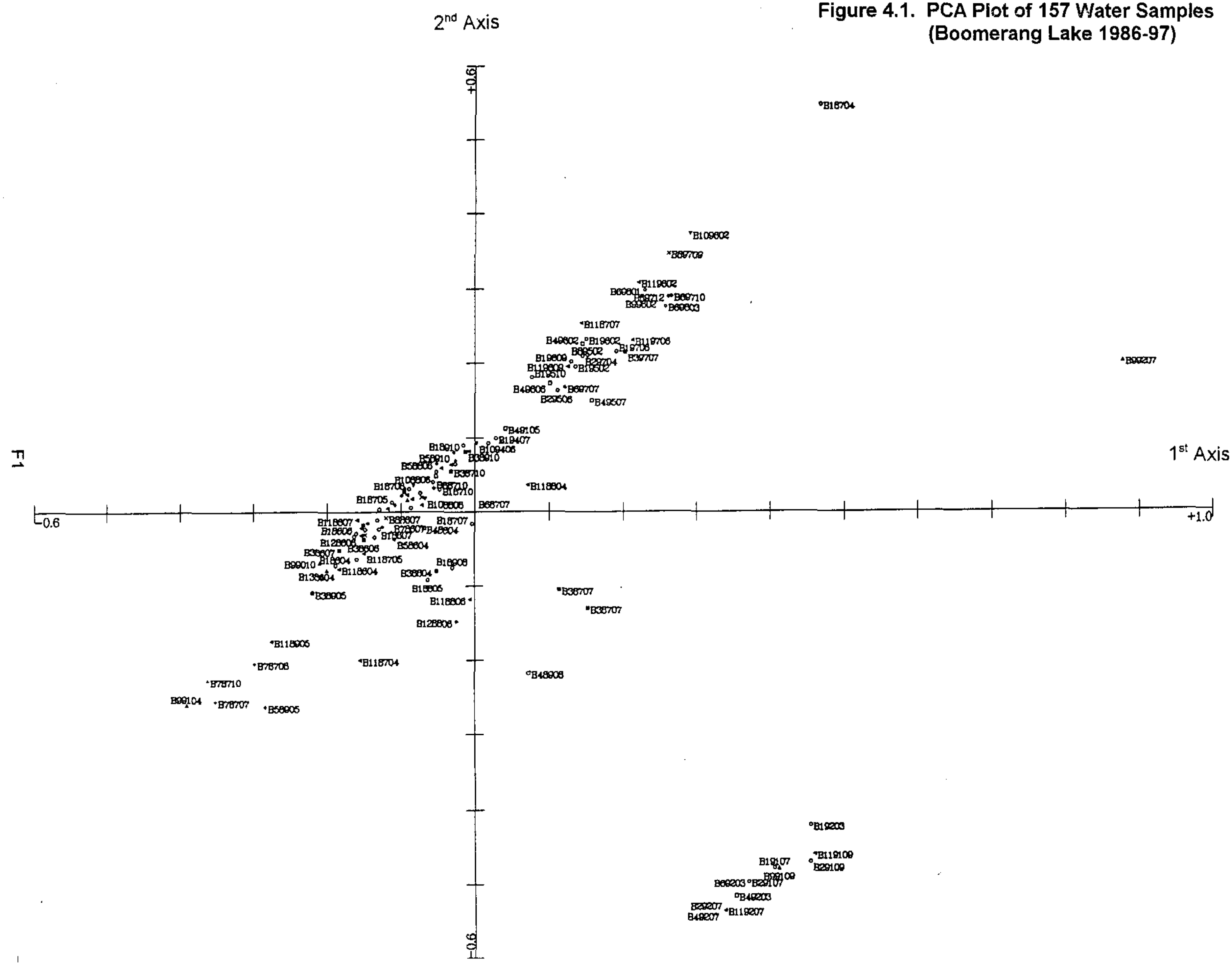
Table 4.5. Occurrence Frequencies of 60 genera/taxa in Boomerang Lake (1986-97)

No.	Frequency %	Genera/taxa	No.	Frequency %	Genera/taxa
1	65.38	*Ochromonas*	30	11.54	*Euglena*
2	61.54	*Chlamydomonas*	31	11.54	*Gymnodinium*
3	57.69	*Staurastrum*	32	11.54	*Oosystis*
4	57.69	Unidentified	33	11.54	*Stauroneis*
		Chlorophyceae-A	34	11.54	*Trachelomonas*
5	53.85	*Pinnularia*	35	11.54	Unidentified
6	53.85	*Ulothrix*			Cyanobacteria
7	50.00	*Eunotia*	36	7.69	*Ankistrodesmus*
8	42.31	*Cryptomonas*	37	7.69	*Cosmarium*
9	42.31	*Euglena*	38	7.69	*Dictyospaherium*
10	42.31	*Sphaerellopsis*	39	7.69	*Glenodinium*
11	38.46	*Navicula*	40	7.69	*Gleoecystis*
12	38.46	*Nitzschia*	41	7.69	*Oscillatoria*
13	38.46	*Peridinium*	42	7.69	*Rhodomonas*
14	34.62	*Chromulina*	43	7.69	*Rhopalodia*
15	34.62	*Euglena*	44	3.85	*Chlorocloster*
16	34.62	*Synedra*	45	3.85	*Chroococcus*
17	30.77	*Lepocinclis*	46	3.85	*Chroomonas*
18	30.77	unidentified	47	3.85	*Cyclotella*
		Chrysophyceae	48	3.85	*Cymbella*
19	30.77	Unidentified Diatom	49	3.85	*Diploneis*
20	26.92	*Fragilaria*	50	3.85	*Epithemia*
21	26.92	*Melosira*	51	3.85	*Kephryon*
22	26.92	*Tabellaria*	52	3.85	*Mallomonas*
23	23.08	*Chlorella*	53	3.85	*Neidium*
24	23.08	*Mougeotia*	54	3.85	*Netrum*
25	19.23	unid-Chrys-1	55	3.85	*Rhizosolenia*
26	15.38	*Asterionella*	56	3.85	*Roya*
27	15.38	Unidentified	57	3.85	*Surirella*
		Cryptophyceae	58	3.85	*Synura*
28	15.38	Unidentifed	59	3.85	Unidentified
		Chlorophyceae-B			Chrysophyceae
29	11.54	*Dinobryon*	60	3.85	*Zygnema*

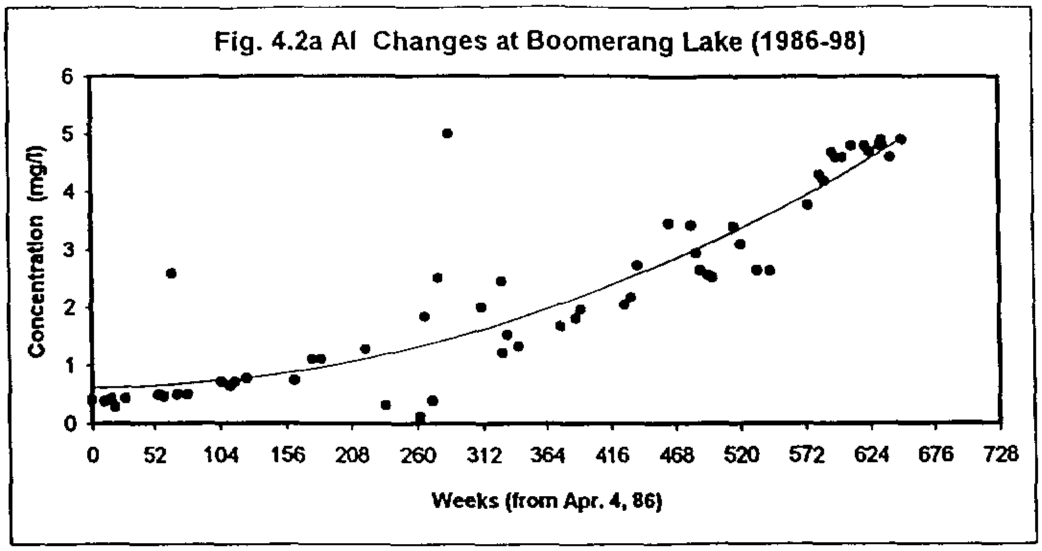
Table 4.6. Sample codes for cluster analysis (Figure 4.5).

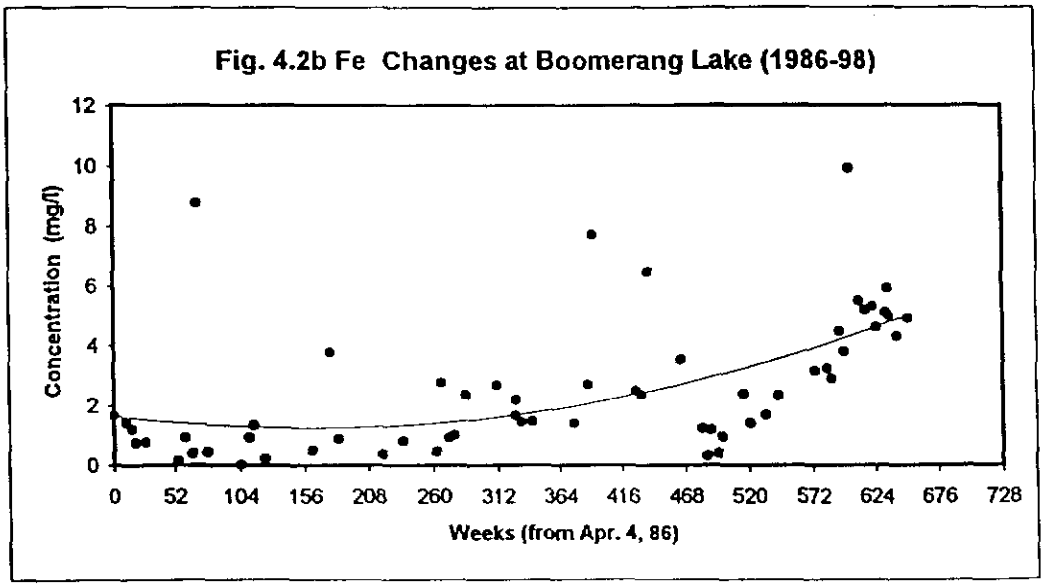
Samples - codes				
Sampling Date	Case		Dates	Case
05-Apr-86	1		18-Jul-92	27
17-Jun-86	2		14-Aug-92	28
25-Jul-86	3		17-Oct-92	29
17-Aug-86	4		17-Jun-93	30
15-Oct-86	5		11-Sep-93	31
27-Apr-87	6		10-Oct-93	32
31-May-87	7		17-Jun-94	33
16-Jul-87	8		19-Jul-94	34
13-Aug-87	9		29-Aug-94	35
06-Oct-87	10		26-Feb-95	36
08-Apr-88	11		29-Jun-95	37
23-May-88	12		27-Jul-95	38
19-Jun-88	13		15-Aug-95	39
23-Aug-88	14		28-Sep-95	40
14-May-89	15		20-Oct-95	41
25-Aug-89	16		23-Feb-96	42
14-Oct-89	17		29-Mar-96	43
23-Jun-90	18		28-Jun-96	44
13-Oct-90	19		09-Sep-96	45
16-Apr-91	20		06-Apr-97	46
16-May-91	21		16-Jun-97	47
25-Jun-91	22		09-Jul-97	48
26-Jul-91	23		25-Aug-97	49
28-Sep-91	24		17-Sep-97	50
24-Mar-92	25		21-Oct-97	51
13-Jul-92	26		16-Dec-97	52

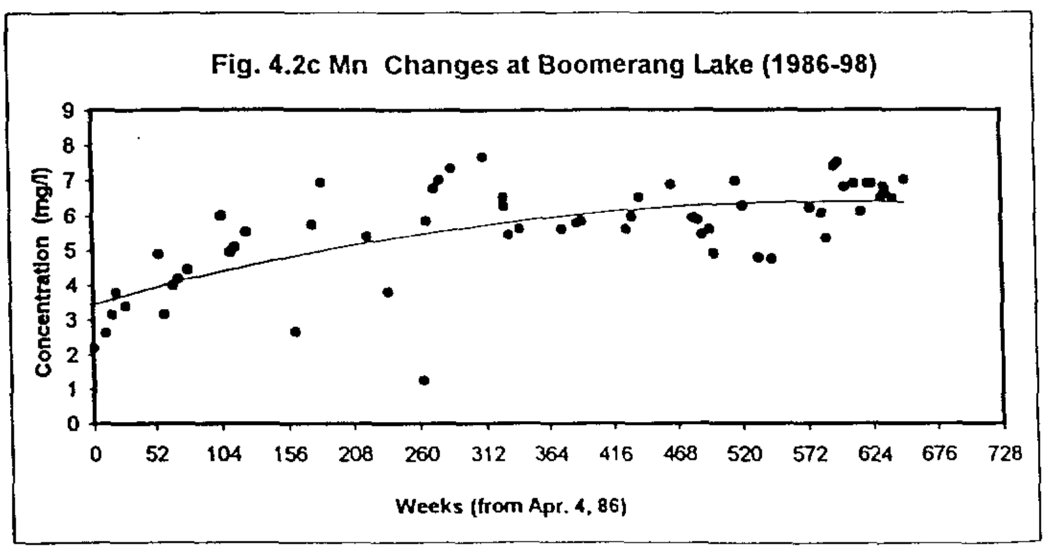
Figure 4.1. PCA Plot of 157 Water Samples (Boomerang Lake 1986-97)



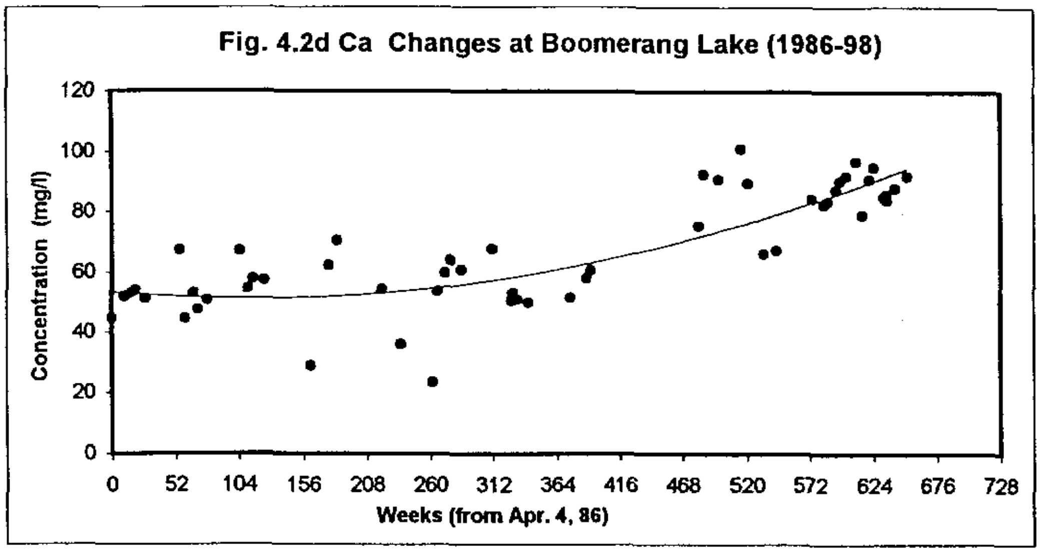
Figures 4.2a-c. Time Trend Analysis of Al, Fe and Mn

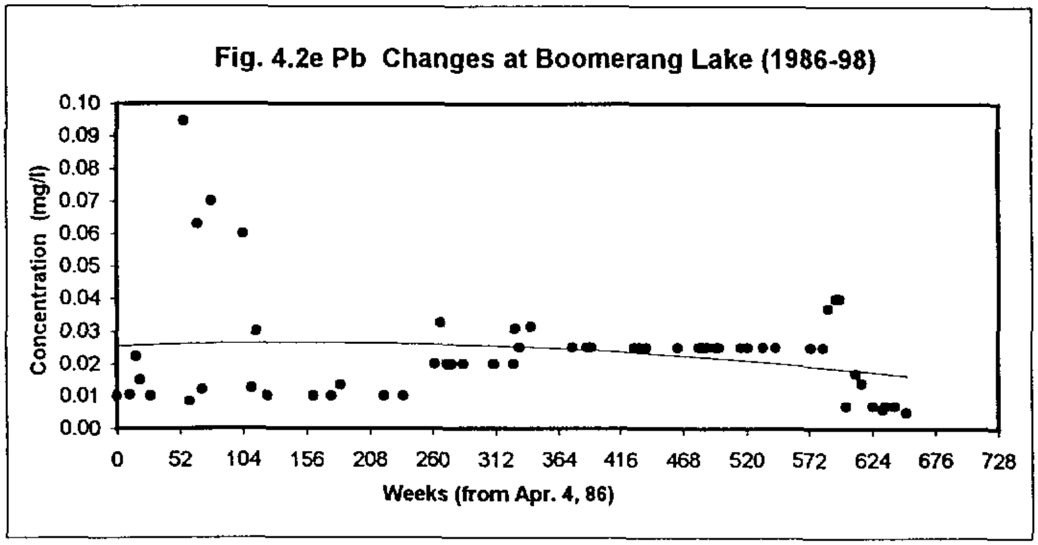


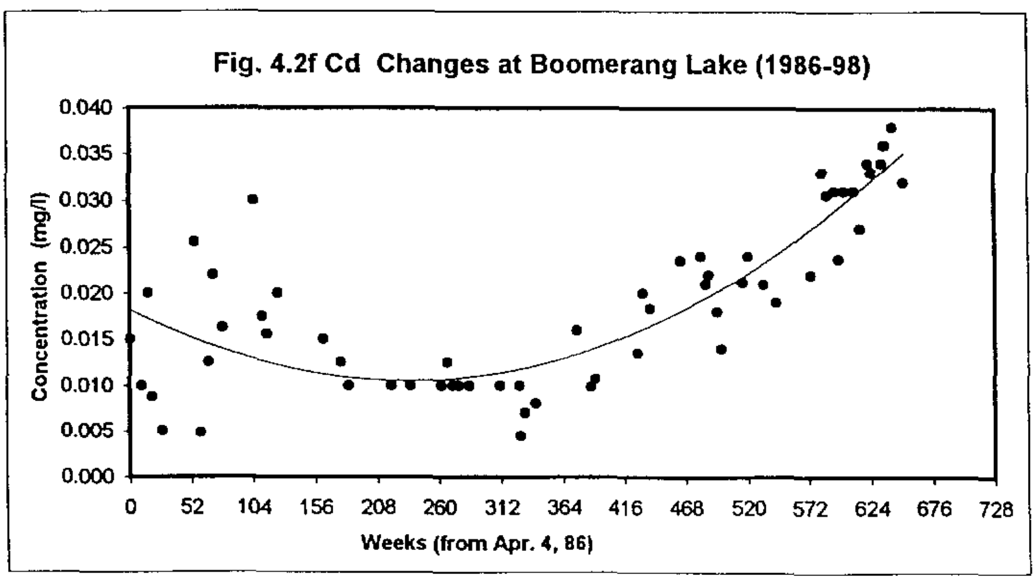




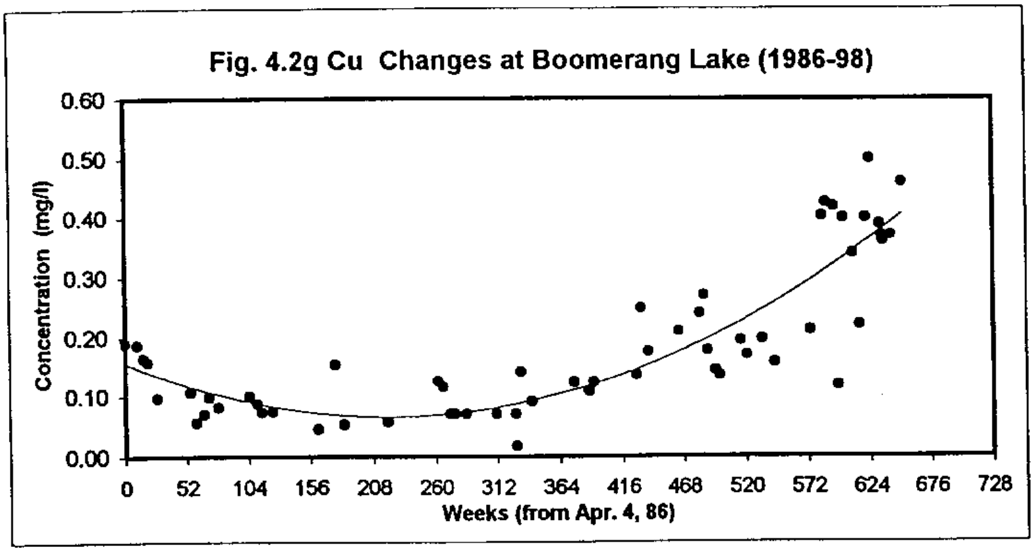
Figures 4.2d-f. Time Trend Analysis of Ca, Pb and Cd

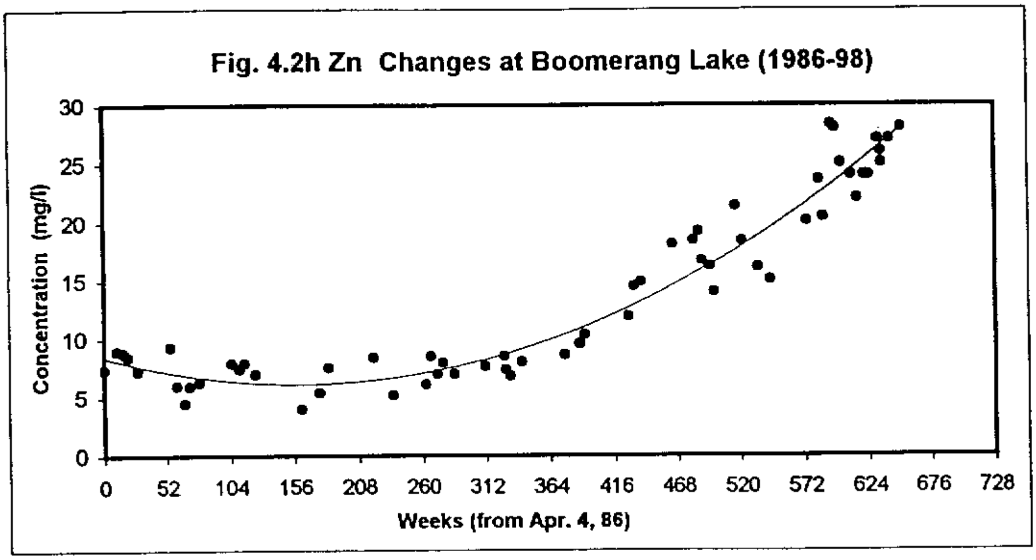


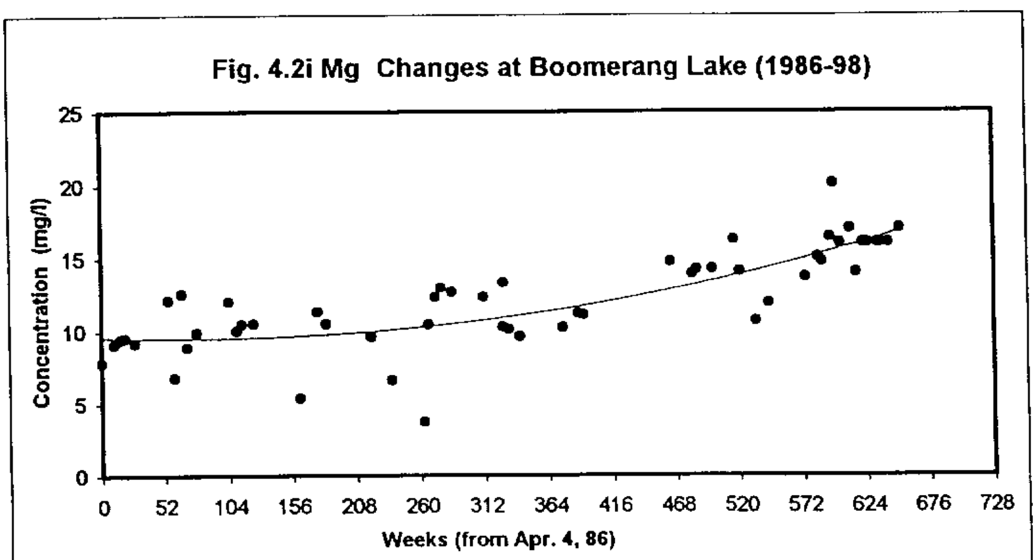




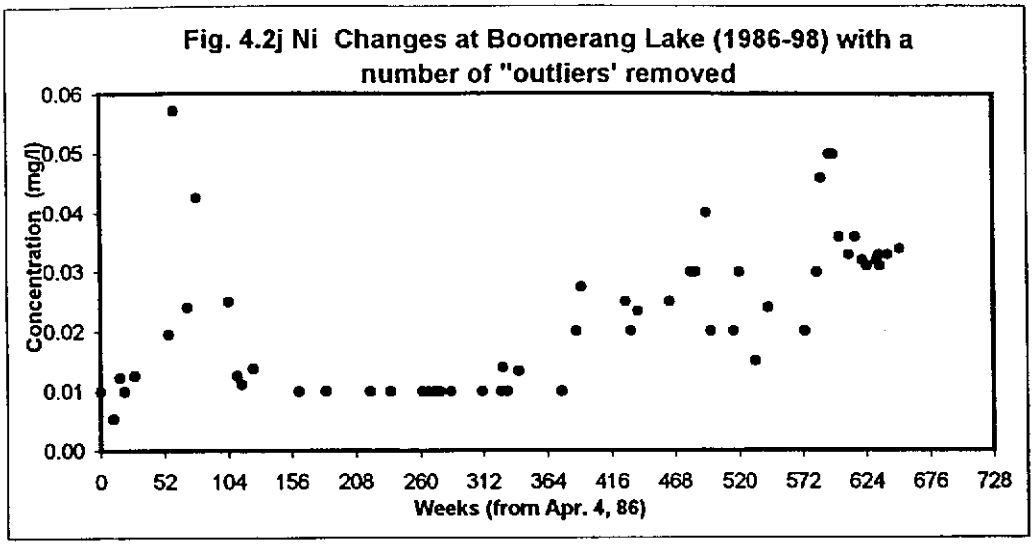
Figures 4.2g-i. Time Trend Analysis of Cu, Zn and Mg

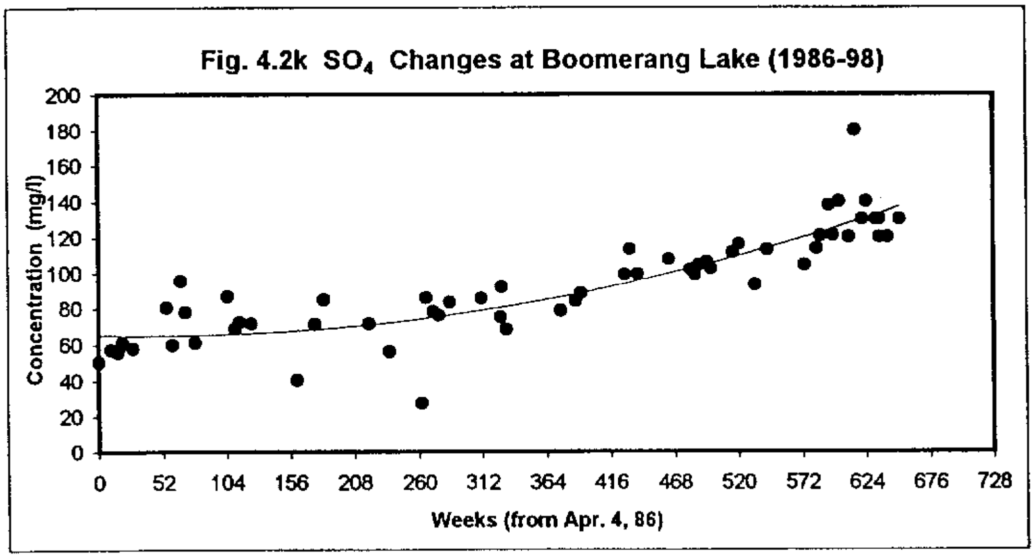


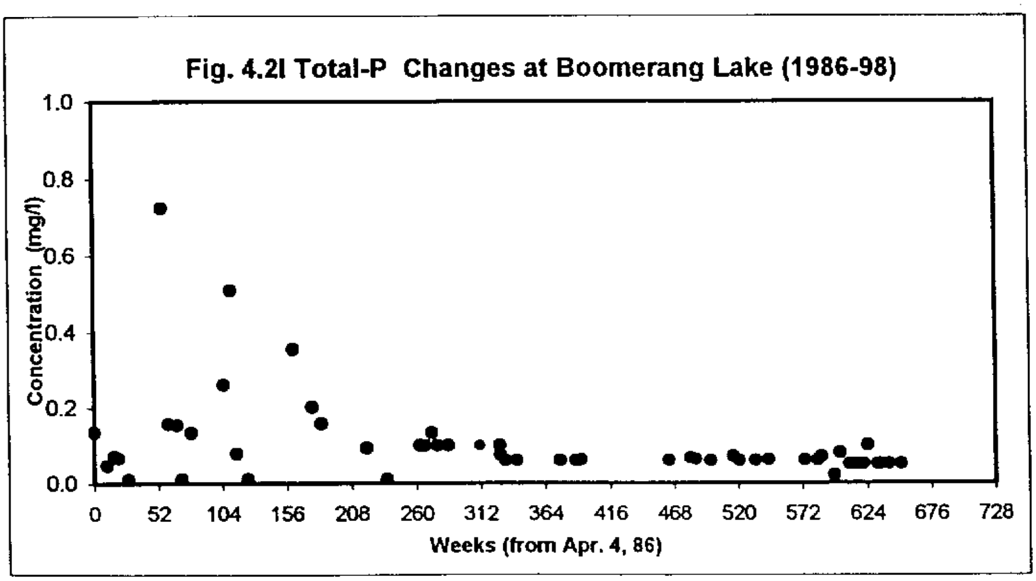




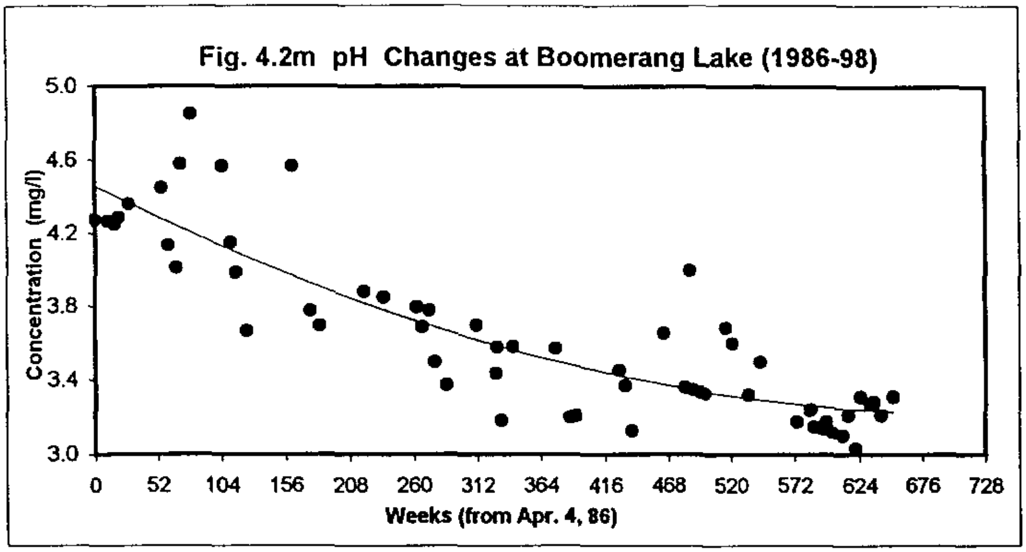
Figures 4.2j-l. Time Trend Analysis of Ni, SO_4 and Total-P

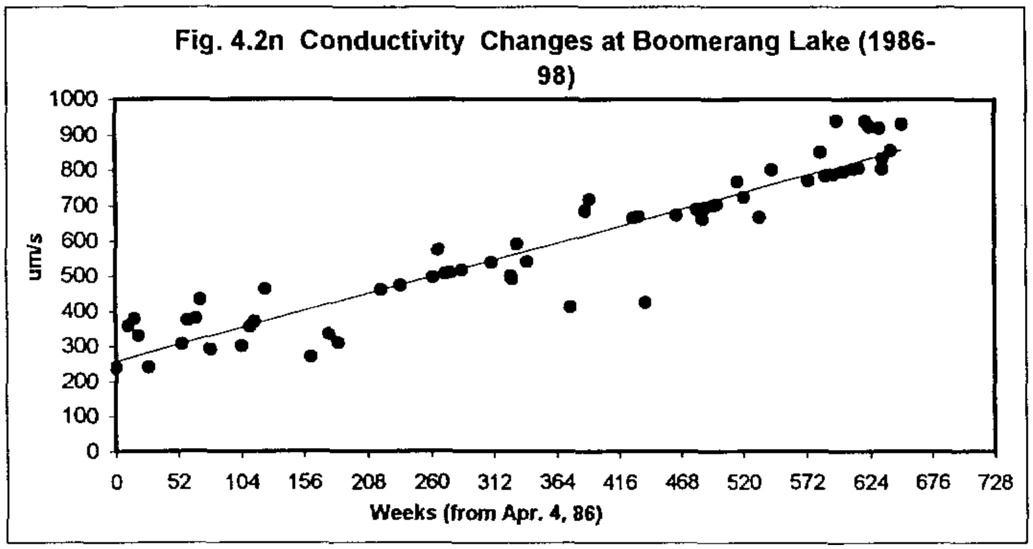


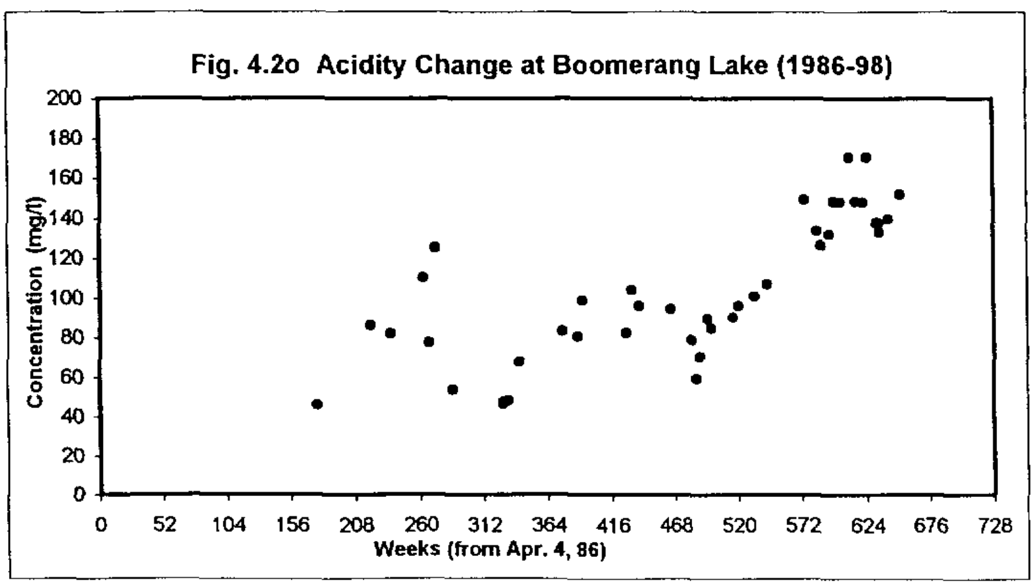




Figures 4.2m-o. Time Trend Analysis of pH, Conductivity and Acidity







Figs. 4.3. PCA Plot of 52 Composite Water Samples
(Boomerang Lake 1986-97)

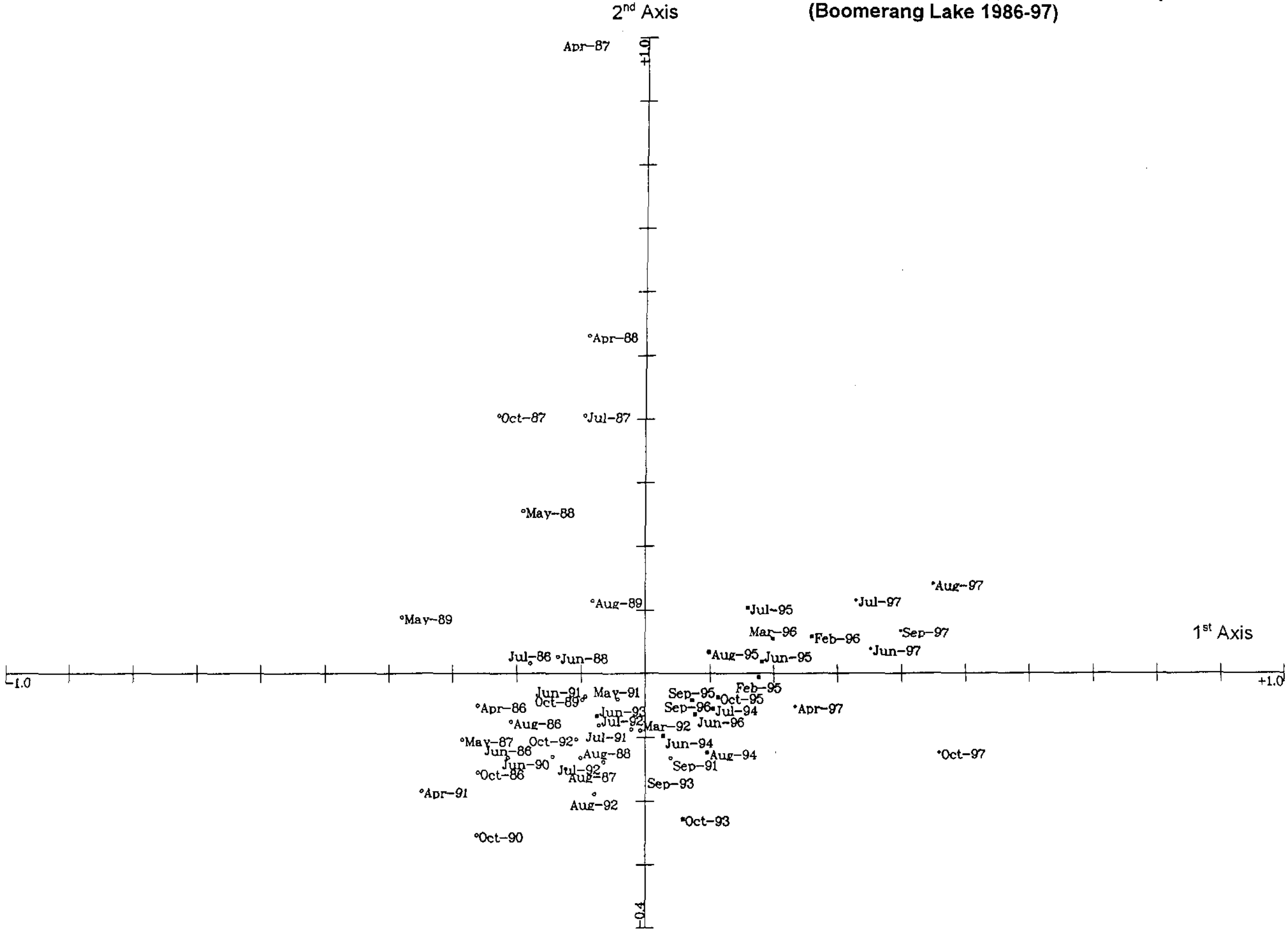
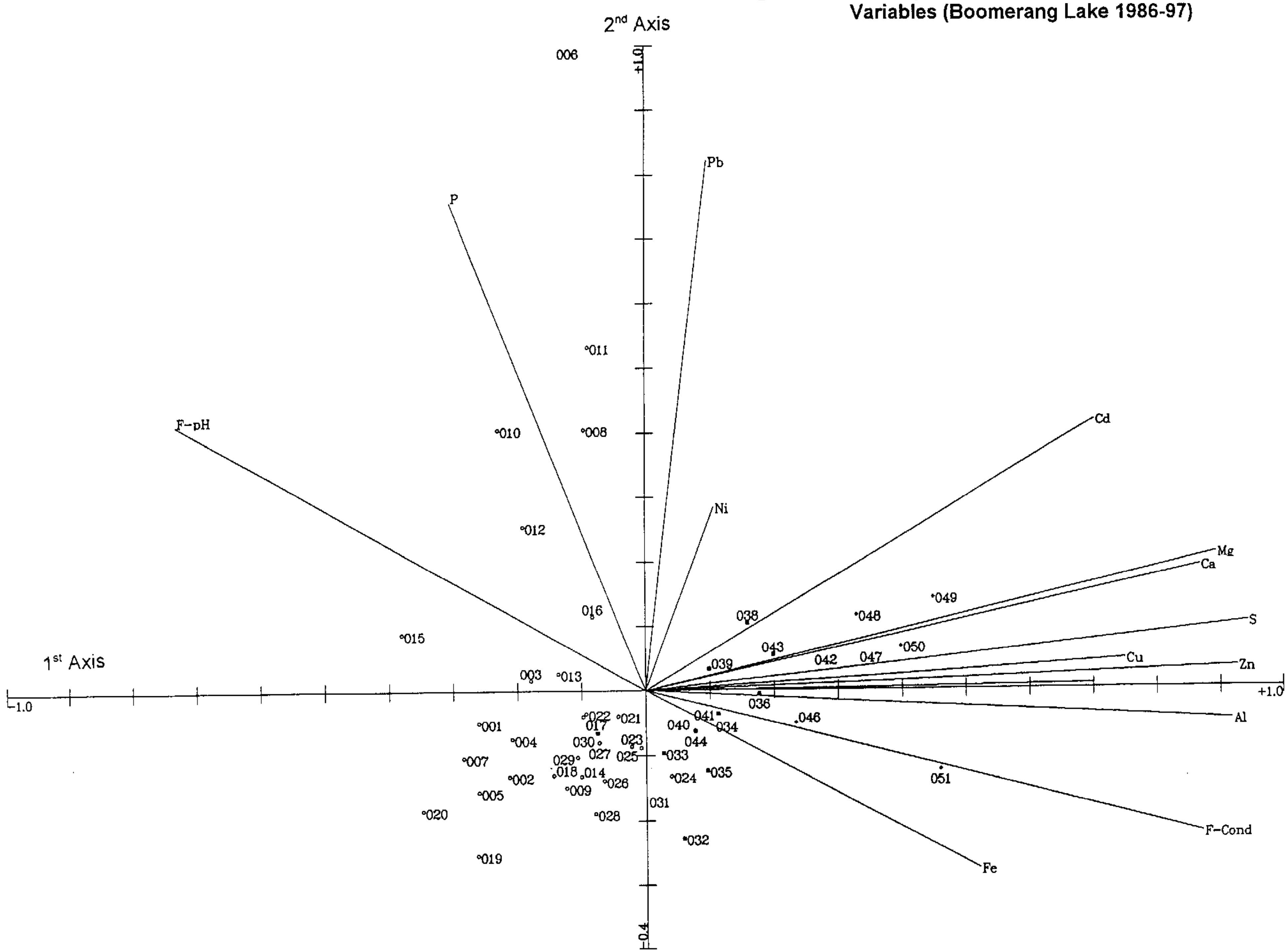


Figure 4.4. PCA Biplot of 52 Samples and 15 Variables (Boomerang Lake 1986-97)



F8

Figure 4.5. Cluster Analysis of Water Samples from Boomerang Lake (1986-97) based on 15 Variables using Ward Linkage

SEE TABLE 4.6 FOR SAMPLE CODES

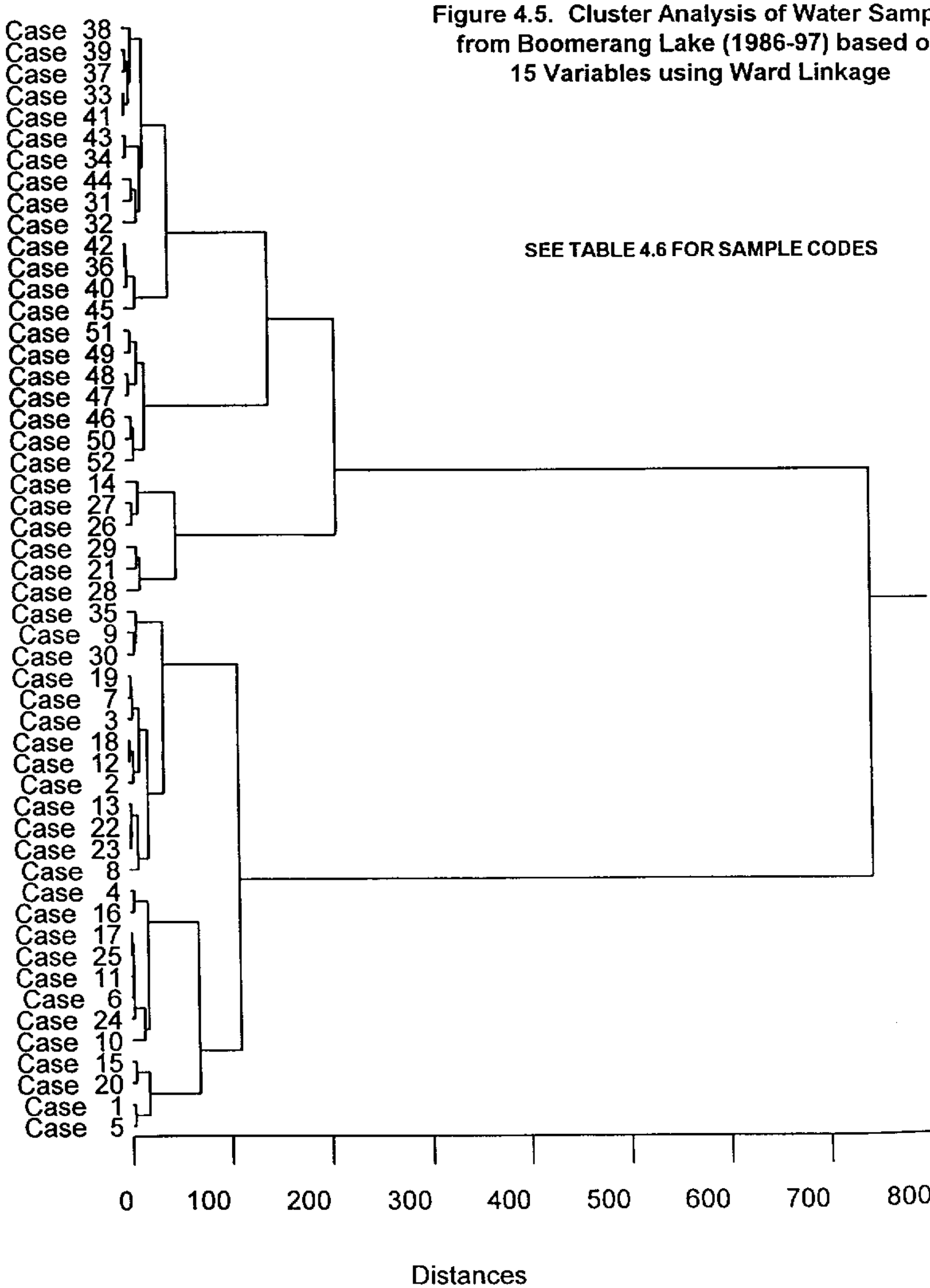
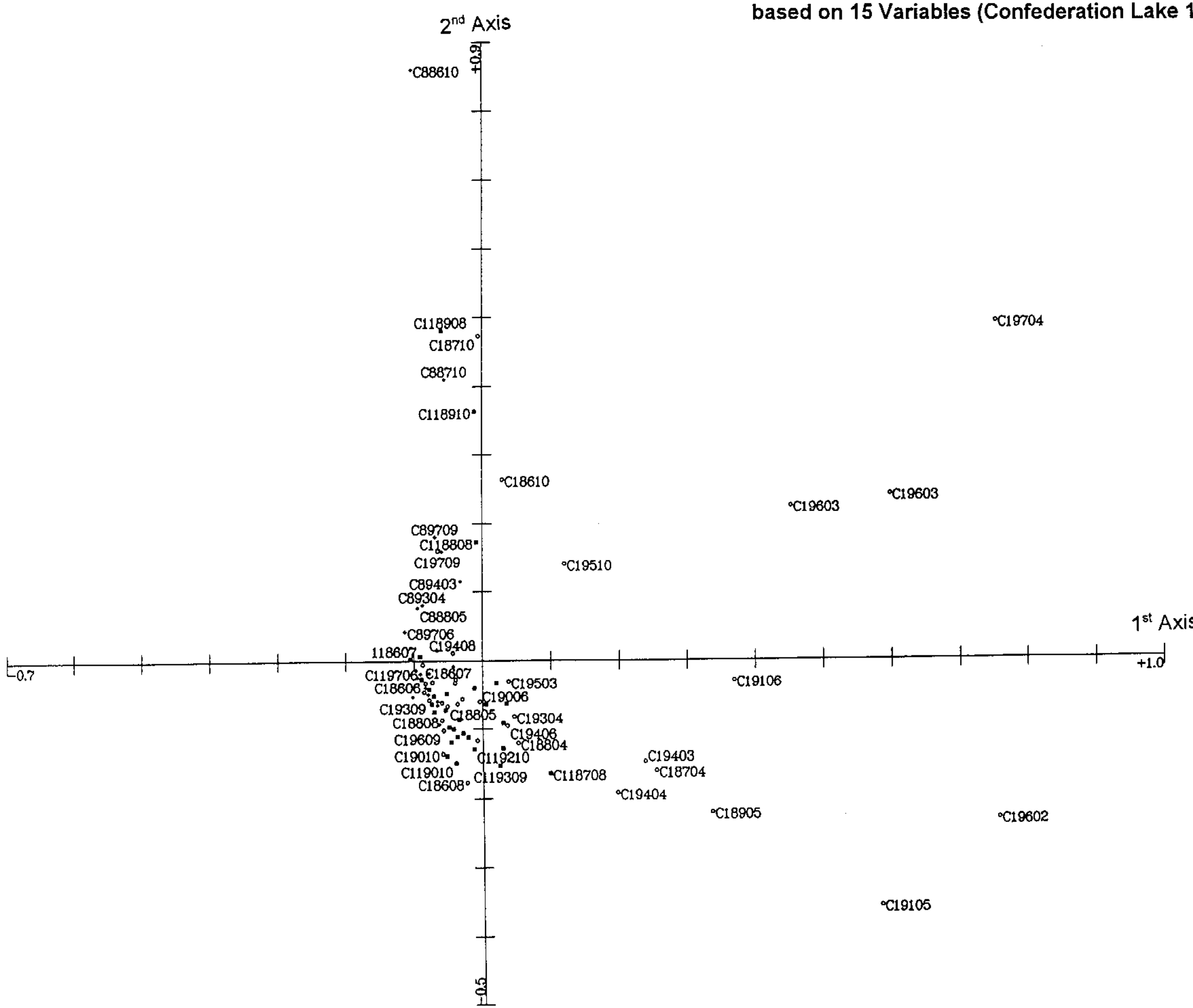


Figure 4.6. PCA Plot of 109 Water Samples from 3 Locations based on 15 Variables (Confederation Lake 1986-97)



F10

Figure 4.7. PCA Plot of 109 Water Samples and 14 Water Quality Variables (Confederation Lake 1986-97)

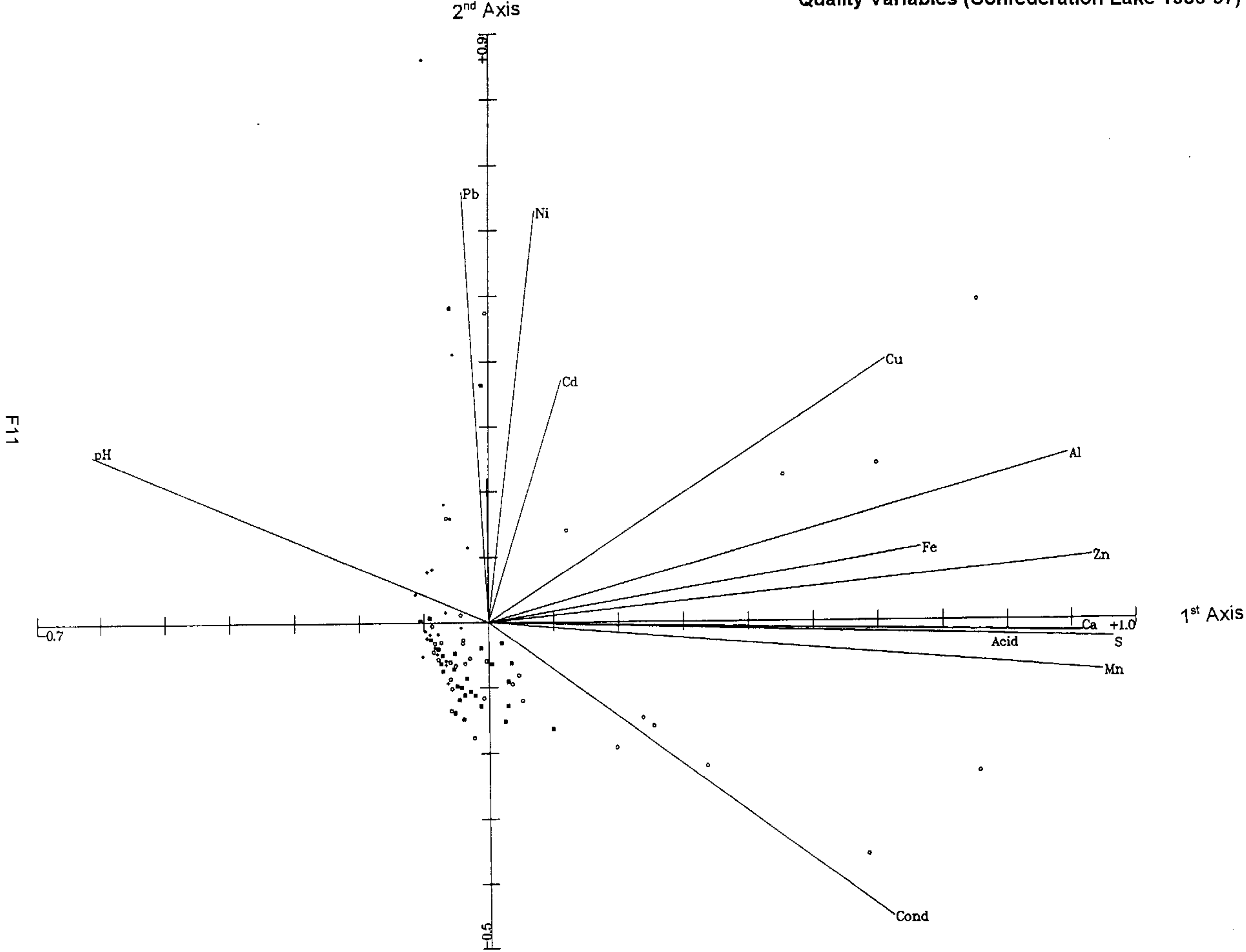
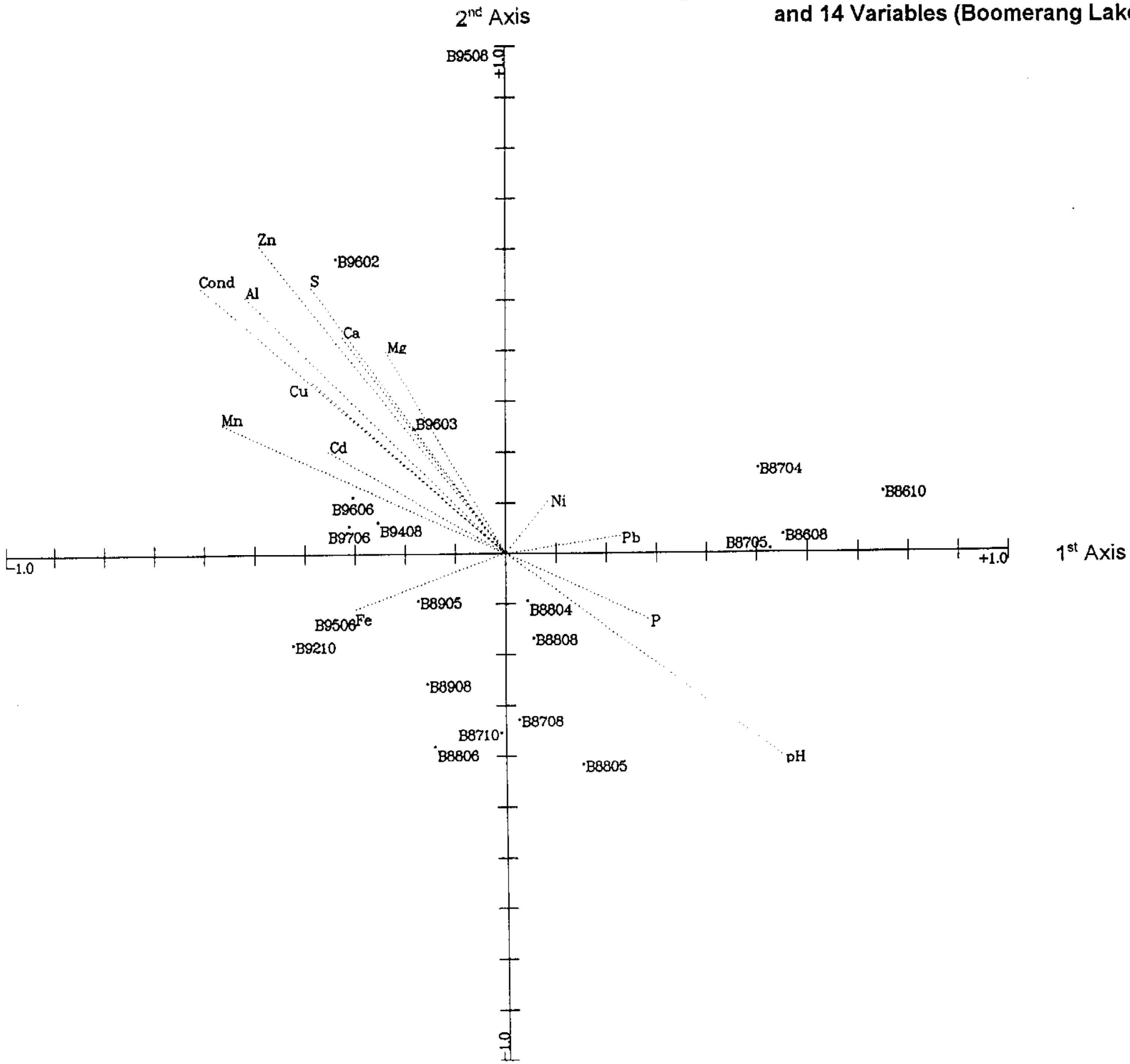
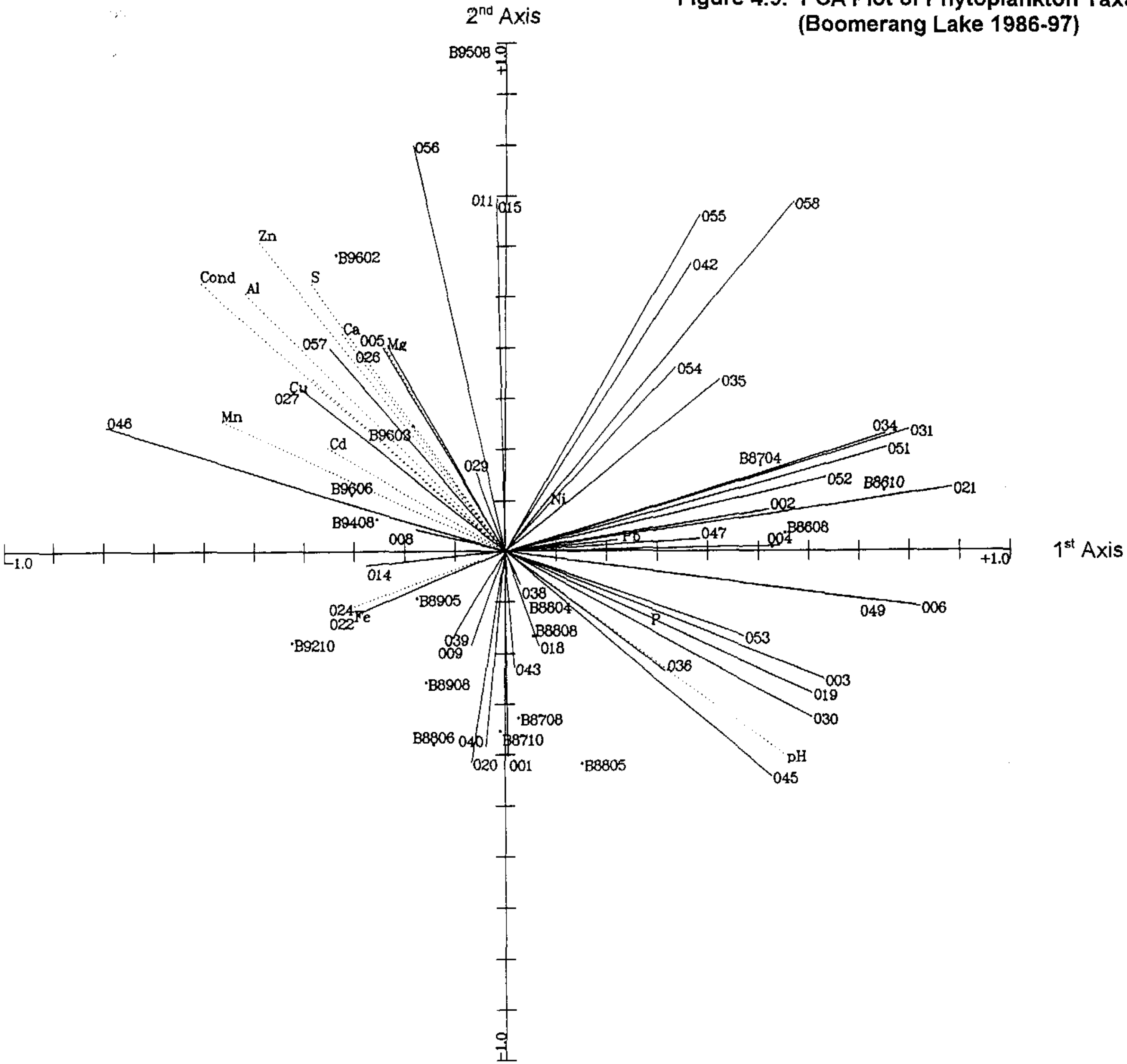


Figure 4.8. PCA Plot of 20 Composite Phytoplankton Samples and 14 Variables (Boomerang Lake 1986-97)



F12

Figure 4.9. PCA Plot of Phytoplankton Taxa and 14 Variables (Boomerang Lake 1986-97)



F13

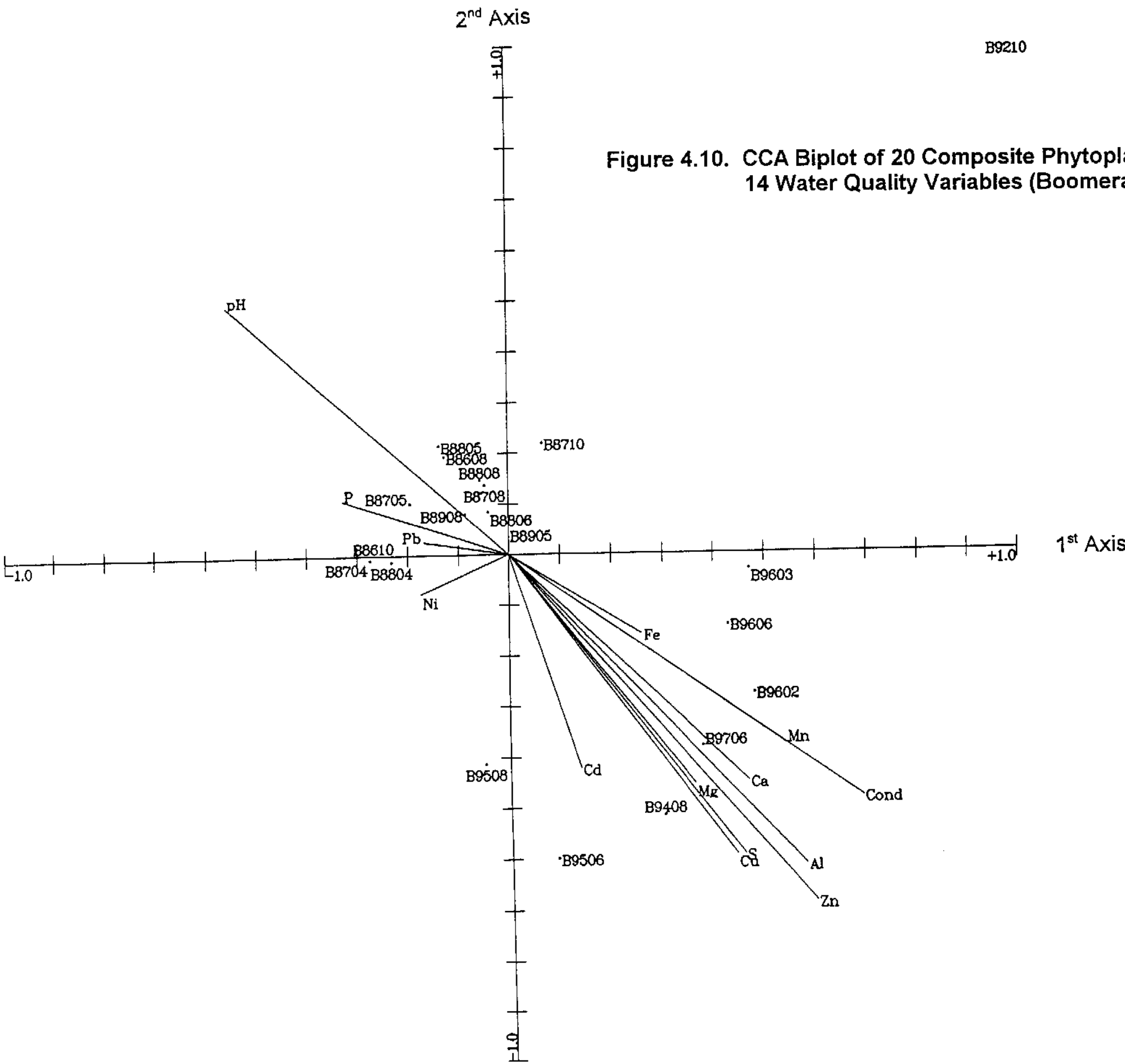


Figure 4.10. CCA Biplot of 20 Composite Phytoplankton Samples and 14 Water Quality Variables (Boomerang Lake 1986-97)

F15

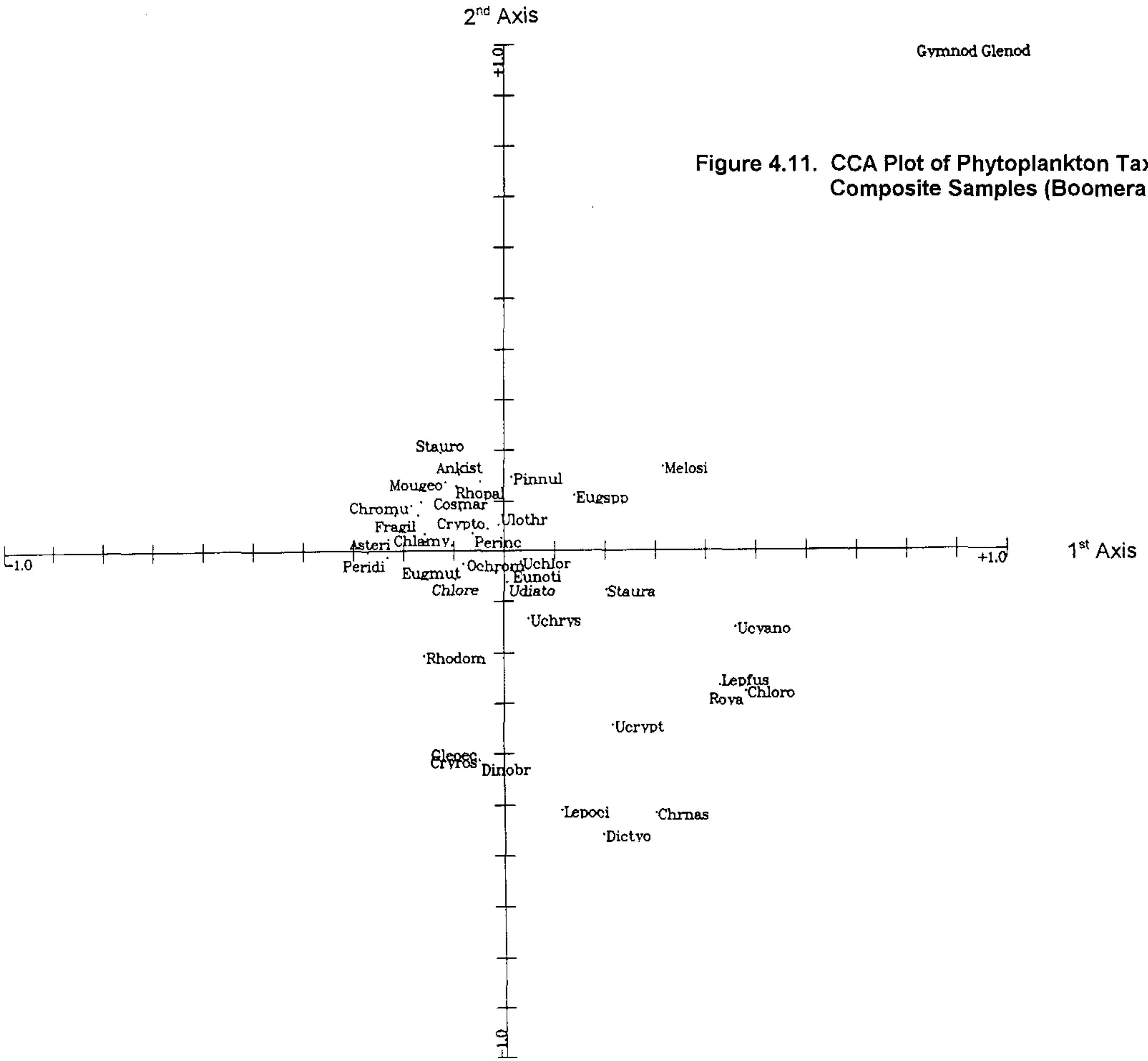
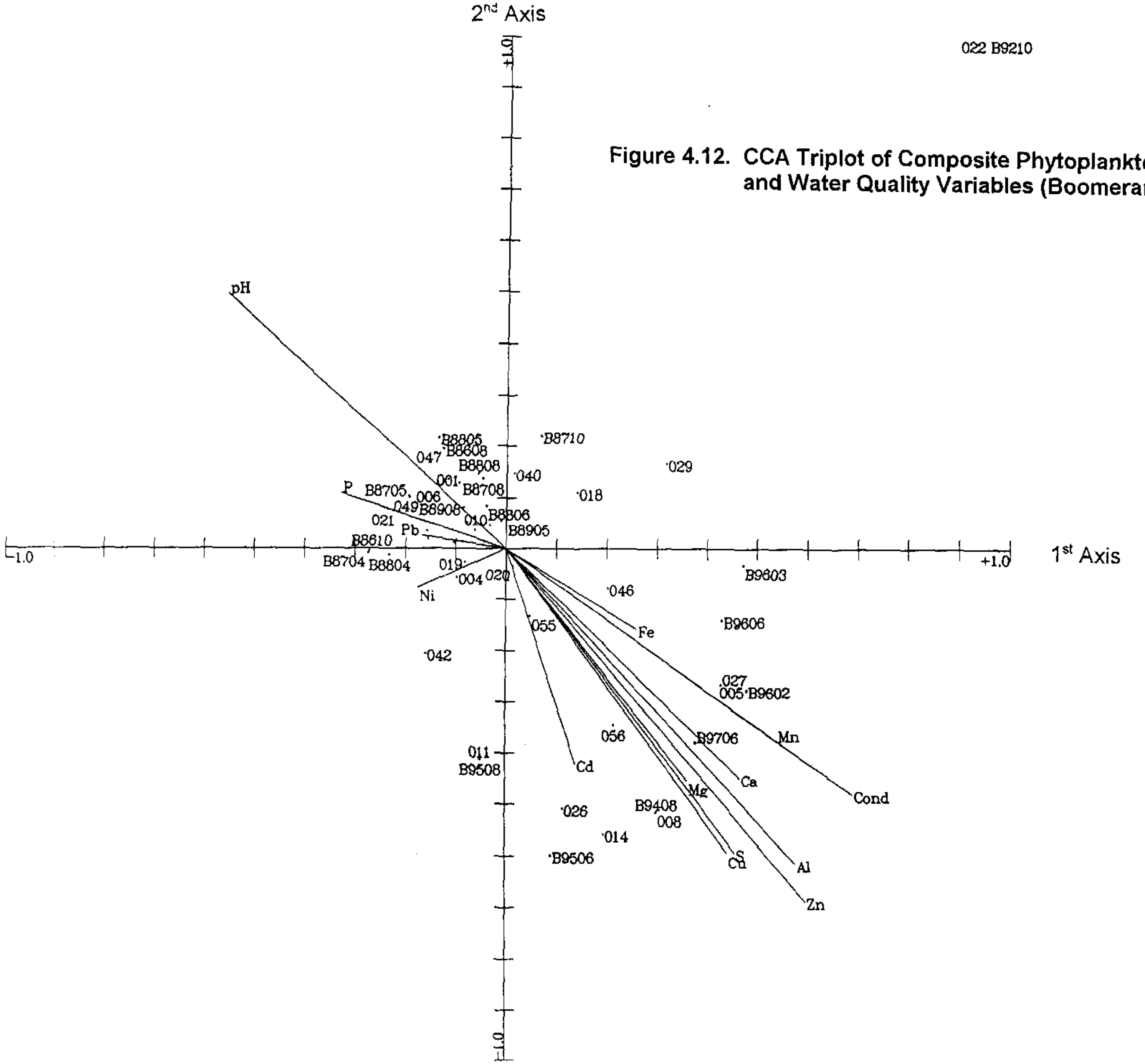


Figure 4.11. CCA Plot of Phytoplankton Taxa based on 20 Composite Samples (Boomerang Lake 1986-97)

Figure 4.12. CCA Triplot of Composite Phytoplankton Samples, Taxa and Water Quality Variables (Boomerang Lake 1986-97)



F16

Fig. 4.13a. pH Changes over Time in Cementation Experiemnt of Boomerang Lake water*

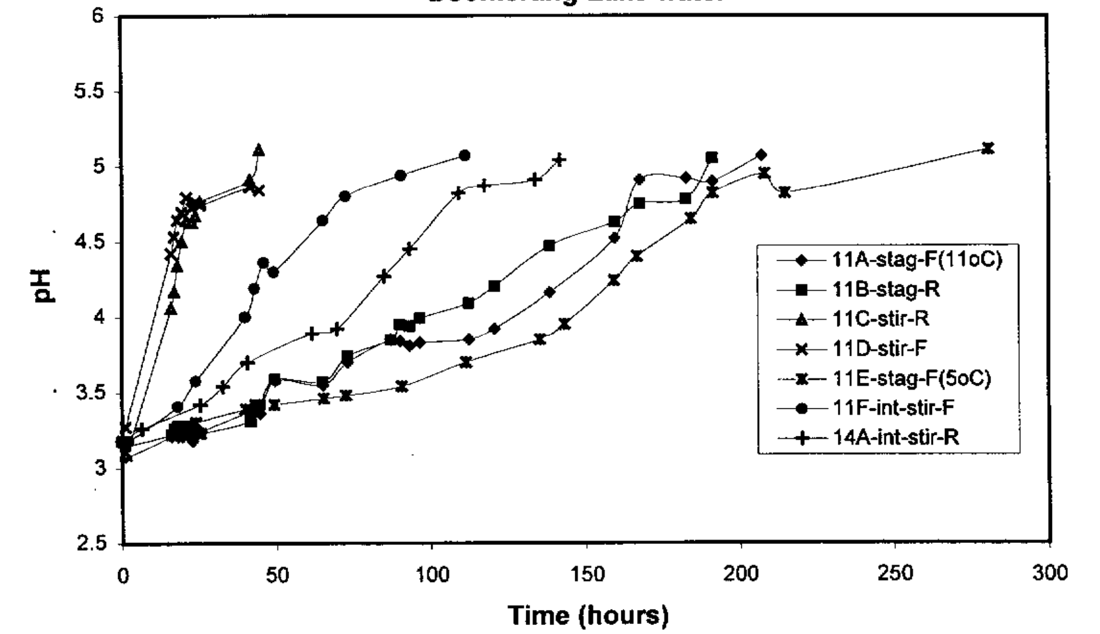
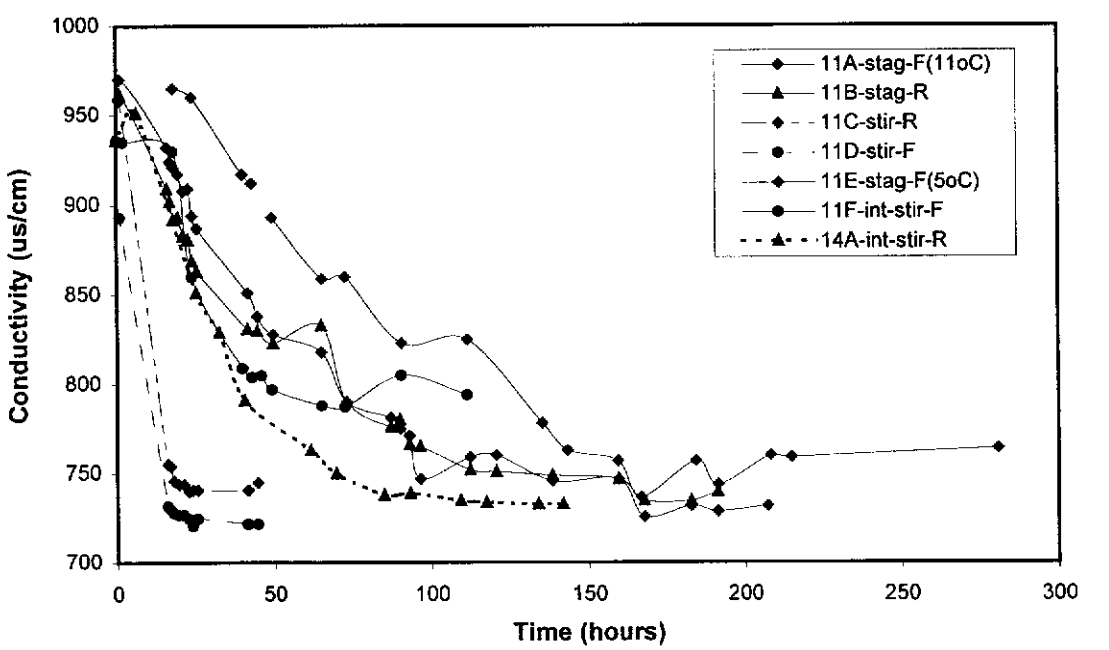


Fig. 4.13b. Conductivity Changes over Time in the cementation experiment of Boomerang Lake water*



*. 11A(B)-stag-F: stangant at 11°C (room temperature); 11C(D)-stir-F(R): stirring in a fridge at 11°C (room temperature); 11E(F)-stag-F (5°C): stagnant (intermediate stirring) at 5oC; 14A-int-stir-R: intermediate stirring at room temperature

PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 5

LINK LAKE

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

5.0 Site Description

Upper and Lower Link Lakes, shown on the Map 4, are located in the Rabbit Lake drainage basin, which is immediately west of Pow Bay on the Wollaston Lake and south of B-Zone, described earlier. Upper Link Lake (ULL) is 22.7 ha and Lower Link Lake (LLL) 22.8 ha. Both lakes are very shallow (approximately 1.4 m on average) and well mixed, receive runoff from the Rabbit Lake waste rock storage area, and drain to Pow Bay.

The wastewater from two waste rock piles discharges into Upper and Lower Link Lakes via a collection ditch. The major elements of concern in the lakes are ^{226}Ra and Uranium. As charophytes are known to concentrate ^{226}Ra, in 1989 twelve tonnes of charo (*Nitella flexils*) were transferred from Lower Link Lake to Upper Link Lake, where the aquatic community had been disturbed during pit development, in order to establish a biological polishing capacity there. Extensive investigations have also been conducted on the hydrology, limnology and water chemistry of the lake, and the results are presented in several reports submitted to CAMECO and the regulatory agencies. More details are given in the publications (Smith and Kalin, 1989, Kalin, 1989 and Boojum, 1996).

5.1 Monitoring Program

5.1.1 Water Quality

Water chemistry has been monitored at twenty-seven locations at the Rabbit Lake mining area (W1-W27, Map 4). Extensive sampling was carried out at four lake-locations, W14 and 15 in Upper Link Lake, W20 and W25 in Lower Link Lake (Table 5.1). The monitoring station W9 was also included in the analysis as it represents the collective runoff from the waste rock piles and the mine site to Upper and Lower Link Lake. This analysis focuses on these five locations. All monitoring data are included for completeness. Water quality monitoring at these locations was initiated between 1980 -1988. The monitoring data cover at least ten years. Although samples were collected at monthly intervals, varying percentages of data are missing at different locations, for different years and parameters. A total of 604 samples were included in this analysis, which is still a very large data set.

Fourteen elements/variables were normally measured. Many of the them were at low levels, such Cu, Pb, Zn and Cl, or within the ranges of natural lakes, such as HCO_3 and SO_4. Consequently, this quality analysis concentrated on the major contaminants, Ra^{226} and Uranium, and pH. The statistics of three variables in table provide only a brief description of the data series for each location and, because different sampling dates, frequencies, and periods are covered at the locations, they cannot be compared directly.

5.1.2 Phytoplankton

Phytoplankton samples were collected from Upper Link Lake and Lower Link Lake by Boojum staff in 1989, 1991 and 1995, and were fixed with Lugol's solution in the field. They were then shipped to Algatax in Toronto for species identification, resulting in a total of 21 samples being compiled for this analysis. The phytoplankton data set is binary, i.e., taxa were recorded by their presence or absence, and identification was trimmed to genus level.

5.2. Data Analysis

A variety of statistical techniques, from simple linear correlation to complex multivariate approaches, are available for long-term trend analysis of water quality. Since these methods have different assumptions, such as data normality and/or linear relationships, the characteristics of the data set were assessed before suitable analysis methods were chosen.

5.2.1 Data Normality

Detailed distribution tests are time-consuming and not the major concern here and so the distribution of variables was diagnosed only briefly, by examining the ratio between the standard deviation and the average. If the standard deviation to average ratio is below 0.25, the data series is likely to be normally distributed (Elliot 1983); otherwise, it may fit a Poisson, Random, or Binomial distribution. Of the five variables examined here, only pH appeared to be distributed normally, but pH already resulted from -log [H^+]. A normal distribution is an assumption for many statistics, particularly for significance tests, but heterogeneity is the nature of environmental systems and non-normal distributions and non-linear relationships are common. In particular, when significant spatial and temporal gradients exist, data series will not follow normality. Data transformation and standardization can improve data approximation to normality, but the environmental implications of such manipulations are complex and generally unjustified. For example, log transformation always reduces the weight of higher concentrations, but contaminants show toxicity only at certain high levels. Whenever applicable, non-parametric or other distribution-insensitive statistics were employed.

5.2.2 Wilcoxon Signed Rank Test

In this analysis, we used Wilcoxon Signed Rank Test (Zar 1984) to examine the differences between locations. This is the non-parametric equivalent of a Paired T-test. Comparing samples collected on the same date removed the daily, weekly or monthly temporal effects and this test is robust with respect to sampling and measuring errors, as it compares the paired samples only qualitatively (which one is higher, ignoring by how much).

The Wilcoxon Test was applied only to the last five years for Ra^{226} and U data because:

i) the data sets at different locations cover varying lengths of sampling period and only a comparison of the same period makes sense and;

ii) changes in the past few years are our major concern. The Wilcoxon Test involves the difference in distribution, rather than average, so averages were not reported here or below.

5.2.3 Trend Analysis

For long-term trend analysis, simple Pearson's Correlation appeared to fit the time-concentration curves best in most cases. Seasonal Kendall Test, another commonly used method for trend analysis (Hirsch et al. 1991), requires equal sampling intervals, which the present data set does not have. Also, Systat (8.0), used in this analysis, gives only the correlation coefficient (Tau-b), without reporting the probability, which makes it impossible to judge the significance of the test. Auto-correlation was used to detect and quantify possible seasonality in the water quality variables.

5.2.4 Auto-correlation

Auto-correlation measures whether two objects closer in time or space are more similar than more distant objects, a phenomenon common in environmental and ecological systems. For example, when two plant samples are collected from adjacent areas, they are usually more similar in species composition and relative abundance than when they originate from more separated locations. Similarly, two water samples collected on consecutive dates frequently have similar water qualities. A key factor in this technique is time lag. Auto-correlation will calculate the correlation coefficient between a data series and itself, with time lag. When lag=0, the auto-correlation will be 1.0, i.e., an exact correlation between the data series and itself. If the auto-correlation at lag 1 is high, then each value is highly correlated with the value at the previous time point. If the auto-correlation at lag 12 is high for monthly data, then each month is highly correlated with the same month the year before, i.e., strong seasonality exists. The technique can, therefore, tell whether periodicity exists and what its cylce is (seasonal or yearly). For more details regarding this technique, see Systat. 8.0 Reference (SPSS 1998).

5.2.5 Cross-correlation

With many environmental processes, a change in one factor may influence other factors only after a certain time has elapsed. Cross-Correlation (SPSS 1998) was used in this study, in order to examine the interactions between two water quality variables. If the time lag = 0, that interaction is simply the Pearson Correlation which, when strongest, means the two variables correspond to each other without a time lag. When the lag is positive (negative), cross-correlation calculates the correlation coefficient between the values in the first series and the subsequent (previous) values in the second series. When a strong correlation occurs at lag = n, one variable corresponds to the other one, with this time lag.

5.2.6 Multivariate Analysis

Principal Component Analysis (PCA) was applied to the binary phytoplankton data in order to examine the temporal-spatial changes in the community structure of Upper and Lower Link Lakes. No attempt was made to relate changes in phytoplankton to water quality variation, as the results indicated that the common species composing the community did not change obviously in relation to the two major contaminants (U and Ra^{226}), which decreased only to a limited extent. Some other, non-water quality factors, appear to contribute to the limited changes that do occur in community composition.

5.3. Results

5.3.1 Long-Term Trends of Water Quality

Since sampling was initiated at different times and was conducted at varying intervals, data for all locations were converted to the number of weeks from 18th April 1980 for comparative purposes. Although samples were always not collected on the same day or week over the locations, this will not influence the potential long-term trends.

Ra^{226}: Changes in Ra^{226} over the sampling periods at the eight locations are shown in Figures 5.1a-h. At the pre-lake location, W9, Ra^{226} ranged more widely (0.06-1.3 Bq/L). Over the longer term (1988 to 1998), the concentration trend increased slowly, but significantly in statistical terms ($r=0.416$, $P<0.05$) (Figure 5.1d). At W14, Ra^{226} (0.12-1.1 Bq/L) was usually higher than at W9, and this difference was significant over the past five years (Wilcoxon Test, $P<0.01$). The higher levels can be attributed to the extra Ra^{226} input from the delta area of Upper Link Lake, where sediment contains high levels of Ra^{226}. Contaminated groundwater discharge at the delta area also contributed to this increase (Boojum 1993). Ra^{226} at this location did not show any significant trend either between 1986 and 1998), ($r=0.170$, $P>0.05$) (Figure 5.1e), nor during the past five years ($r=0.06$, $P>0.1$).

At the next location downstream, W15, where the concentration is even lower (0.14-0.8 Bq/L), there has been a significant decrease during the past five years ($P<0.01$), but no significant long-term trend ($r=0.03$, $P>0.1$), even though some variation was observed (Figure 5.1f). At W20, Ra^{226} decreased further (0.07-0.90 Bq/L) (Figure 5.1g), and for the last five years the concentration has been significantly lower than at W15 (Wilcoxon Test, $P<0.01$). No significant time-trend was observed over the longer sampling period (1988-98) at this location ($r=0.09$, $P>0.1$).

Ra^{226} concentrations at the outflow of Lower Link Lake, W25, were the lowest (0.01-0.60

Bq/L) of any recorded and, during the last five years, they were significantly lower than at location W20 (Wilcoxon Test, P<0.01). This location also exhibited a significant decreasing trend over 1980 - 1998 (r=-0.310, P<0.001), which continued over the last five years (r=-0.323, P<0.05) (Figure 5.1h).

Uranium: The long-term changes in uranium concentrations at all eight locations are shown in Figures 5.2a-h, in which extremely high records have been removed to better illustrate the basic trends. The concentration at W9 showed slow but detectable declines (r=-0.368, P<0.01) over the past ten years (1987-98), but were fairly stable in the past five years (r=-0.04, P>0.1). At the outflow of Upper Link Lake W14, U concentrations (0.34-4.4mg/L) were generally lower than at W9 and, for the last five years, the differences were significant (P<0.01). This is contrary to the changes that were observed for Ra^{226}, which increased significantly at W14, suggesting that the sediment and contaminated groundwater in the Delta area affect Ra^{226} rather than Uranium. Over the whole sampling period (1986-98), the concentration trend increased slowly, but statistically detectable (r=0.328, P<0.01). In the past five years, the trend became less significant (r=0.271, P>0.05) (Figure 5.2e).

At the outflow of Upper Link Lake, W15, U concentrations were even lower (0.28-3.7 mg/L) than at W14 and these differences were highly significant (P<0.001) during the last five years. At W15 itself, the long-term trend (1987-98) showed slow, but significant increases (r=0.333, P<0.05) - a trend that persisted over the last five years (r=0.442, P>0.05) (Figure 5.2f).

At W20 (Figure 5.2g), U concentration was typically below 2.5 mg/L, despite a few high observations. Data from the past five years showed concentrations significantly lower than at W15 (Wilcoxon Test, P<0.01). Between 1988 and 1998, the concentrations at W15 varied and showed no significant trend (r=0.101, P>0.1), and this was also true for the past five years. At the outflow of the system, W25, U concentrations decreased further (0.06-2.21 mg/L) (Figure 5.2h). The Wilcoxon Test on the last five years of data indicated significantly lower U concentrations than at W20 ($P<0.01$), but no significant long-term trend, although some variation was observed. The past five years of data yielded a similar result.

5.3.2 Seasonal Trends

Ra^{226}: The concentrations of Ra^{226} and Uranium at all eight locations were originally plotted against sampling date in relation to pH and temperature. It was found that, although there was always some variation among the different months, the variations appear to be particularly regular at W14 and W15, suggesting strong seasonal trends. Auto-correlation was used to detect and quantify this possible seasonality and the results are shown in Figures 5.3a-h for Ra^{226} and Figures 5.4a-h for U.

In these figures, lag unit is a sampling event, although samples were collected typically at monthly intervals. The two lines indicate the 95% confidence bands, and the peak crossing above them demonstrates a significant correlation over time.

Auto-correlation is very weak and not periodical W9 (Figures 5.3a), meaning no seasonal trends existed. At W9, the auto-correlation does not show any periodicity, but it significantly decayed over time (Fig. 5.3b), implying Ra^{226} concentrations did change seasonally, but with a long-term trend. The latter point agrees with the earlier description (see Figure 5.1d).

The Ra^{226} auto-correlation is strong, however, at locations W14 and W15, with a periodicity of approximately 10-12 sampling events - a typical seasonal trend (Figures 5.3e-f). By examining the raw data, it is clear that Ra^{226} concentrations reached their minimum in the summers of each year (June-Aug.) and peaked in winter/spring. It was also noted that periodicity slowly decayed as time lag increased. Possible reasons for this include:

i) the difference from non-monthly intervals accumulated;

ii) the periodicity is relatively weak at the early phase.

Two sub-series, containing only 1980-1989 and 1990-1998 samples, were drawn from the original data. The auto-correlation for 1980-1989 shows no periodicity, but the 1990-98 data show a periodicity similar to that in Figures 5.3e-f. This means that the seasonality developed only from 990, which coincides with the time when a macrophyte, *Nitella* sp., was transferred into Upper Link Lake in 1989 (Boojum 1993).

The interaction of Ra^{226} with pH was examined using Cross-Correlation, the results of which are shown for locations W14 and W15 in Figures 5.3e-f. Clearly, Ra^{226} and pH are strongly and negatively correlated, and both show strong seasonal trends. In other words, when pH was high in summer months, Ra^{226} was low, and Ra^{226} was high when pH was low in winter.

The auto-correlation plot of Ra^{226} at W20and W25 shows no periodicity. The cross-correlation with pH yielded similarly negative results, meaning that the seasona trends in both Ra^{226} and pH had disappeared at these two locations. Auto-correlation at W20 was fairly high when the lag was small, but became very low as time lag increased. This suggests a weak or non-existent time trend. Auto-correlation at W25 rapidly decayed as time lag increased, implying a strong time-trend, as Figure 5.1h shows.

As with Ra^{226}, uranium auto-correlation does not show any periodicity W9. Since its cross-correlation with pH at these locations is also weak (Figures 5.4d), neither uranium nor pH were seasonal here. And, whereas the behaviour of uranium at W14 and W15 was very similar to Ra^{226}, in that the auto-correlation is strong and very periodic, there is a significant and negative cross-correlation between U and pH in a cyclical manner. These results show that uranium at these two locations was highly seasonal and strongly and negatively related to pH. Again, this seasonality was weak or non-existent before 1990, but it became strong in the past few years. Uranium concentrations were also seasonal at W20 (Figure

5.4g), although less strong and consistent. Cross-correlation indicates a strong interaction between Uranium and pH, although this pattern disappears at W25, which is located in the channel to the Pow Bay.

5.3.3 Ra^{226}, Uranium and TSS

As a water quality parameter, TSS is important in the way that it relates to the fate of contaminants and the functioning of biological polishing systems. TSS data are very scattered, with mostly very low records but a few that are very high. The high records are believed to be the result of rapid runoff after precipitation although, at this time, TSS data have not been related to rainfall data. How Ra^{226} and U correlated with TSS was examined for all five major locations over the entire sampling period. The results are presented in Table 5.2.

Ra^{226} is significantly correlated with TSS at W9, W14 and W25, while U is closely correlated with TSS only at W20. It appears, therefore, that Ra^{226} is more closely associated with TSS than U. It was also noted that at W15 and W20, where TSS was usually very low, Ra^{226} and TSS were not significantly correlated.

An attempt was made to test the differences between TSS levels among the locations, even though TSS data depend strongly on the date and time the sample was taken - meaning data from the same month or week at different locations are not comparable. Removing all data that did not match in terms of the time recorded caused heavy data loss, however, so, as a result, no significance tests could be carried out. TSS levels at W14, W15, and W20 were, however, generally lower than at other locations.

5.3.4 Phytoplankton Community

Seventy-two genera, plus three unidentified taxa, were recorded in the samples. *Cryptomonas rostriformis* was differentiated from other species of the genus because it appeared to be different in its distribution and tolerance to contamination. The occurrences of the genera/taxa are shown in Table 5.3, where forty-two genera/taxa occur no more than three times in all 21 samples and a further twenty genera/taxa occur less than 10 times. The top ten genera/taxa were *Scenedesmus* (85.7%), *Cryptomonas* (81%), Unidentified Chlorophyte (76.2%), *Euglena* (71.4%), *Asterionella* (66.7%), *Planktospheria* (61.9%), *Tabellaria* (61.9%), Unidentified Chrysophyte, *Oscillatoria* (57.1%), *Cryptomonas rostriformis* (52.4%), *Staurastrum* (52.4%), *Dinobryon* (47.6%), *Mougeotia* (47.6%) and *Synedra* (47.6%).

The average number of genera/taxa recorded in a sample is 19.1, with a standard deviation of 8.62. The changes in the number of genera/taxa in the two lakes in three sampling years are given in Figure 5.5, which shows the richness did not change obviously over the period, although the highest was recorded in Lower Link Lake in 1991.

The PCA plot of the 21 samples on the first two dimensions is presented in Figure 5.6. The first two axes explain 30.6% of the total variance, with Axis 1 alone accounting for 18%. It is evident that the phytoplankton community compositions in Upper and Lower Link Lake were different in 1989, with Upper Link Lake samples on the left in the plot, and the Lower Link Lake samples on the right. In 1991 and 1995, however, this pattern disappears and the samples are heavily mixed. The 1989 samples are located lower down than the 1991 and 1995 samples, indicating their difference in genera/taxa composition.

Genera/taxa are plotted in Figure 5.7, in which it can be seen how they are associated with different locations and times the samples were collected. It was noted that *Scenedesmus*, *Cryptomonas*, *Euglena*, *Planktospheria*, *Oscillatoria*, *Navicula*, *Nitzschia* and an unidentified Chlorophyte were recorded in both lakes all the time. *Tabellaria*, *Cryptomonas rostriformis*, *Staurastrum*, *Dinobryon*, *Mougeotia*, *Synedra*, *Aphanizomenon*, *Asterionella* and an unidentified Chrysophyte were the second most common. Those genera/taxa that are strongly associated with particular samples are either rare or less abundant, implying that phytoplankton composition in the two lakes remained relatively stable over the sampling period.

No attempt was made to relate changes in phytoplankton community composition to water quality in this study. Firstly, temporal changes in phytoplankton community composition were relatively minor, particularly in Upper Link Lake, where the most common genera/taxa in 1989 remained dominant until 1995. Secondly, the changes in Ra^{226} and U at W15 and W25 during the 1989-1995 period were quite limited. The compositional changes observed here, therefore, appear to be related to environmental factors other than water quality. The phytoplankton communities of the two Link Lakes are compared with those of the other lakes/pits.

5.4 Discussion

Whereas most long-term monitoring programs focus on organic pollution/ eutrophication, acidification and other metal contamination (Stow et al. 1998, Stoddard et al. 1998), this analysis documents long-term trends in two uncommon metals, Ra^{226} and U, and relates these trends to biological systems.

As with other contaminants, Ra^{226} and U decreased in a downstream direction, probably due to both natural purification and the biological polishing system in the lakes. Over the long term, Ra^{226} trended to slowly decrease while U trended to increase. In the past five years, the input of both metals was relatively constant, however, which suggests that the biogeochemical processes in the waste rock piles have stabilized. It was also noted that Ra^{226} concentrations decreased gradually, but continuously at the output (W25) and, during the last five years, it decreased significantly between W14 to W25. These results indicate that Ra^{226} was being removed from the water as it flowed through the system, and that its removal was improved over time. A more quantitative assessment of this process will be made elsewhere. Uranium is somewhat different in that, while the concentrations at each location tended to be relatively stable over the past five years, those concentrations decreased as one progressed downstream. This suggests that the removal of U in the system was fairly stable.

Auto- and cross-correlation shows that Ra^{226} and U changed seasonally in both lakes (W14, W15), but the seasonality disappeared at all non-lake locations. Levels of both metals were lower in summer and higher in winter, while pH was higher in summer and lower in winter. This pattern appears to correspond to the growth of macrophyte and phytoplankton very well. The higher pH in summer appears to be the result of photosynthesis. It is known that *Nitella* sp. established a healthy population in Upper Link Lake around 1990, reaching a biomass as high as 250 g dry /m^2 (Boojum 1993). Boojum also observed that *Nitella* could absorb Ra^{226} from the water column and that phytoplankton also contributed to the removal (Boojum 1993).

Accordingly, the seasonal patterns observed in both Ra^{226} and U can be explained as follows. In summer, *Nitella* sp. and phytoplankton remove Ra^{226} and U from the water column, probably by various mechanisms that significantly reduce the concentrations. In winter, when the plants stop growing, Ra^{226} and U levels increase. The initial occurrence of this seasonality, in approximately 1990, matches the time when Nitella was transferred to Upper Link Lake, seemingly providing field evidence for the functioning of ecological engineering.

Since *Nitella sp.* also grows in Lower Link Lake, a similar seasonal pattern should be seen but, unfortunately, the sampling location best representative of this lake (W23) was not visited regularly and few data are available. At the outflow channel of Lower Link Lake, W25, neither Ra^{226} nor U showed any seasonality, suggesting that the effects of *Nitella* were restricted to the lake where the biomass was adequate to influence water quality.

Phytoplankton communities in Ra^{226}- and U-contaminated waters are poorly, if at all, reported in the literature. This study documents the genera/taxa composition in two uranium-mining impacted lakes. In 1998, St-Cyr et al. published a summary of the literature on the general tolerance of phytoplankton species to metals, in which three that are listed as "resistant" (*Scenedesmus*, *Euglena*, and *Synedra*) occur in the top 14 common genera in the Link Lakes. However, four other common genera found in the lakes (*Asterionella*, *Cryptomonas*, *Staurastrum*, and *Tabellaria*) are listed as "sensitive" in the review article. *Dinobryon* has been reported as both sensitive and resistant, depending on the species and researchers, and the other genera common in the lakes, including *Planktospheria*, *Mougeotia* and *Oscillatoria*, do not appear to be cited in the literature regarding their responses to metal contamination. It is perfectly possible that the tolerance of a genus varies from one metal to another, and that it also sometimes depends on the species of the genus. For example, the most common genus in the Link Lakes, *Scenedesmus*, was reported to be very sensitive to arsenic (Voke et al. 1980), but it is clearly extremely tolerant of Ra^{226} and U. How applicable the results from laboratory toxicity tests to the real world are, is another question that needs to address.

Table 5.1. Sampling dates and total number of samples for all stations.

Stations	Description	Sampling Period		Samples
		From	to	
W3	1.1 North drainage ditch	18-Apr-80	4-Sep-98	199
W4	1.2 south drainage ditch	18-Apr-80	4-Sep-98	189
W5	1.2.5 Airport road	21-Jan-85	5-Sep-97	191
W9	ULL inflow	30-Jul-88	8-Sep-98	83
W14	1.4.1 ULL at narrows	17-Nov-86	8-Sep-98	126
W15	1.4 sedimentation dam	18-Jan-87	8-Sep-98	143
W20	1.413 bad, beaver house	01-Feb-88	8-Sep-98	111
W25	1.4.5 LLL outflow over..	23-Mar-80	8-Sep-98	141

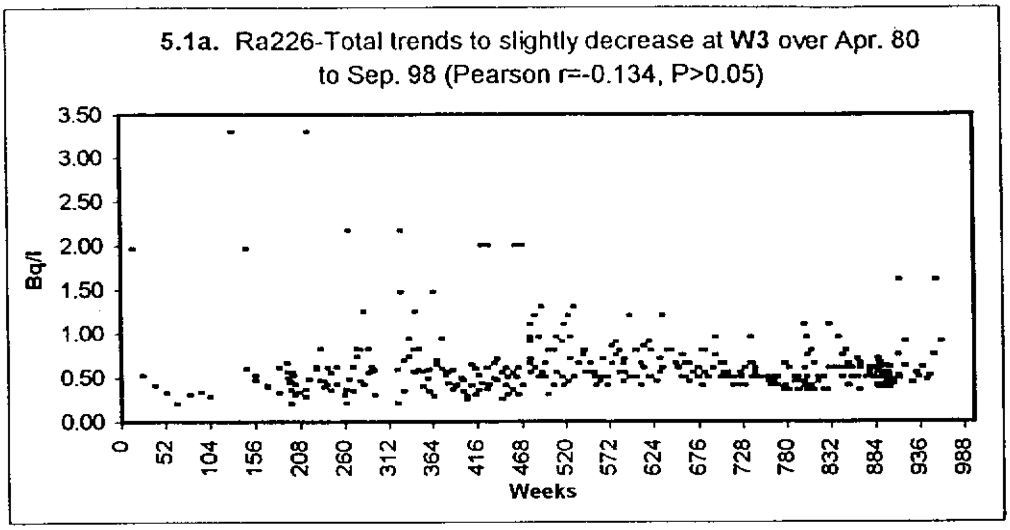
Table 5.2. Descriptive Statistics of four major water quality parameters at eight locations.

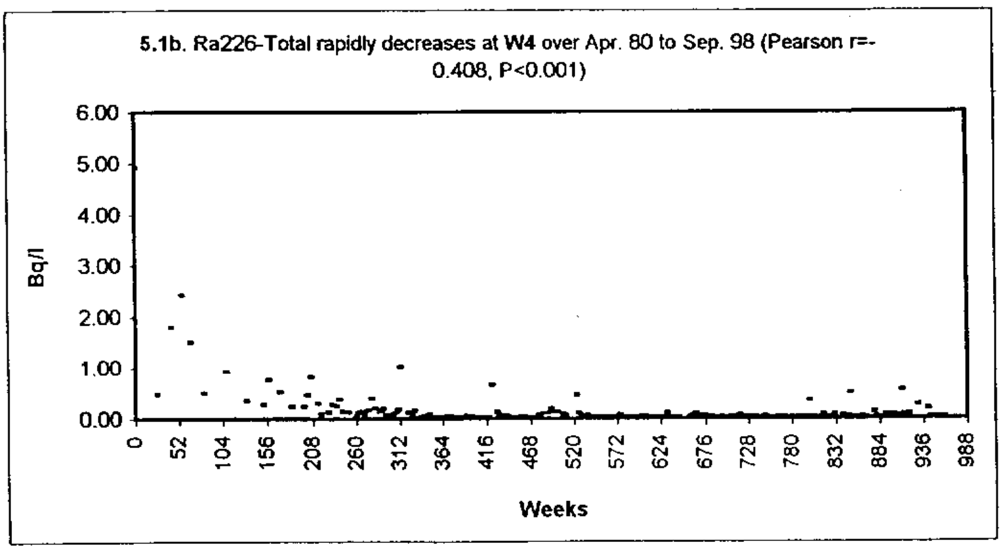
W3	N	Data Missing %	Max	Min	Average(A)	StDev(S)	S/A	Medians
Ra226-total	194	2.51	3.30	0.20	0.62	0.36	43.50	0.54
U-total	195	2.01	28.00	0.05	7.42	4.10	7.41	6.85
pH	190	4.52	8.70	6.10	7.04	0.36	0.05	7.10
TSS	193	3.02	74.00	0.20	2.62	6.31	0.08	1.00
W4	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	185	2.63	4.93	0.01	0.16	0.45	2.78	0.04
U-total	185	2.63	9.20	0.00	0.16	0.80	5.08	0.03
pH	179	5.79	10.50	6.10	6.95	0.53	0.08	7.20
TSS	181	4.74	389.00	0.40	11.42	42.44	3.72	2.00
Temp(C)	159	16.32	24.00	-0.50	7.89	6.28	0.80	6.00
W5	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	186	2.11	1.60	0.01	0.30	0.23	0.77	0.25
U-total	186	2.11	27.00	0.01	3.71	3.19	0.86	3.36
pH	167	12.11	8.40	6.00	7.05	0.36	0.05	7.20
TSS	183	3.68	210.80	0.60	7.44	23.67	3.18	2.00
W9	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	81	2.41	1.30	0.06	0.24	0.20	0.83	0.18
U-total	80	3.61	18.40	0.20	2.55	2.56	1.00	1.64
pH	55	33.73	7.90	6.20	6.73	0.31	0.05	6.80
TSS	76	8.43	2030.00	1.00	31.84	232.52	7.30	2.00
W14	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	123	2.38	1.10	0.12	0.41	0.22	0.52	0.35
U-total	123	2.38	4.40	0.34	1.12	0.77	0.69	0.86
pH	109	13.49	9.30	5.90	6.75	0.76	0.11	7.00
TSS	122	3.17	7.00	0.60	2.44	1.29	0.53	2.00
W15	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	139	2.80	0.80	0.13	0.36	0.14	0.40	0.30
U-total	139	2.80	3.25	0.28	1.00	0.62	0.62	0.75
pH	128	10.49	9.60	5.80	6.71	0.66	0.10	7.00
TSS	135	5.59	28.00	0.40	3.11	3.39	1.09	2.00
W20	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	107	2.73	0.90	0.06	0.20	0.12	0.63	0.17
U-total	107	2.73	22.10	0.10	1.16	3.16	2.71	0.42
pH	80	27.27	9.20	5.90	6.72	0.48	0.07	6.85
TSS	99	10.00	1110.00	1.00	15.27	112.41	7.36	1.50
W25	N	Data Missing	Max	Min	Average	StDev	S/A	Medians
Ra226-total	139	1.42	0.60	0.01	0.11	0.10	0.87	0.08
U-total	137	2.84	2.21	0.06	0.30	0.32	1.08	0.17
pH	122	13.48	8.70	5.90	6.67	0.55	0.08	6.90
TSS	132	6.38	18.00	0.20	3.30	3.39	1.03	2.00

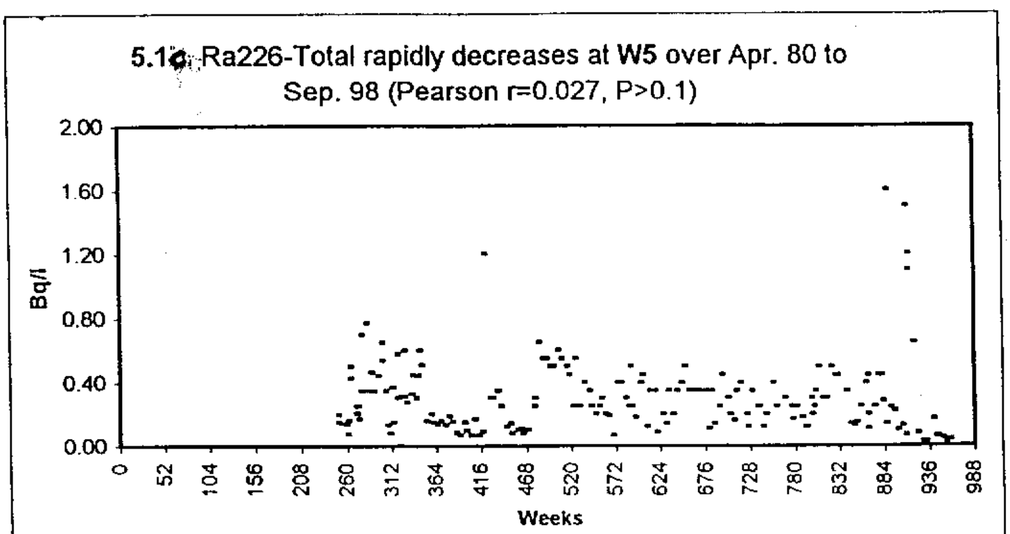
Table 5.3. Occurrences of Phytoplankton Genera/taxa in Link Lakes (1989-1995).

Taxa	Occurrences	%	Taxa	Occurrences	%
Scenedesmus	18	85.7	Melosiria islandica	4	19.0
Cryptomonas	17	81.0	Oedogonium	4	19.0
Unidentified chlorophyte	16	76.2	Coelosphaerium	3	14.3
Euglena	15	71.4	Stigeoclonium	3	14.3
Asterionella formosa	14	66.7	Trachelomonas	3	14.3
Planktospheria	13	61.9	Chroomonas	2	9.5
Tabellaria	13	61.9	Eudorina	2	9.5
Unidentified chrysophyte	13	61.9	Gloeocystis	2	9.5
Oscillatoria tenuis	12	57.1	Gomphosphaeria	2	9.5
Cryptomonas rostriformis	11	52.4	Mallomonas	2	9.5
Staurastrum	11	52.4	Oocystis submarina	2	9.5
Dinobryon	10	47.6	Rhizosolenia longiseta	2	9.5
Mougeotia	10	47.6	Schroederia setigera	2	9.5
Synedra	10	47.6	Selenastrum	2	9.5
Aphanizomenon	9	42.9	Spondylosium	2	9.5
Navicula	9	42.9	Stauroneis	2	9.5
Nitzschia	9	42.9	Acnanthes linearis	1	4.8
Pinnularia	9	42.9	Amphirora	1	4.8
Ankistrodesmus	8	38.1	Bitrichia chodatii	1	4.8
Ochromonas	8	38.1	Botryococcus braunii	1	4.8
Ceratium hirundinella	7	33.3	Carteria	1	4.8
Chlamydomonas	7	33.3	Chlorogonium	1	4.8
Pediastrum	7	33.3	Chromulina	1	4.8
Peridinium	7	33.3	Crucigenia	1	4.8
Closterium	6	28.6	Euastrum humerosum	1	4.8
Cosmarium	6	28.6	Frustulia rhomboides	1	4.8
Gymnodinium	6	28.6	Gonium	1	4.8
Quadrigula lacustris	6	28.6	Hyalotheca dissiliens	1	4.8
Rhodomonas	6	28.6	Lagerheimia	1	4.8
Spirogyra	6	28.6	Lyngbya	1	4.8
Unidentified cyanophyte	6	28.6	Merismopedia tenuissi	1	4.8
Anabaena	5	23.8	Micractinium	1	4.8
Phacus	5	23.8	Micropora	1	4.8
Synura	5	23.8	Neidium	1	4.8
Coelastrum microporum	4	19.0	Nephrocytium obesum	1	4.8
Cymbella	4	19.0	Netrium	1	4.8
Dictyosphaerium pulchellu	4	19.0	Pandorina	1	4.8
Eunotia	4	19.0	Zygogonium/Zygonem	1	4.8

Figures 5.1a-c. Long-Term Changes in Ra^{226} Concentrations at W

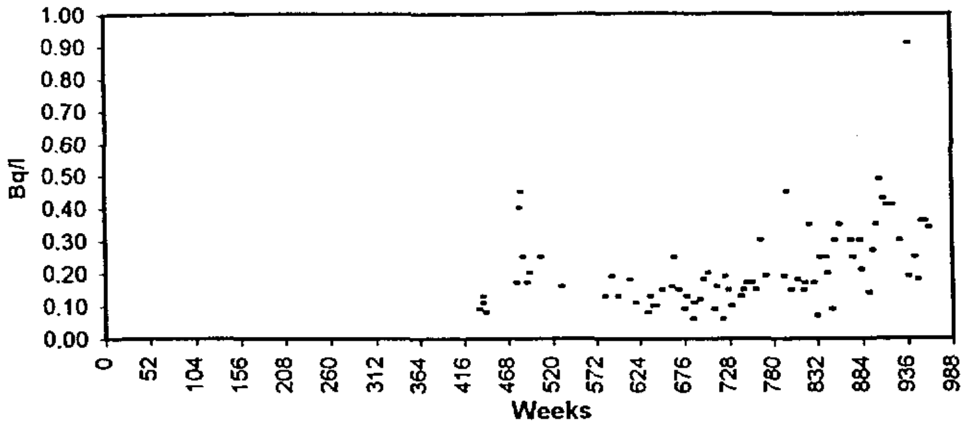




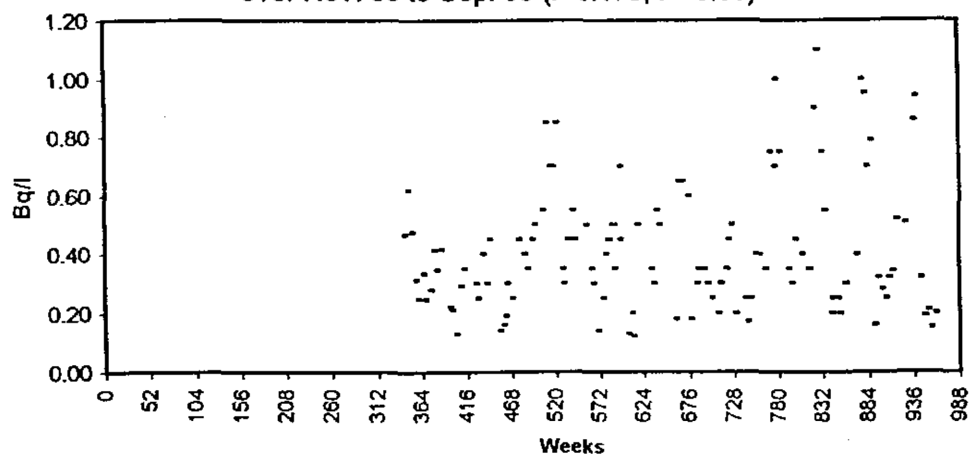


Figures 5.1d-f. Long-Term Changes in Ra^{226} Concentrations at W

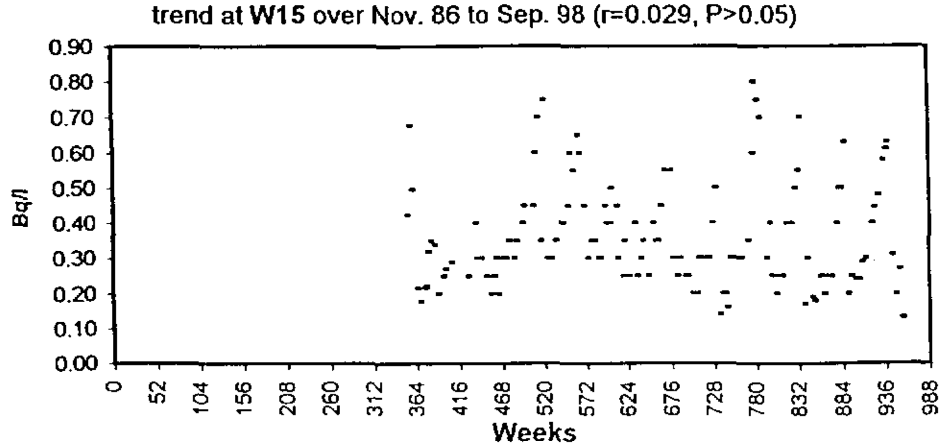
5.1d. Ra226-Total increases slightly, but significantly at **W9** over Jul. 88 to Sep. 98 (r=0.416, P<0.01)



5.1e. Ra226-Total increases slightly, but significantly at **W14** over Nov. 86 to Sep. 98 (r=0.170, P>0.05)

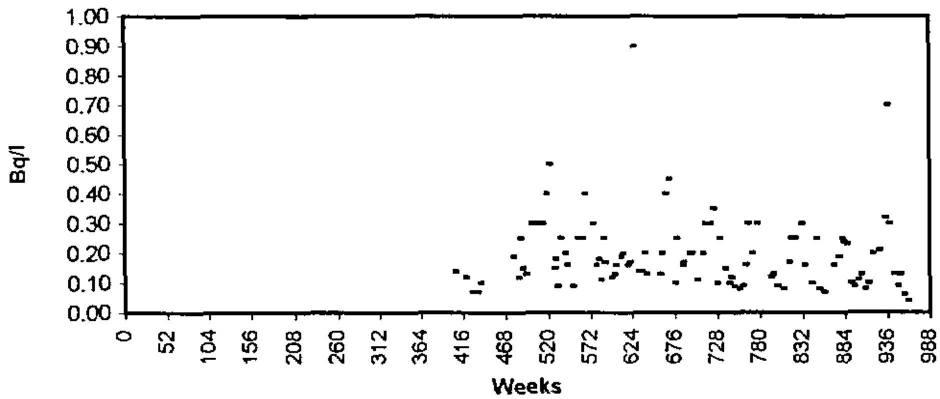


5.1f: Ra226-Total varies irregularly, without a significant time-trend at **W15** over Nov. 86 to Sep. 98 (r=0.029, P>0.05)

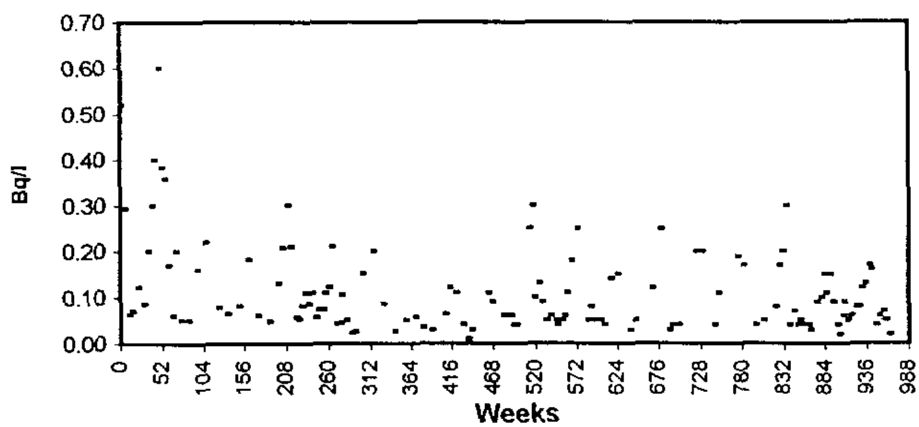


Figures 5.1g and h. Long-Term Changes in Ra^{226} Concentrations at W

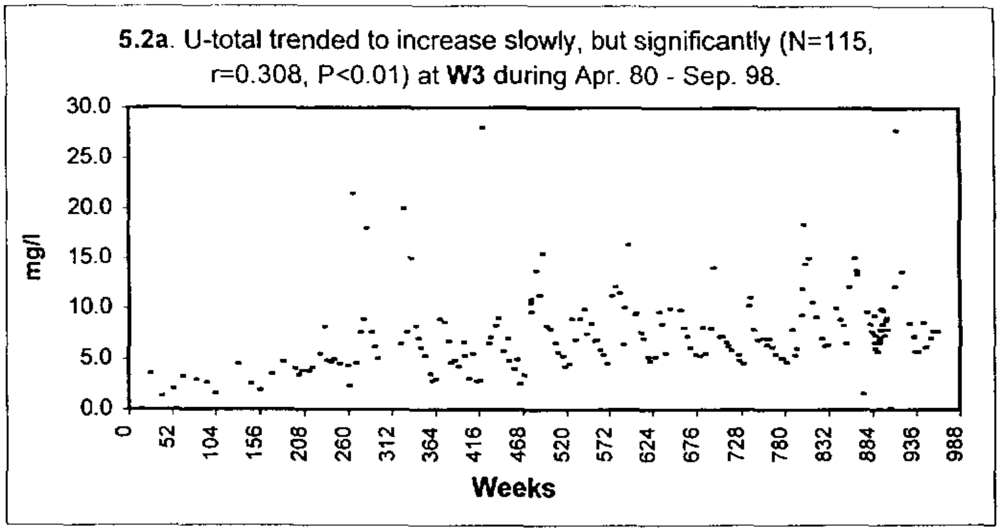
5.1g. Ra226-Total was relatively stable, without a significant time-trend at **W20** over Feb. 88 to Sep. 98 (r=-0.09, P>0.1)

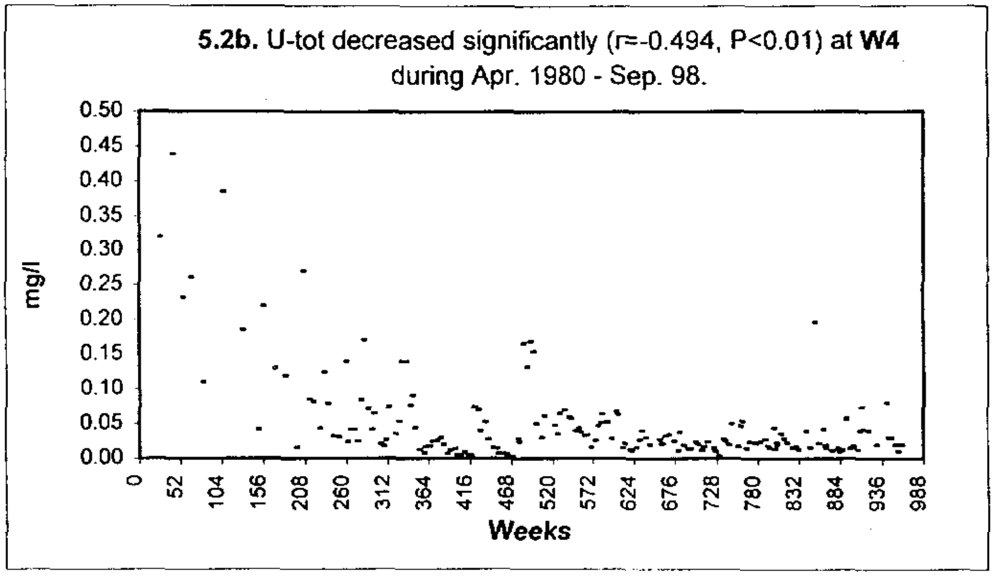


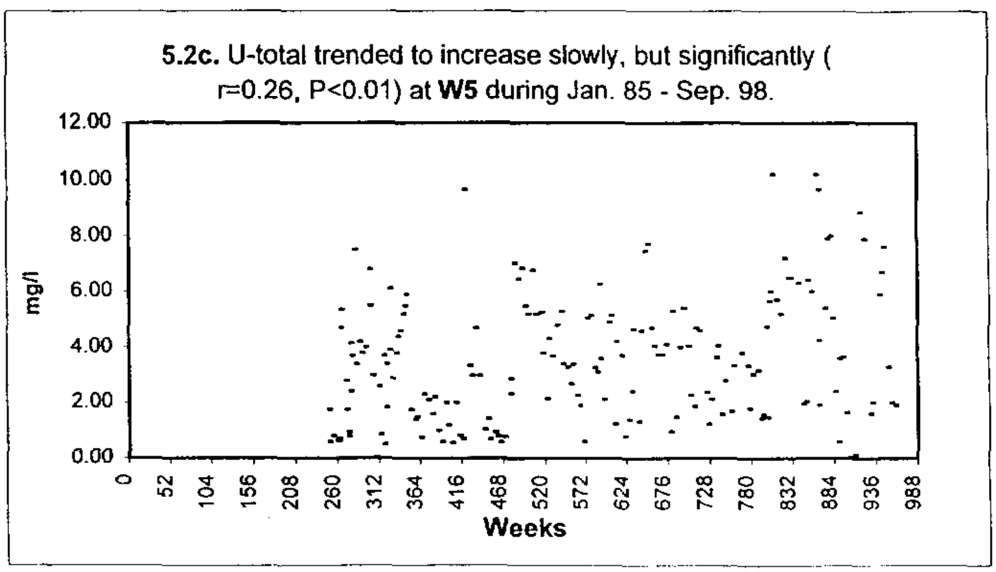
5.1h. Ra226-Total decreased slightly, but significantly at **W25** over Mar. 80 to Sep. 98 (r=-0.306, P<0.001)



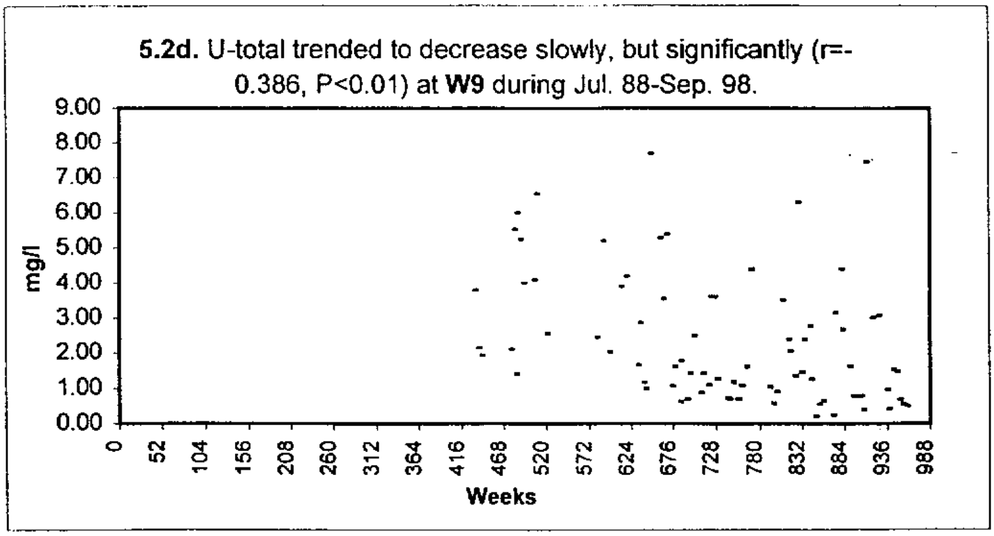
Figures 5.2a-c. Long-Term Changes in U Concentrations at W3-5, Link Lake

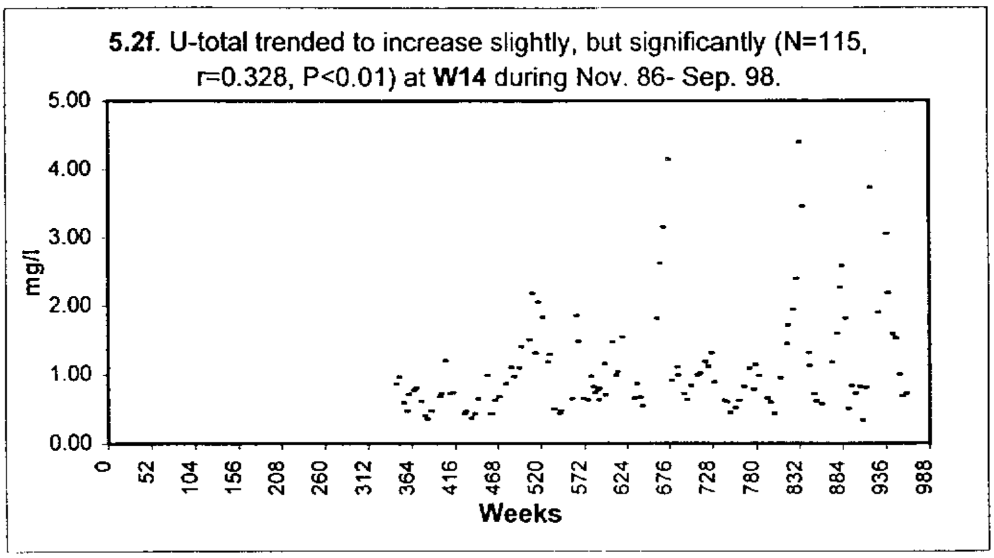


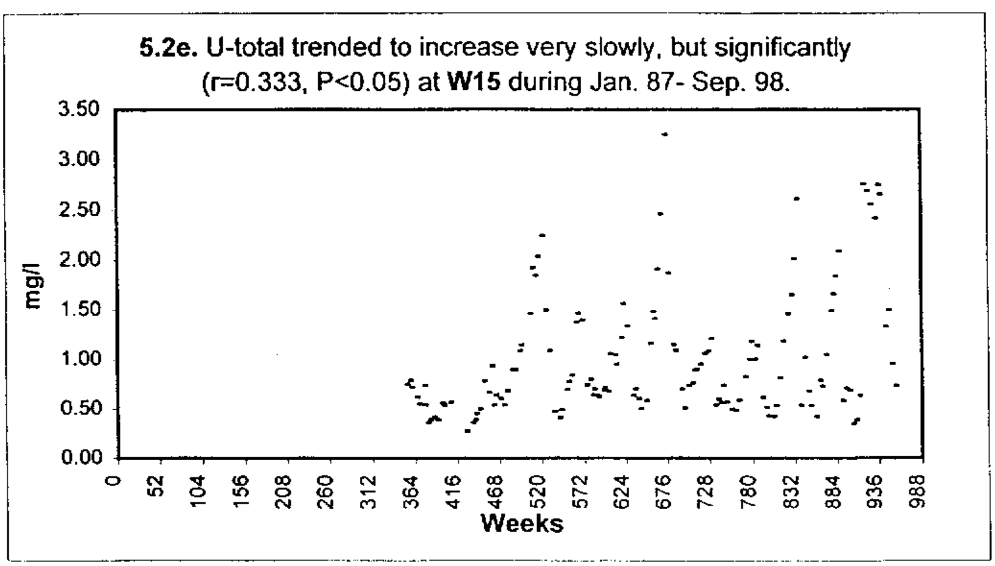




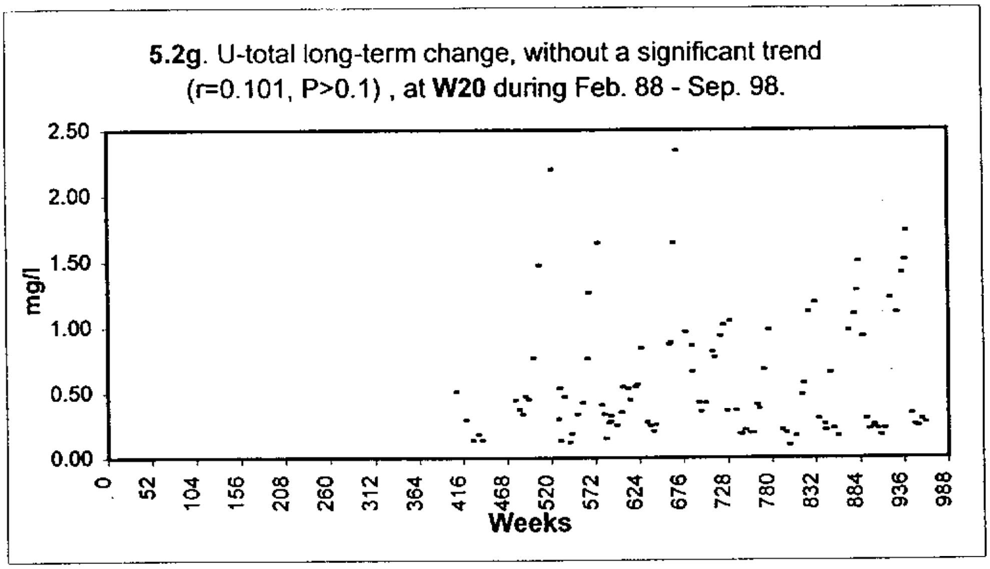
Figures 5.2d-f. Long-Term Changes in U Concentrations at W9, W14, W15, Link Lake







Figures 5.2g and h. Long-Term Changes in U Concentrations at W20, W25, Link Lake



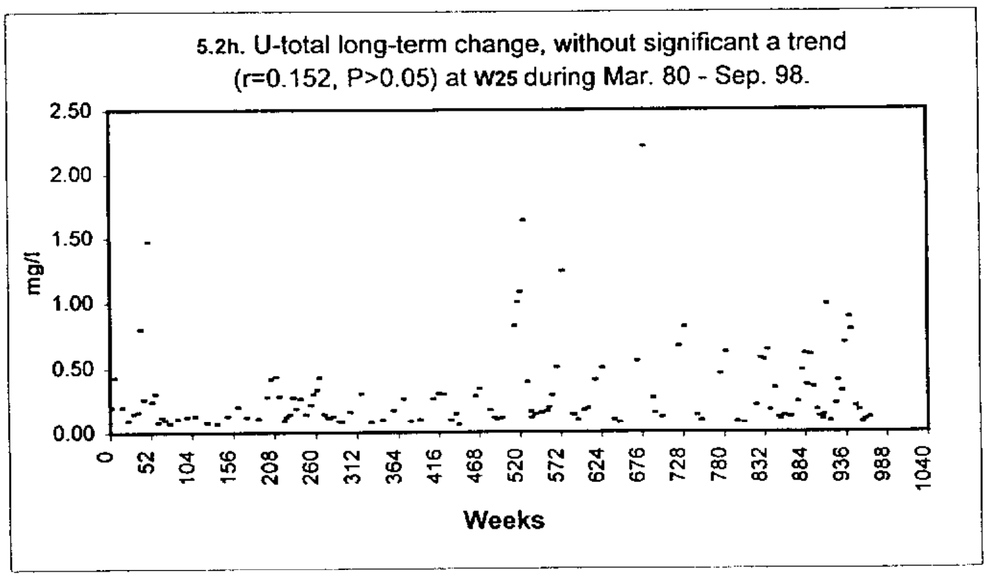
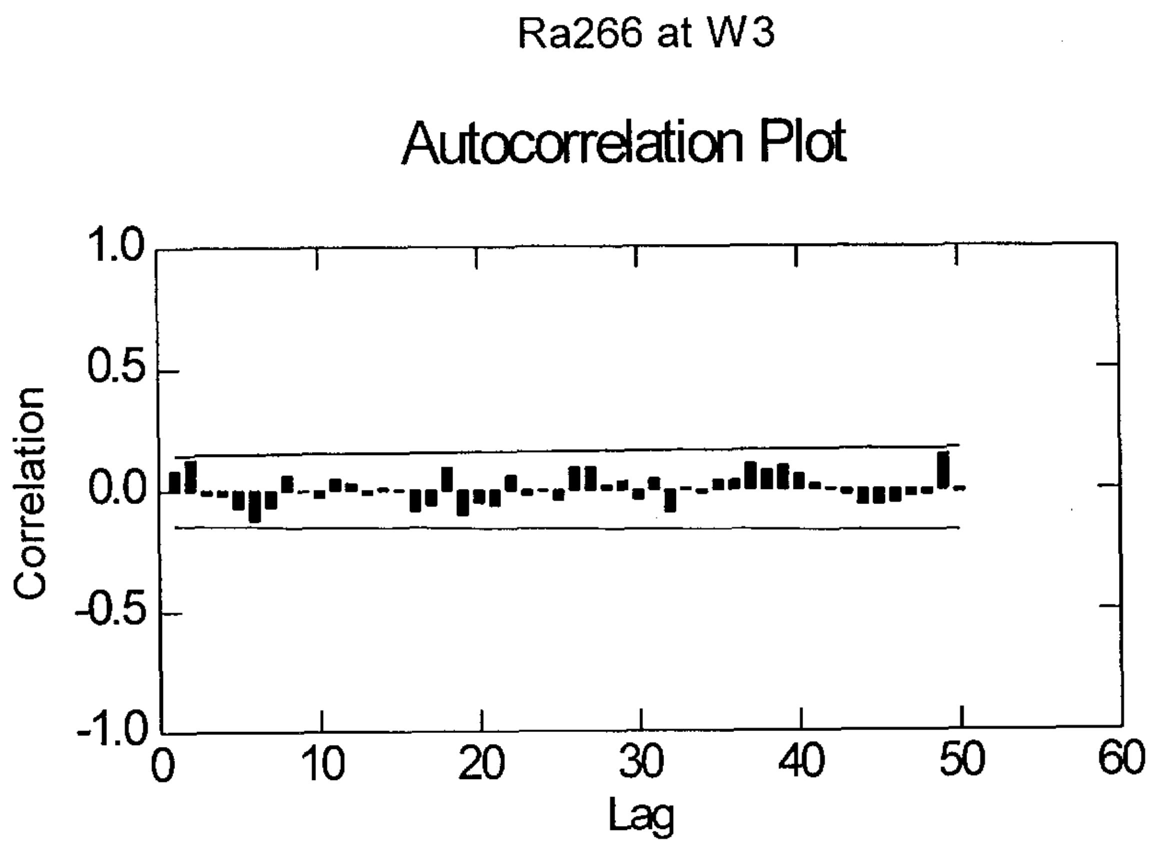


Figure 5.3a. Ra^{226} Auto-correlation and its Cross-Correlation with pH



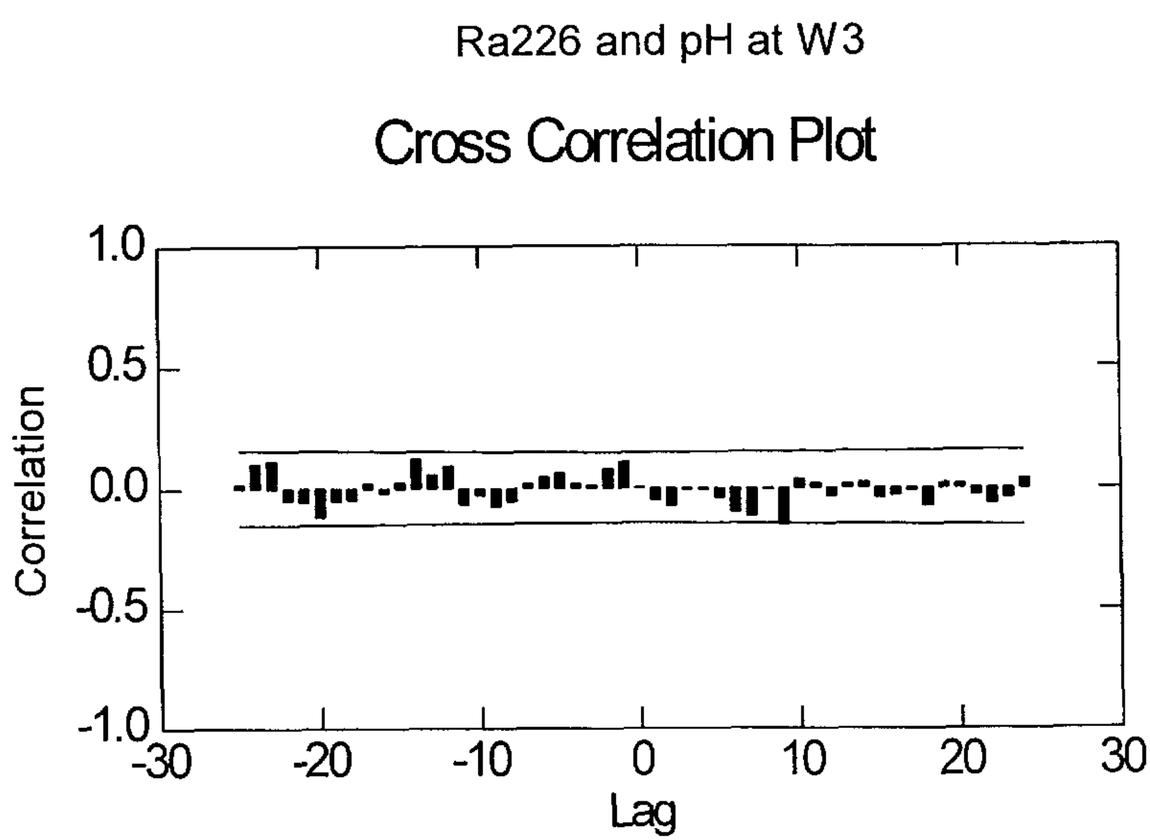
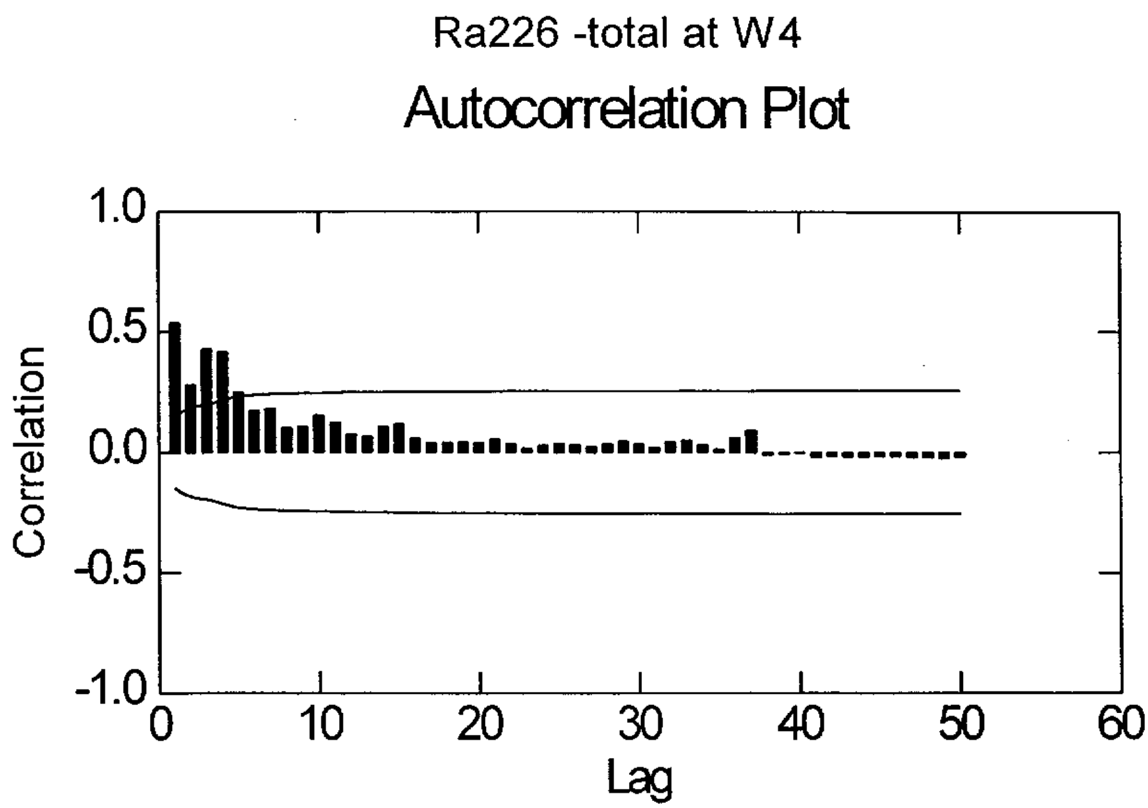


Figure 5.3b. Ra^{226} Auto-correlation and its Cross-Correlation with pH



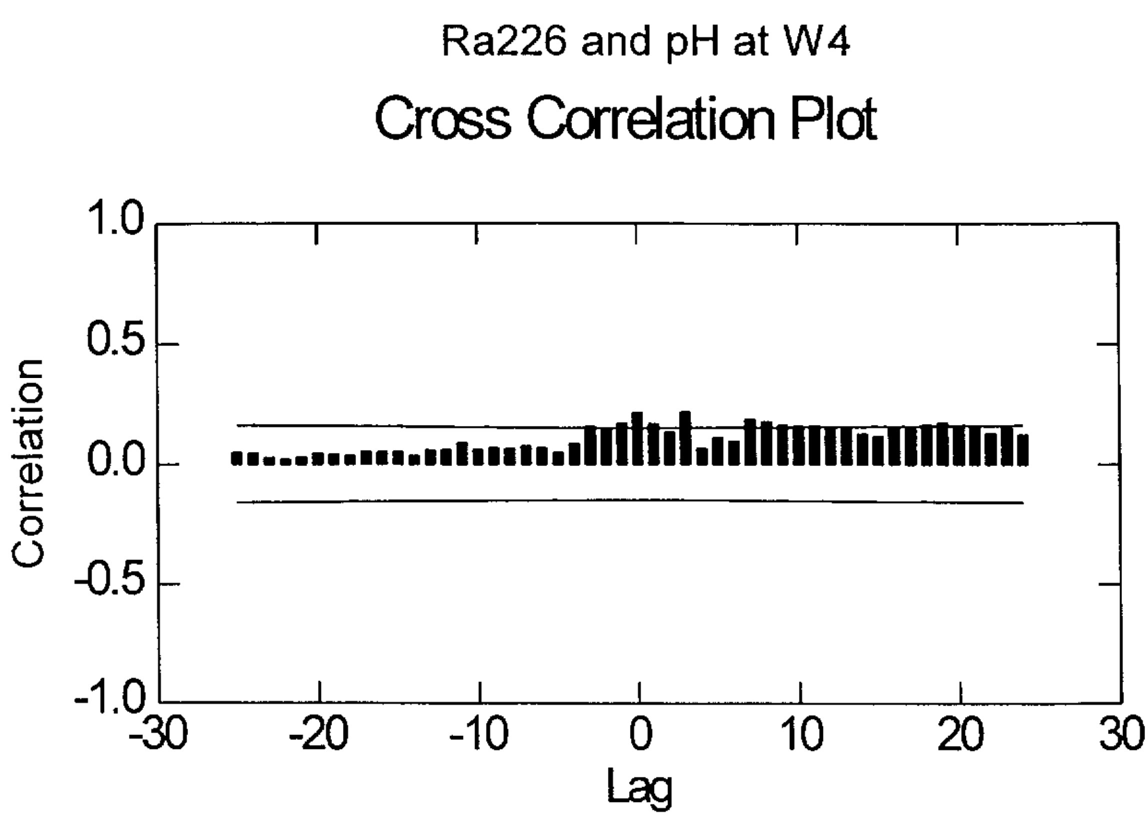
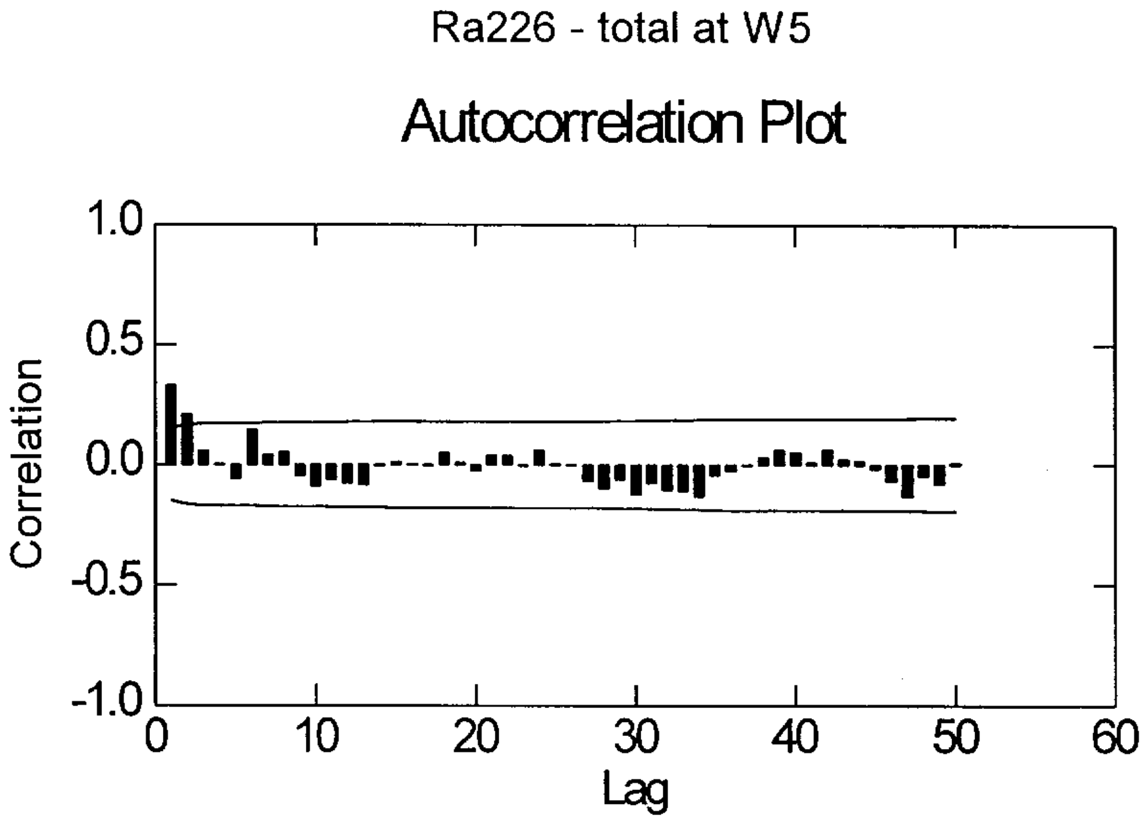


Figure 5.3c. Ra^{226} Auto-correlation and its Cross-Correlation with pH



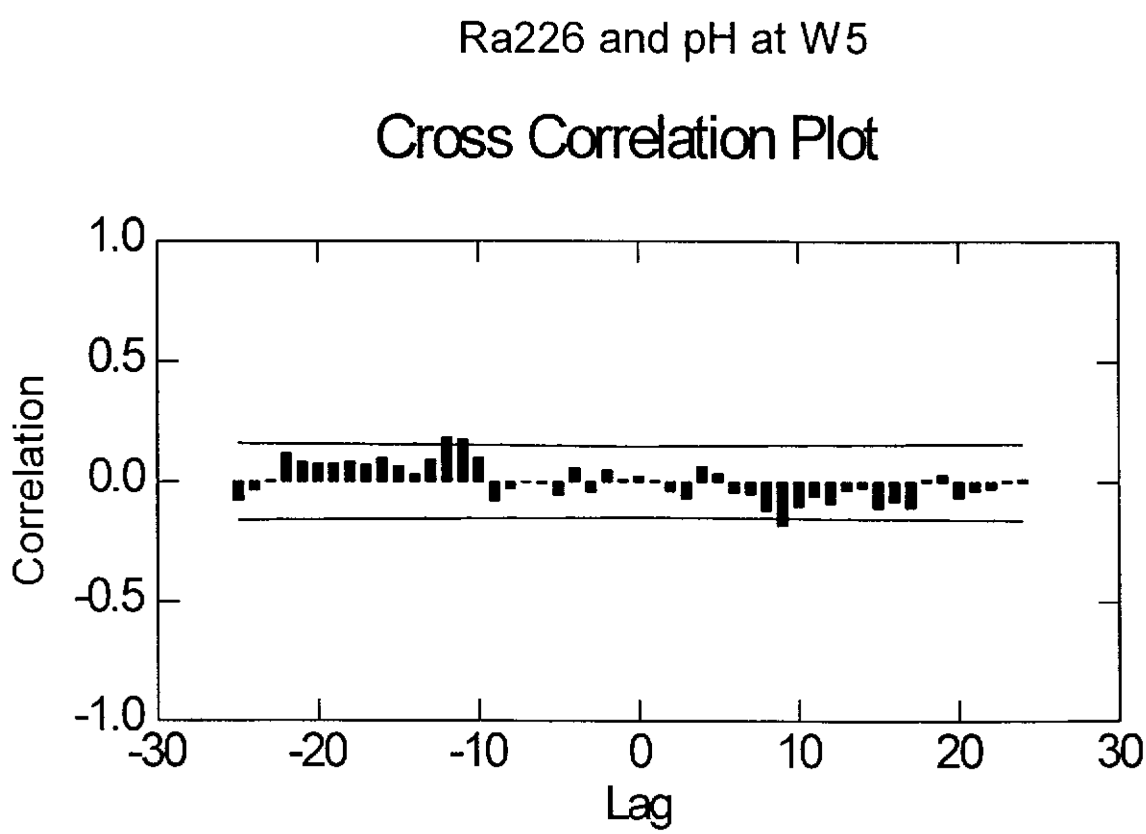
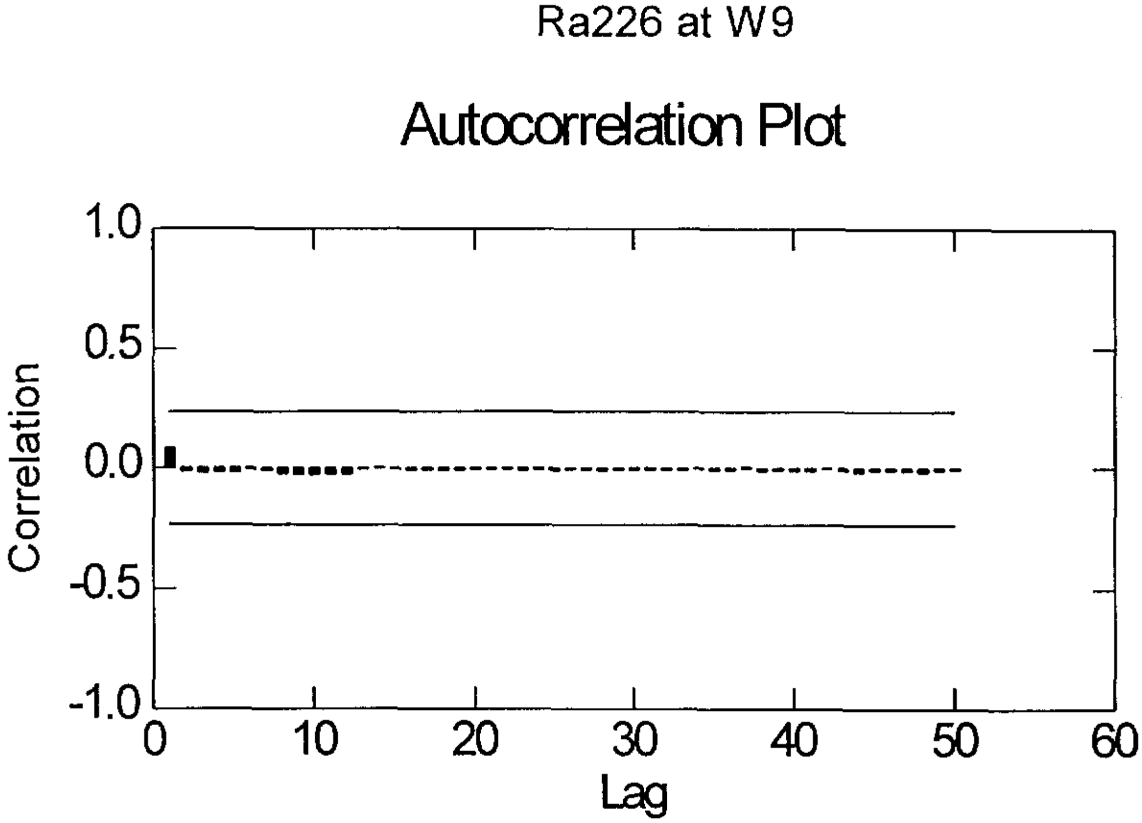


Figure 5.3d. Ra^{226} Auto-correlation and its Cross-Correlation with pH



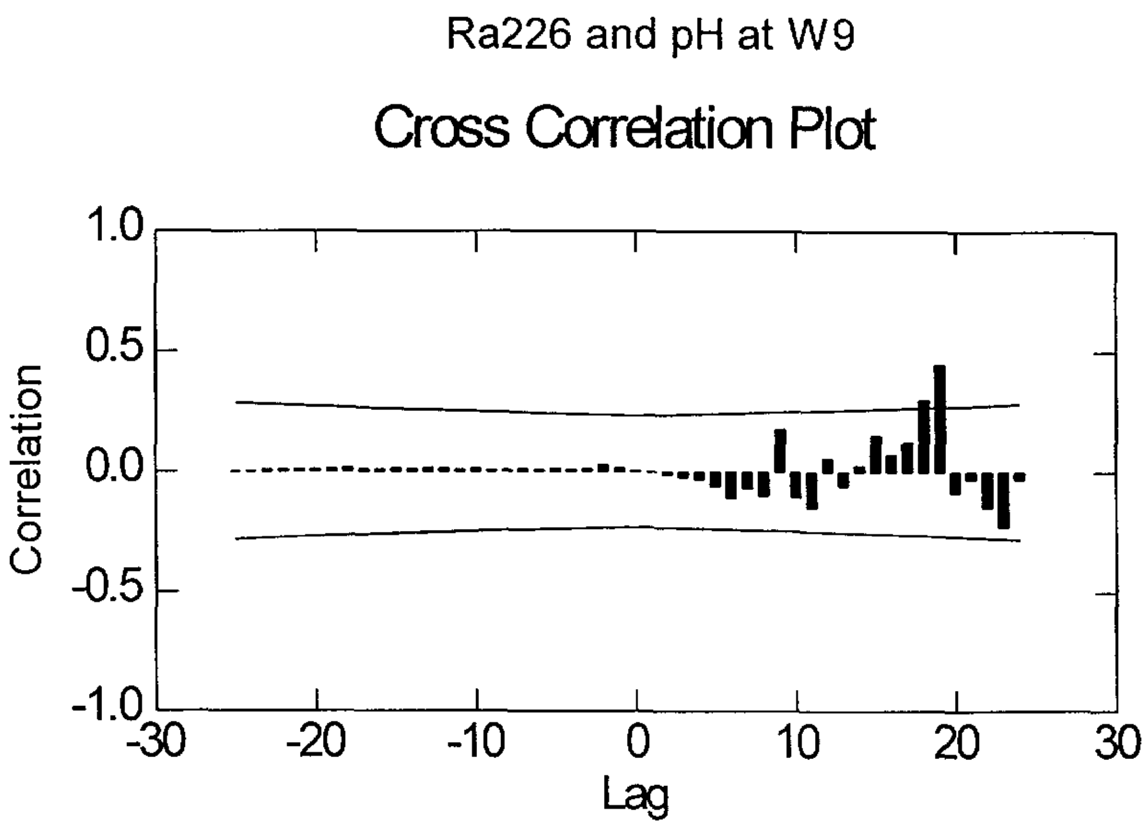
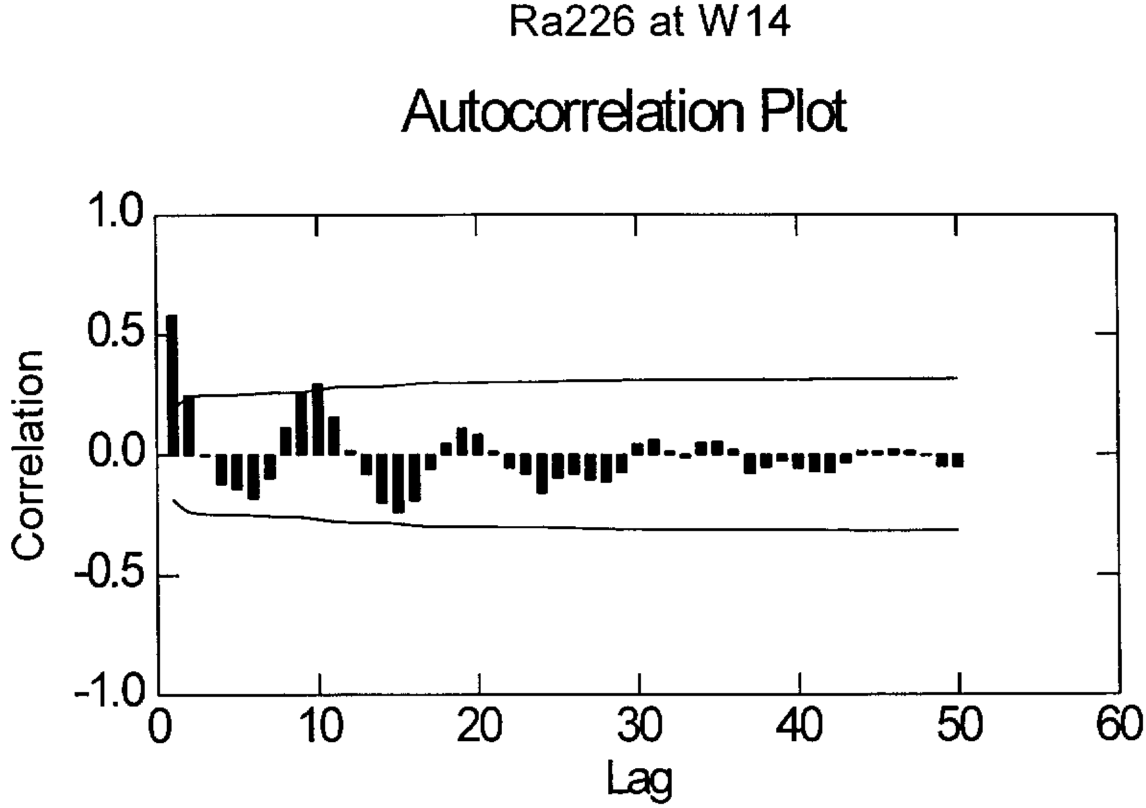


Figure 5.3e. Ra^{226} Auto-correlation and its Cross-Correlation with pH



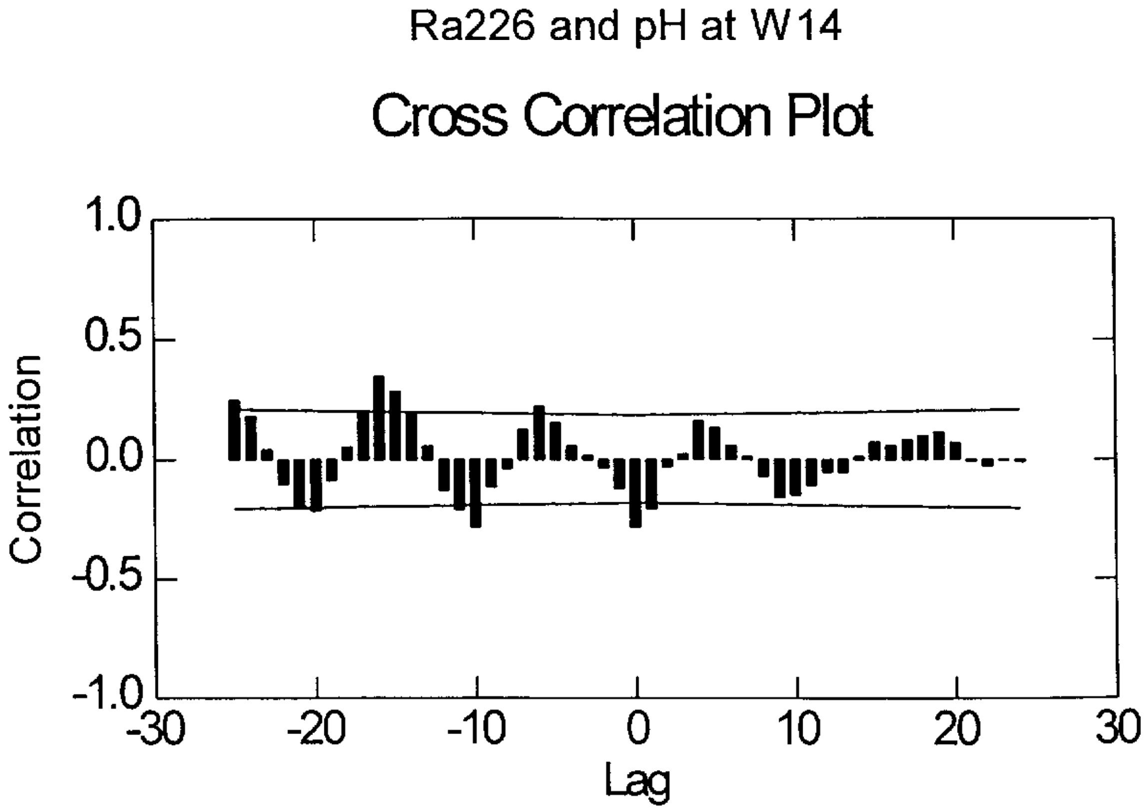
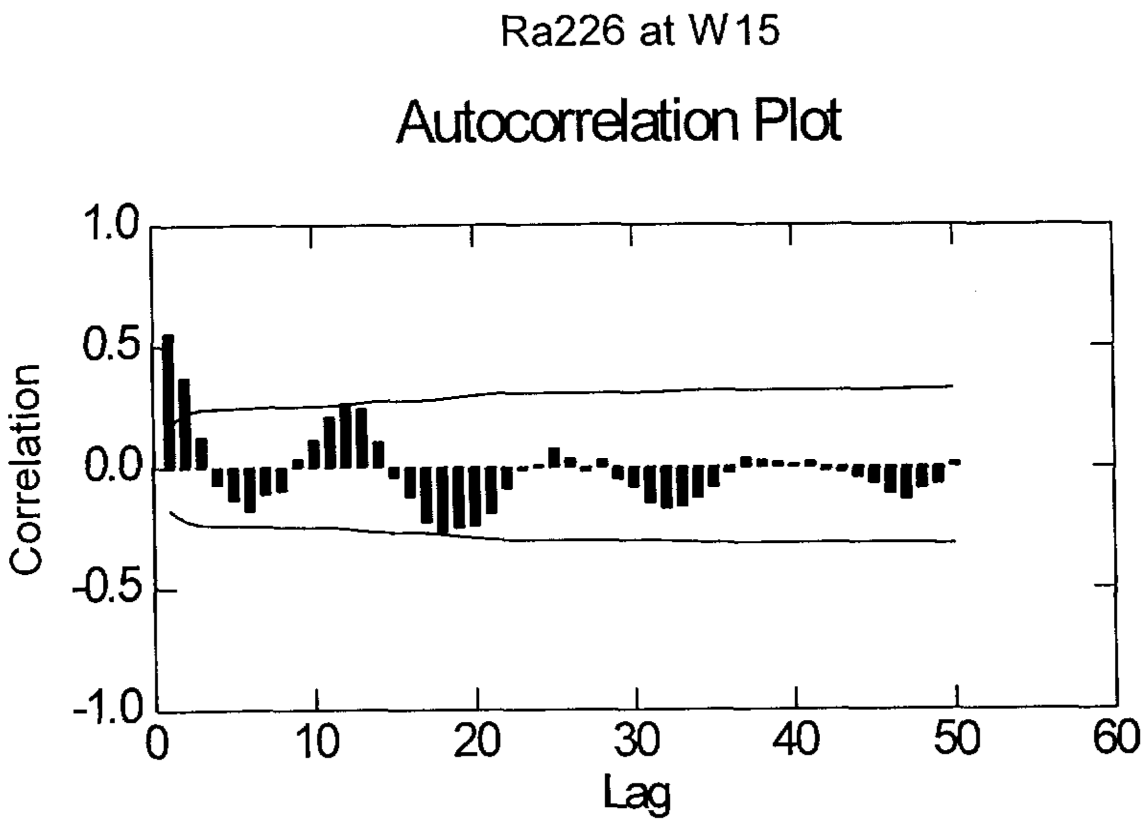


Figure 5.3f. Ra^{226} Auto-correlation and its Cross-Correlation with pH



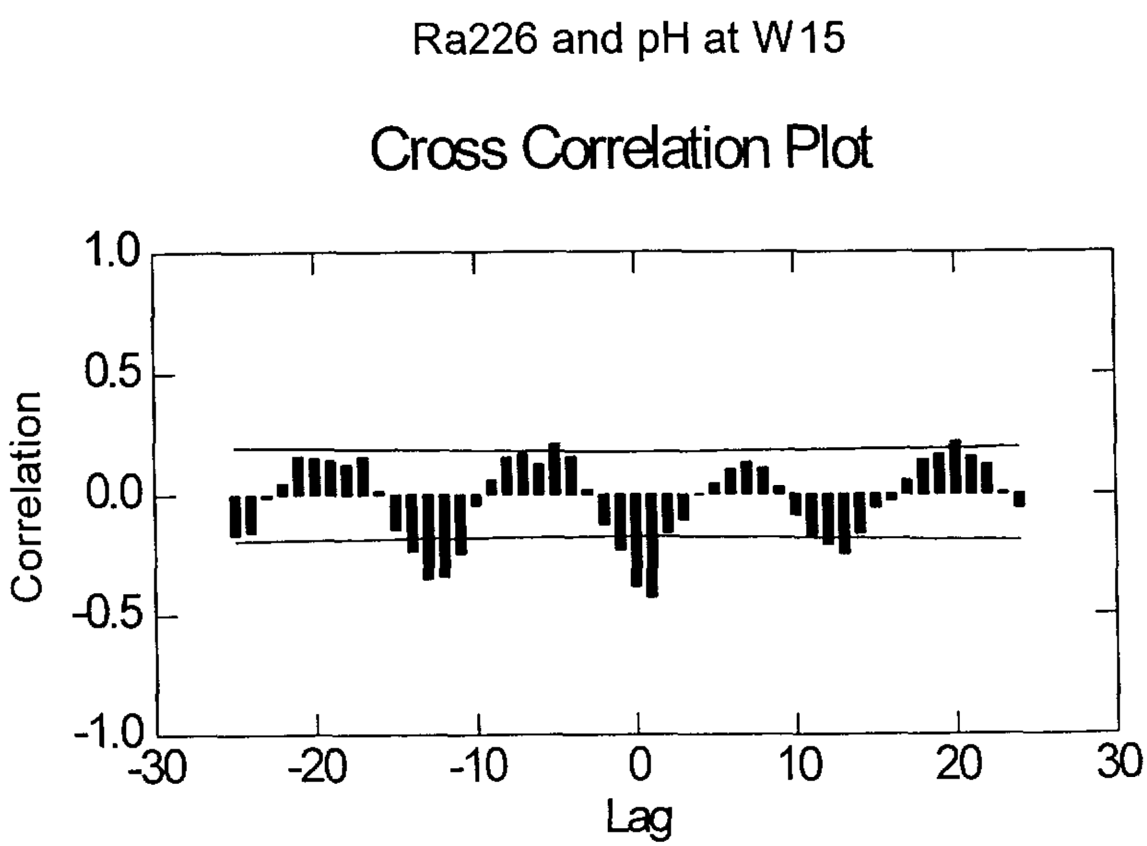
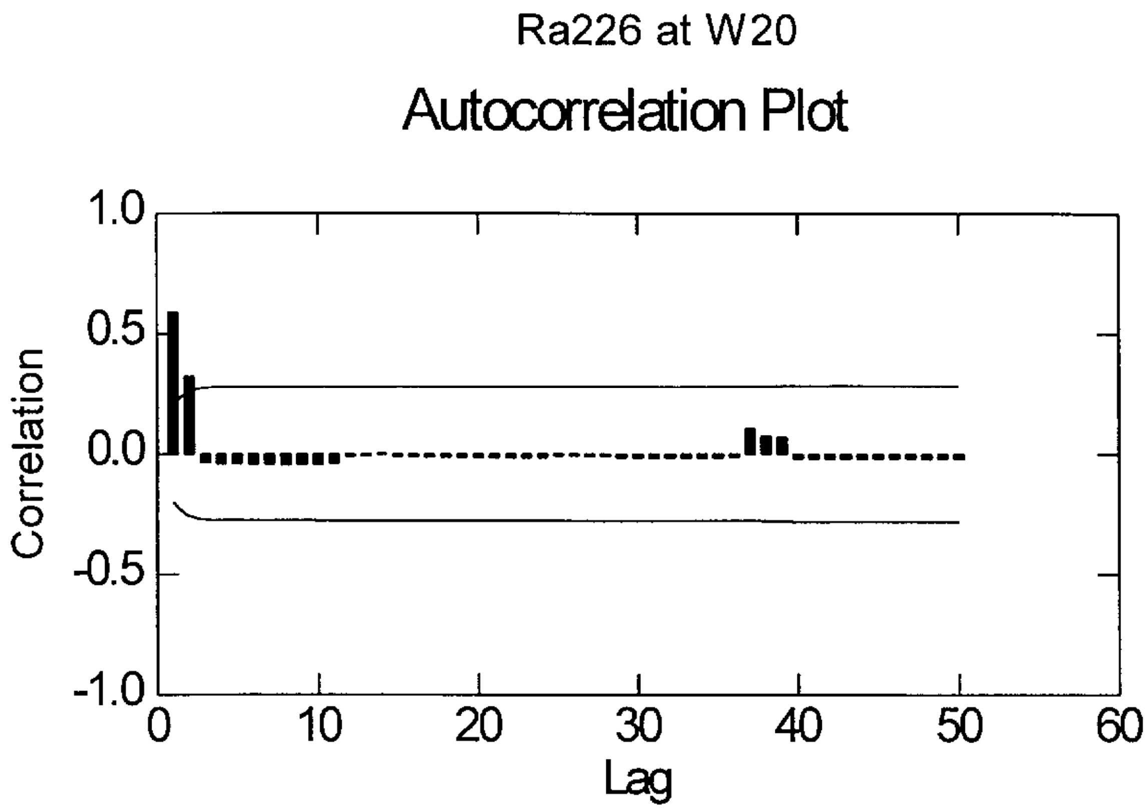


Figure 5.3g. Ra^{226} Auto-correlation and its Cross-Correlation with pH



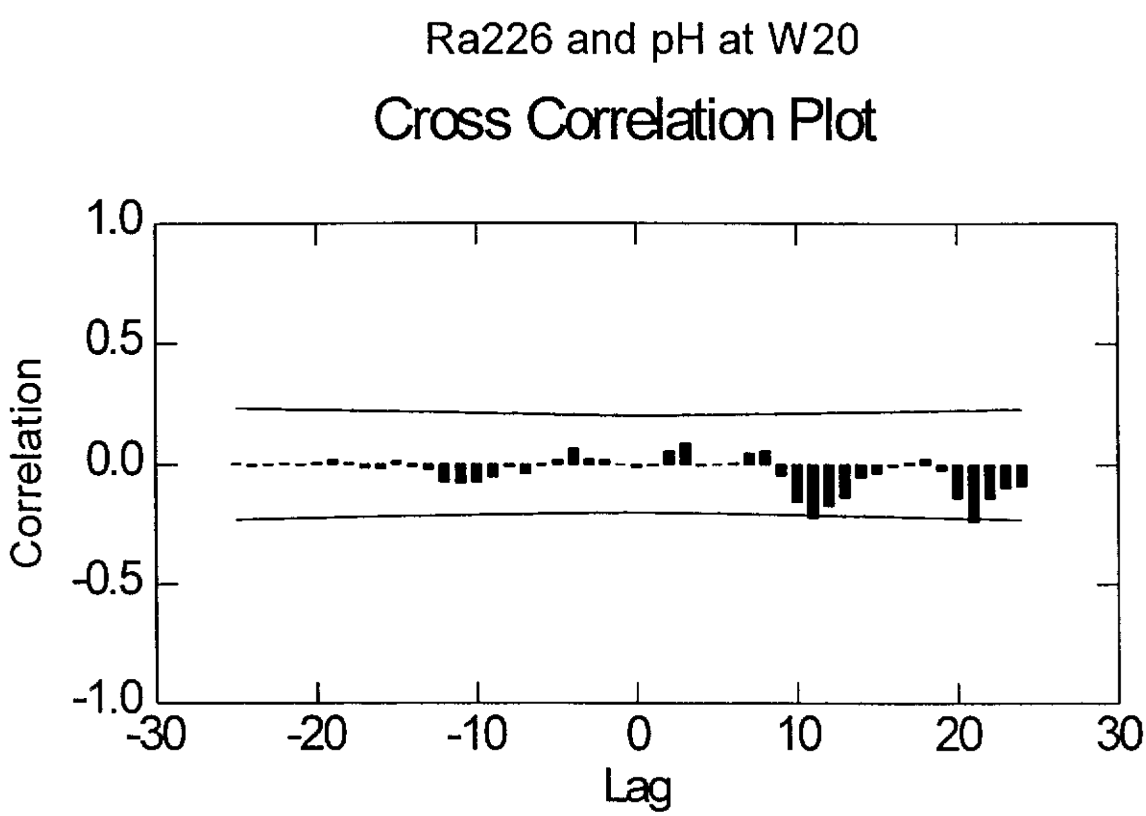
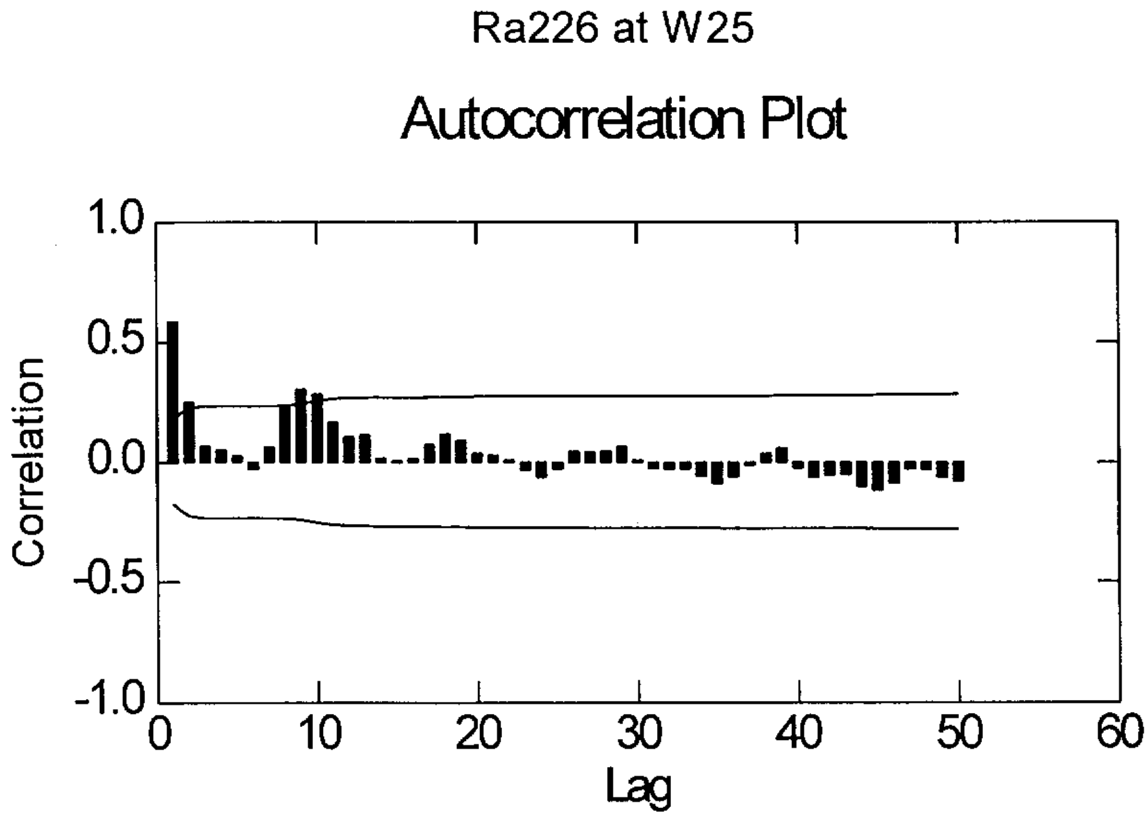


Figure 5.3h. Ra^{226} Auto-correlation and its Cross-Correlation with pH



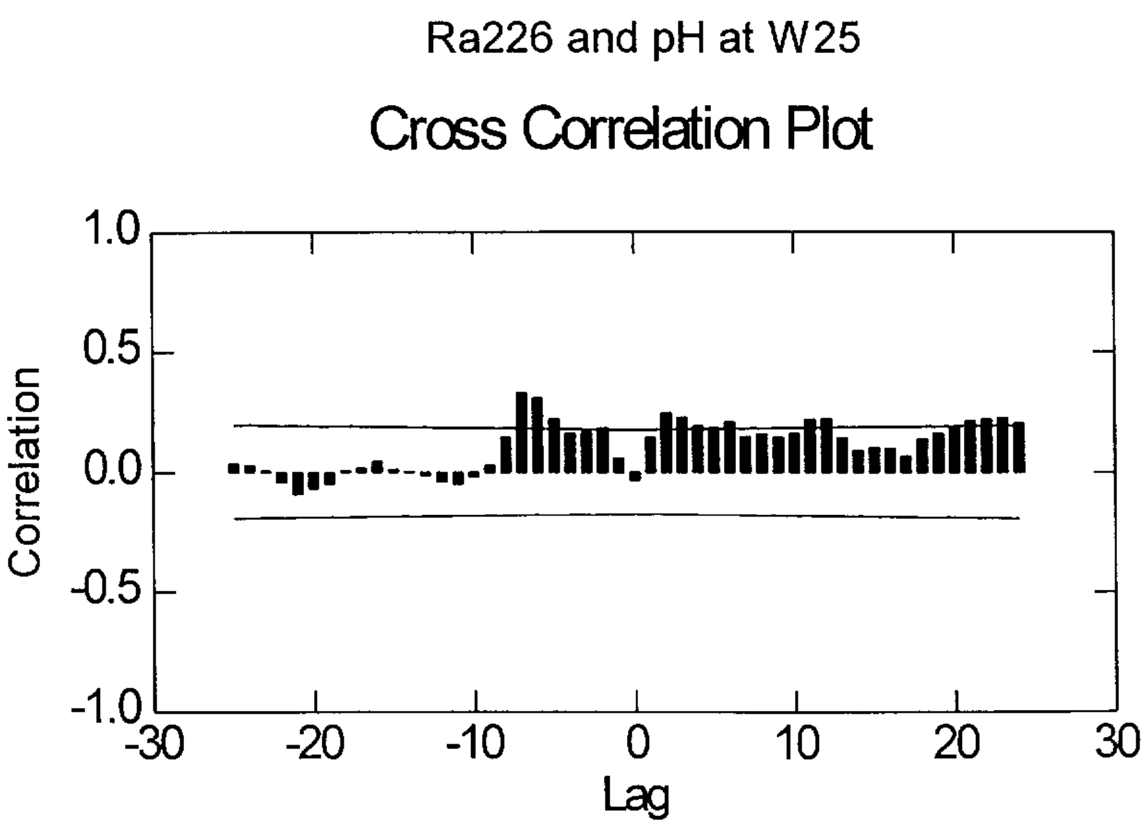
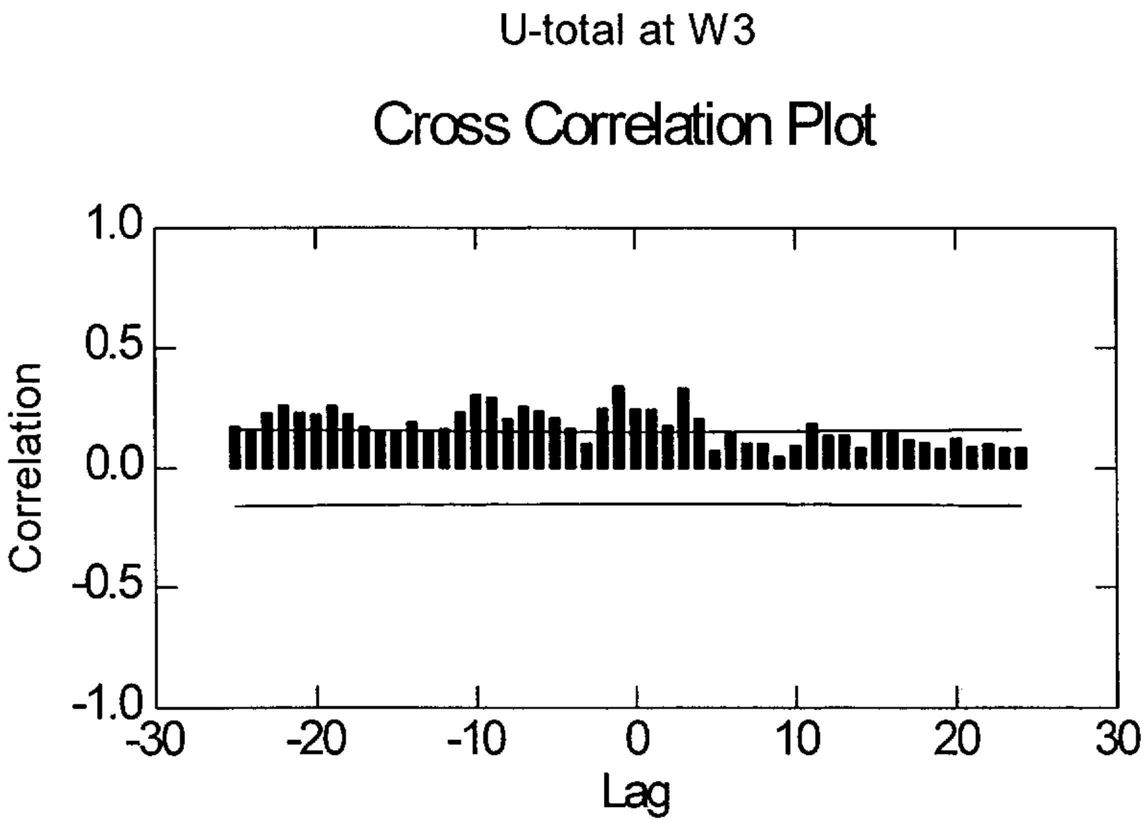


Figure 5.4a. Uranium Auto-Correlation and its Cross-Correlation with pH



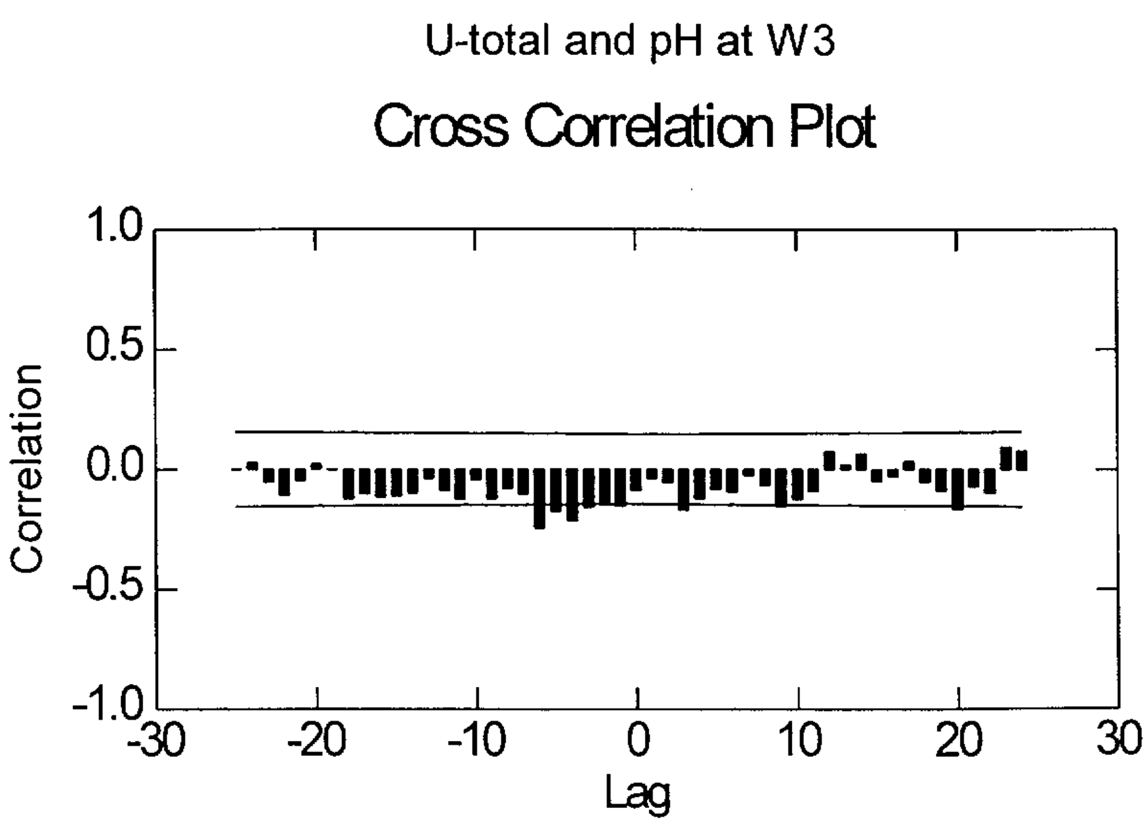
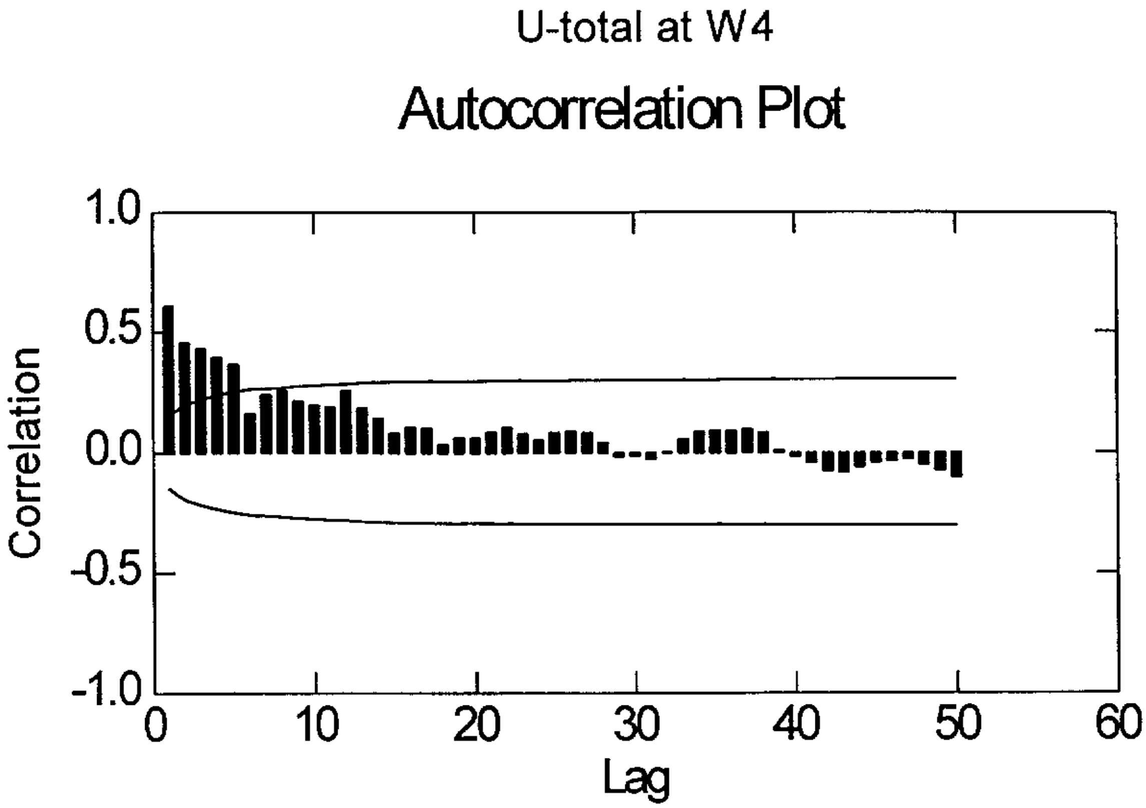


Figure 5.4b. Uranium Auto-Correlation and its Cross-Correlation with pH



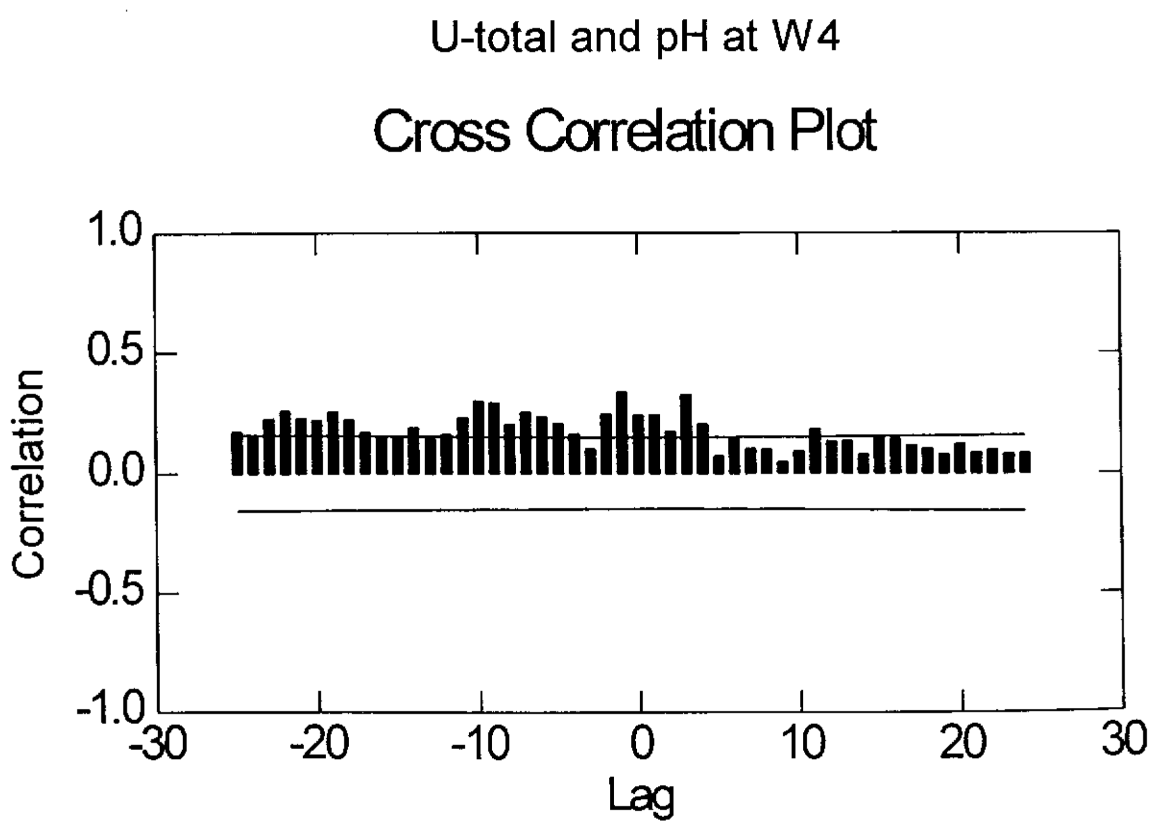
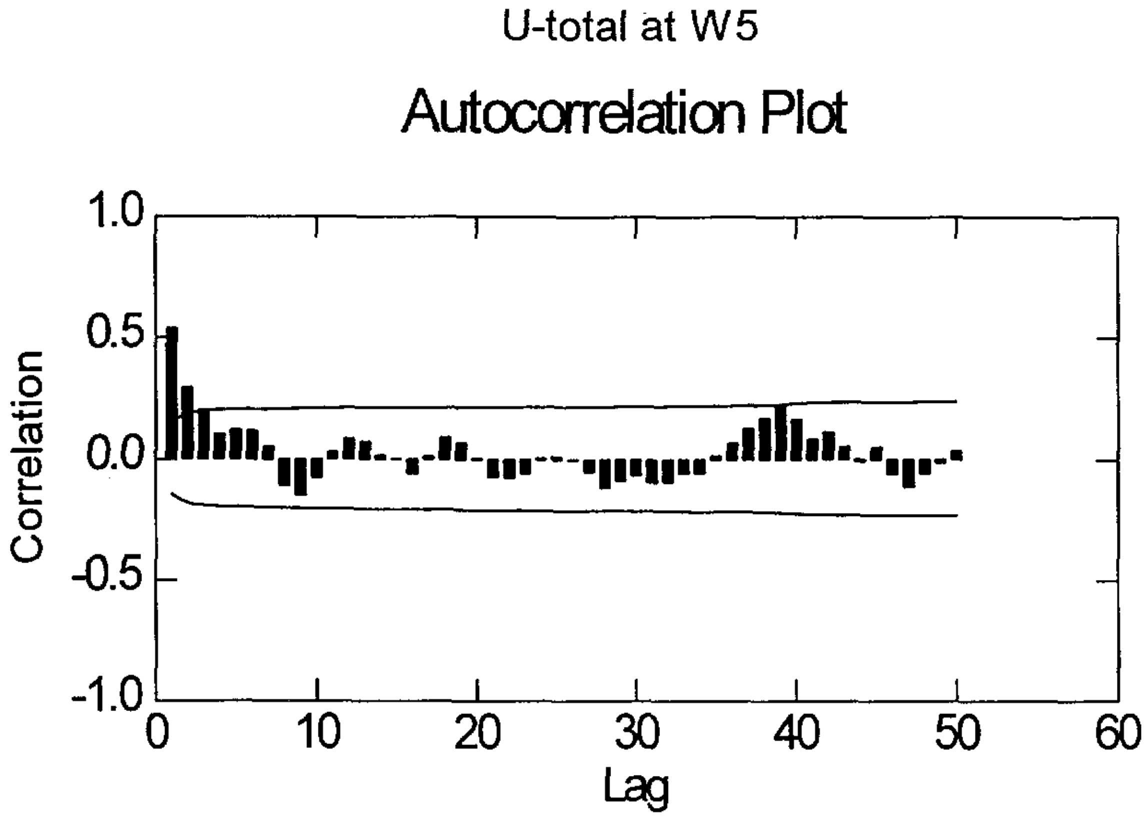


Figure 5.4c. Uranium Auto-Correlation and its Cross-Correlation with pH



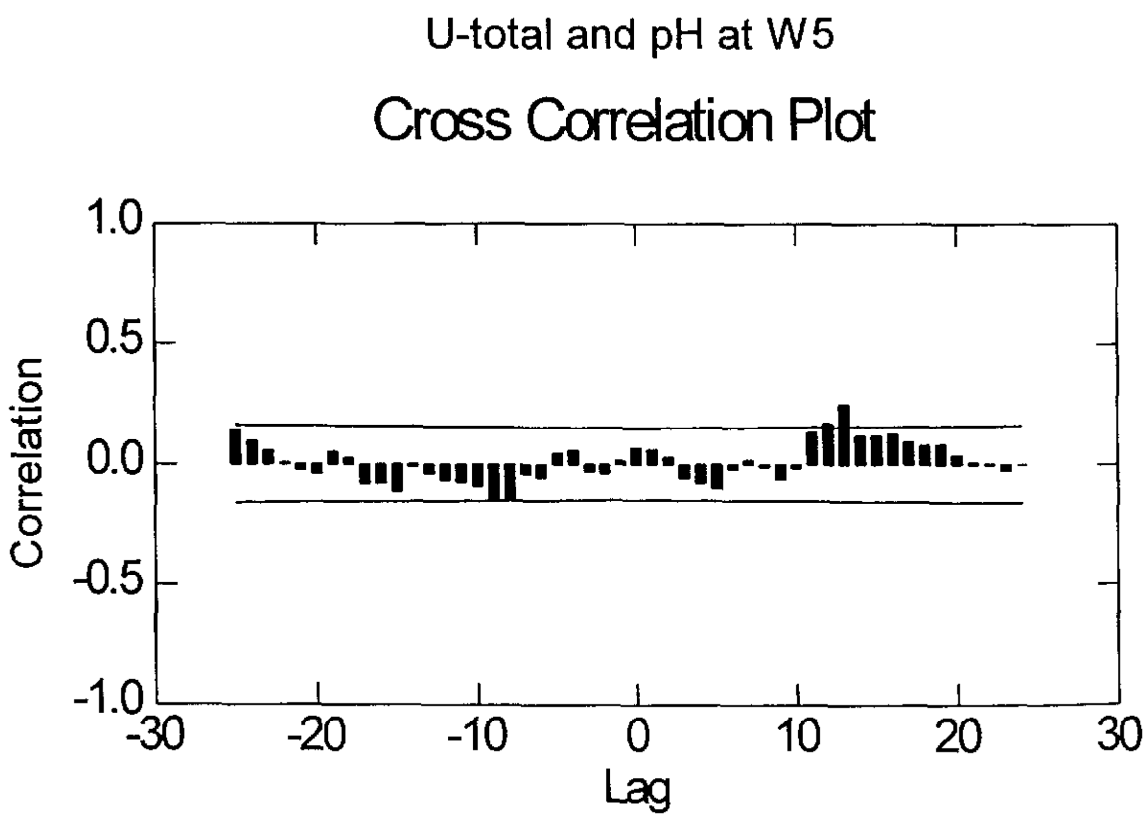
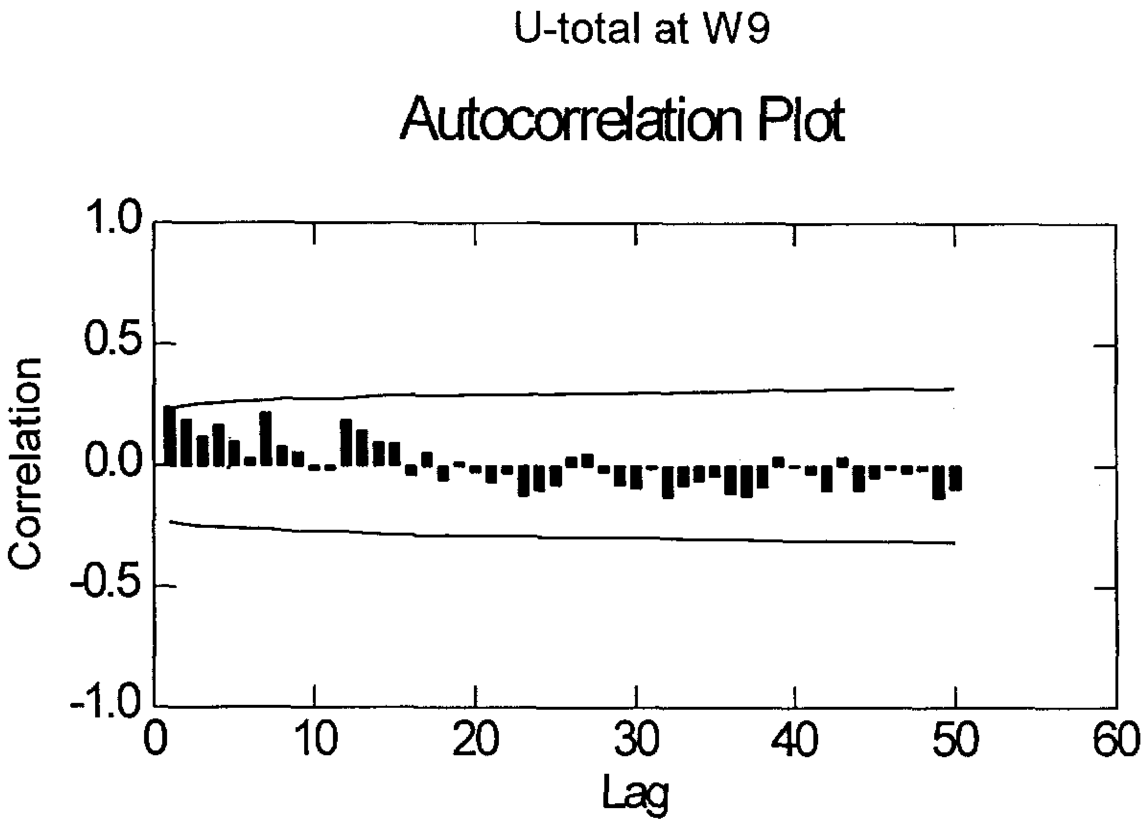


Figure 5.4d. Uranium Auto-Correlation and its Cross-Correlation with pH



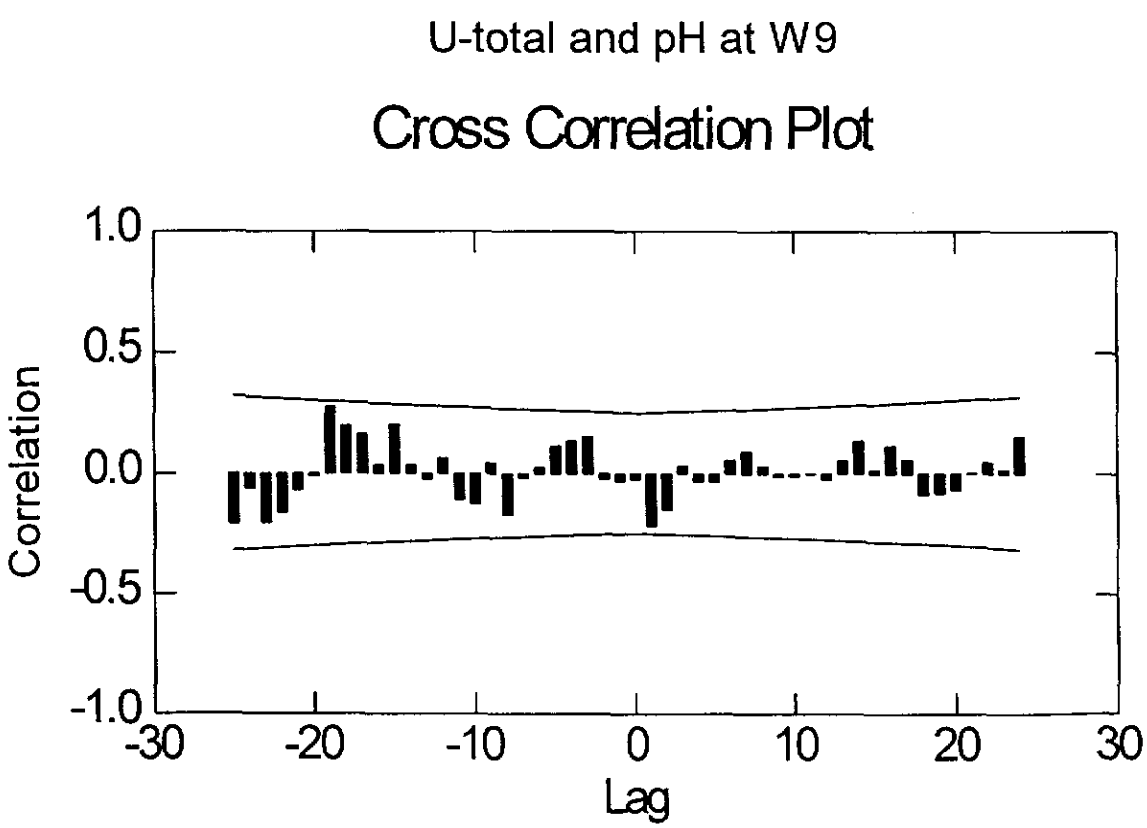
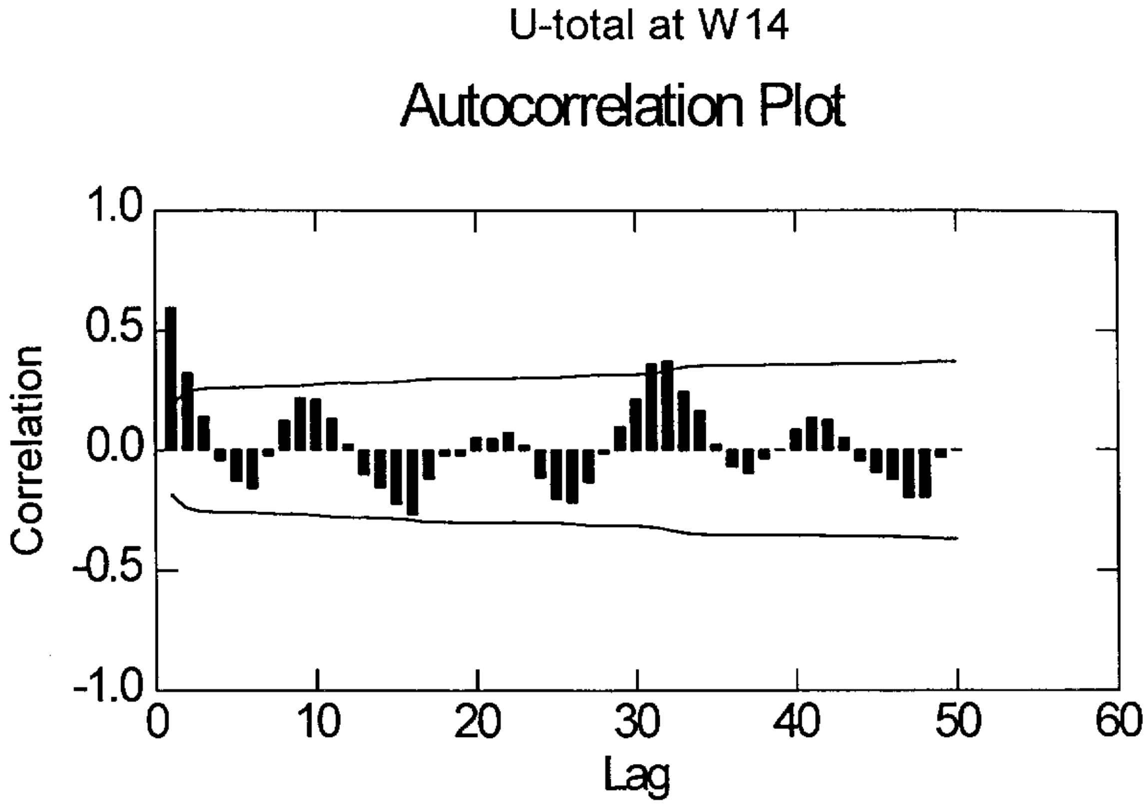


Figure 5.4e. Uranium Auto-Correlation and its Cross-Correlation with pH



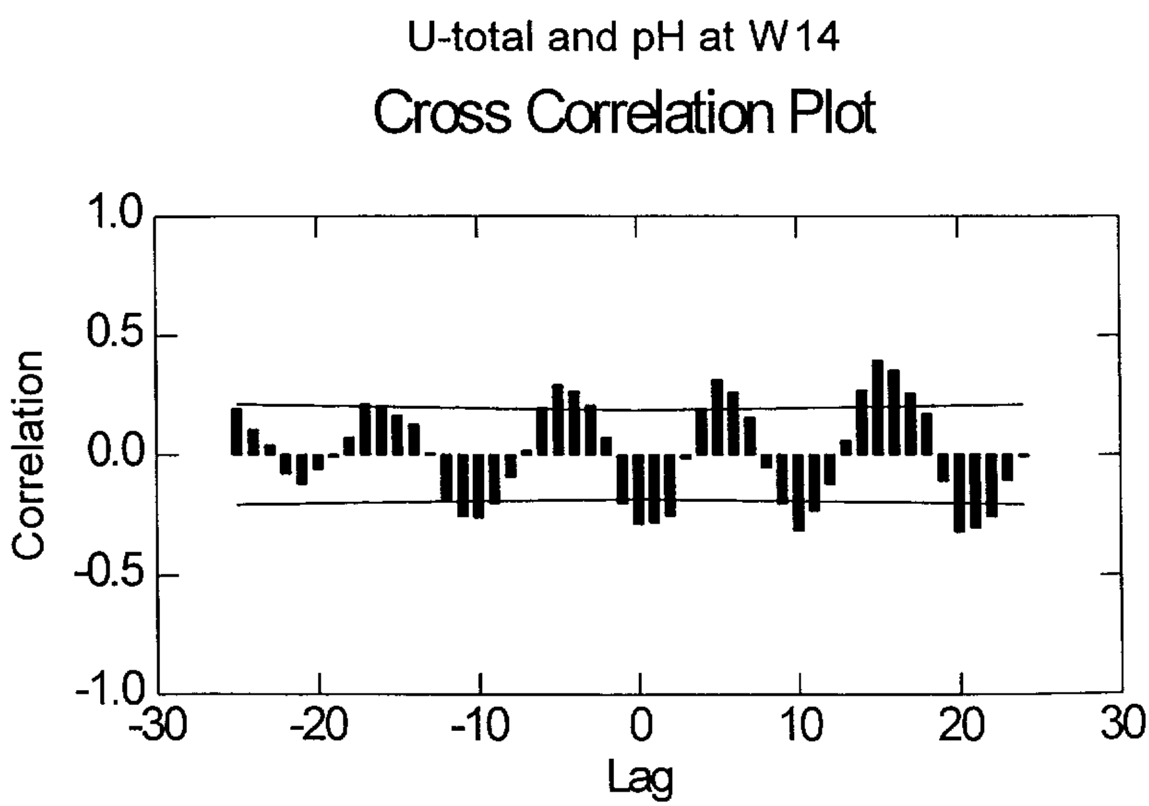
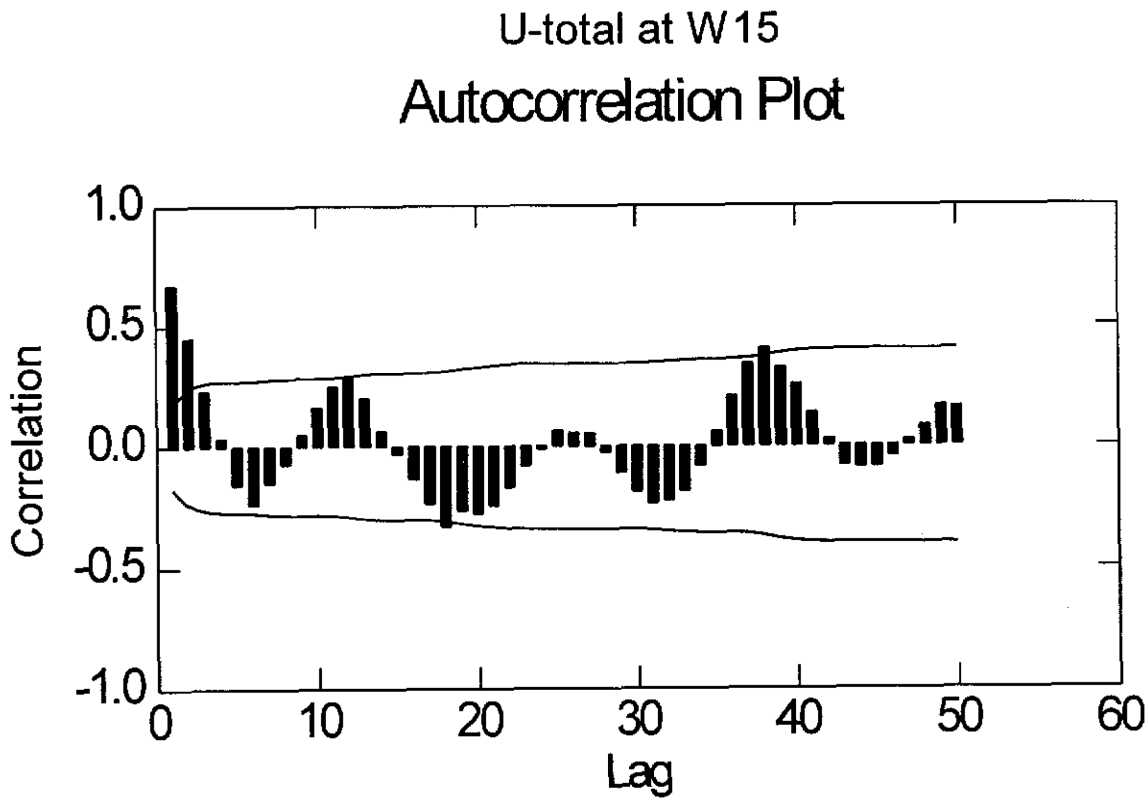


Figure 5.4f. Uranium Auto-Correlation and its Cross-Correlation with pH



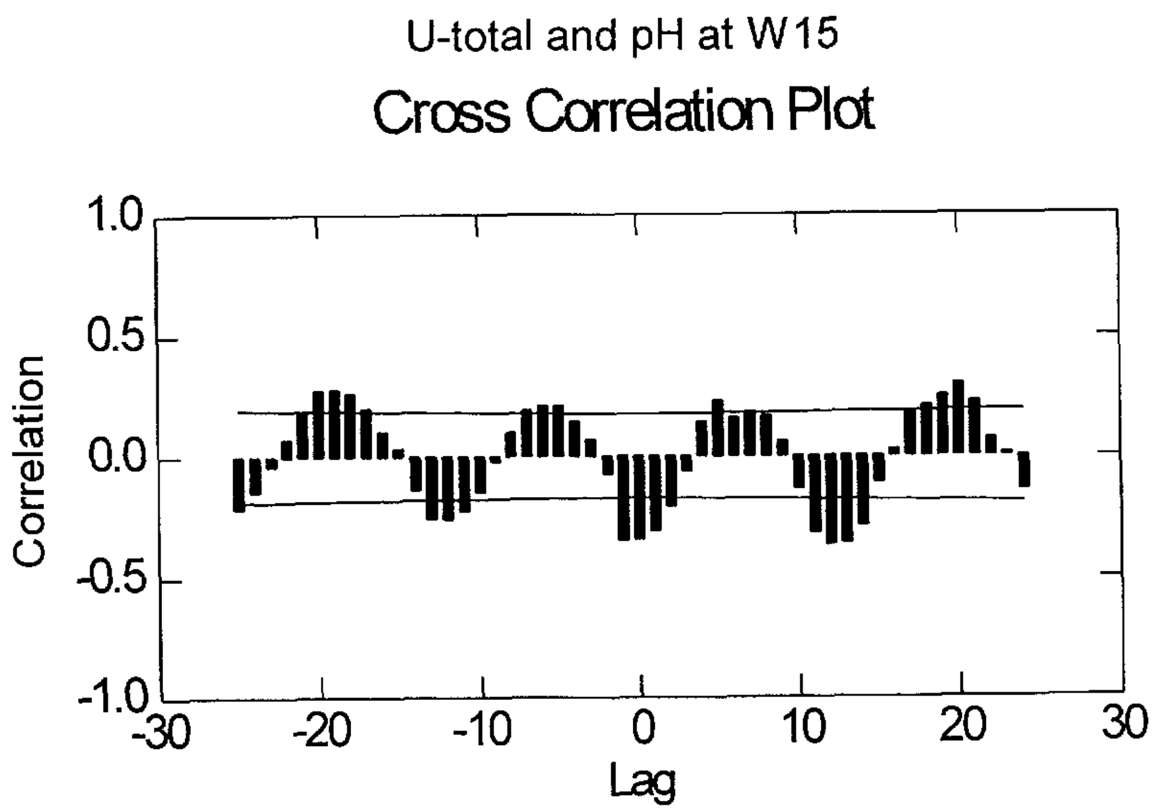
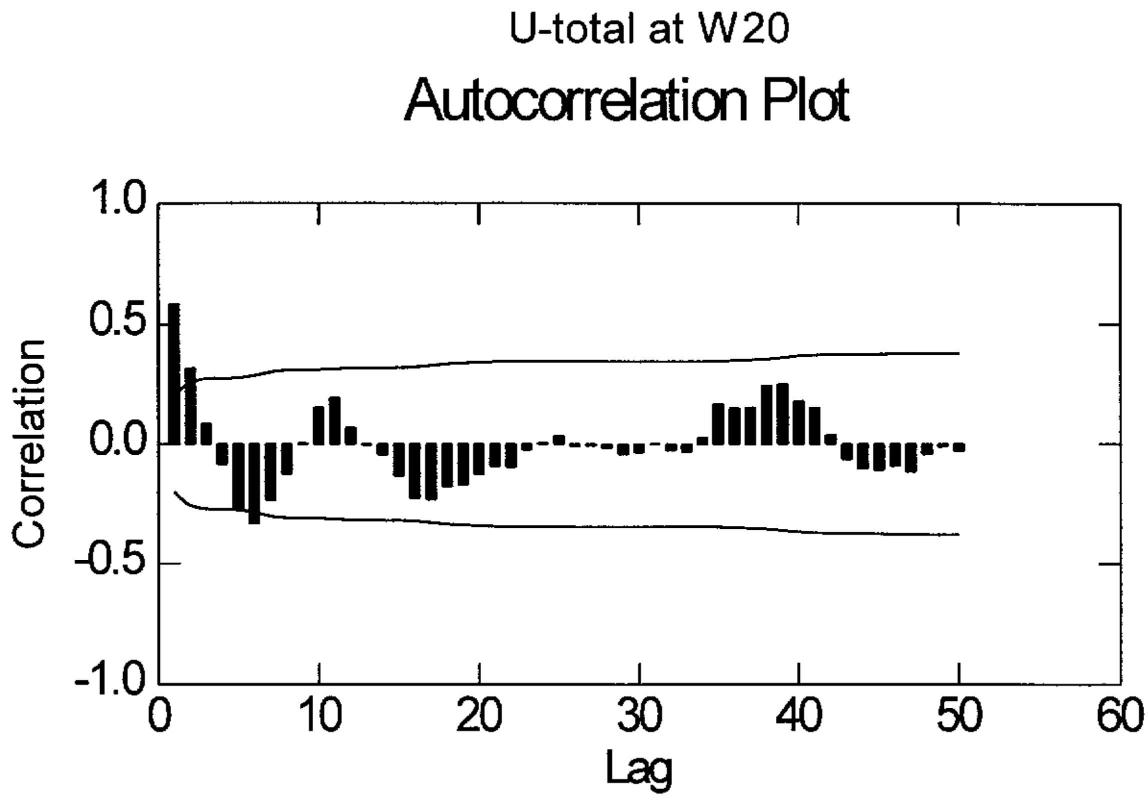


Figure 5.4g. Uranium Auto-Correlation and its Cross-Correlation with pH



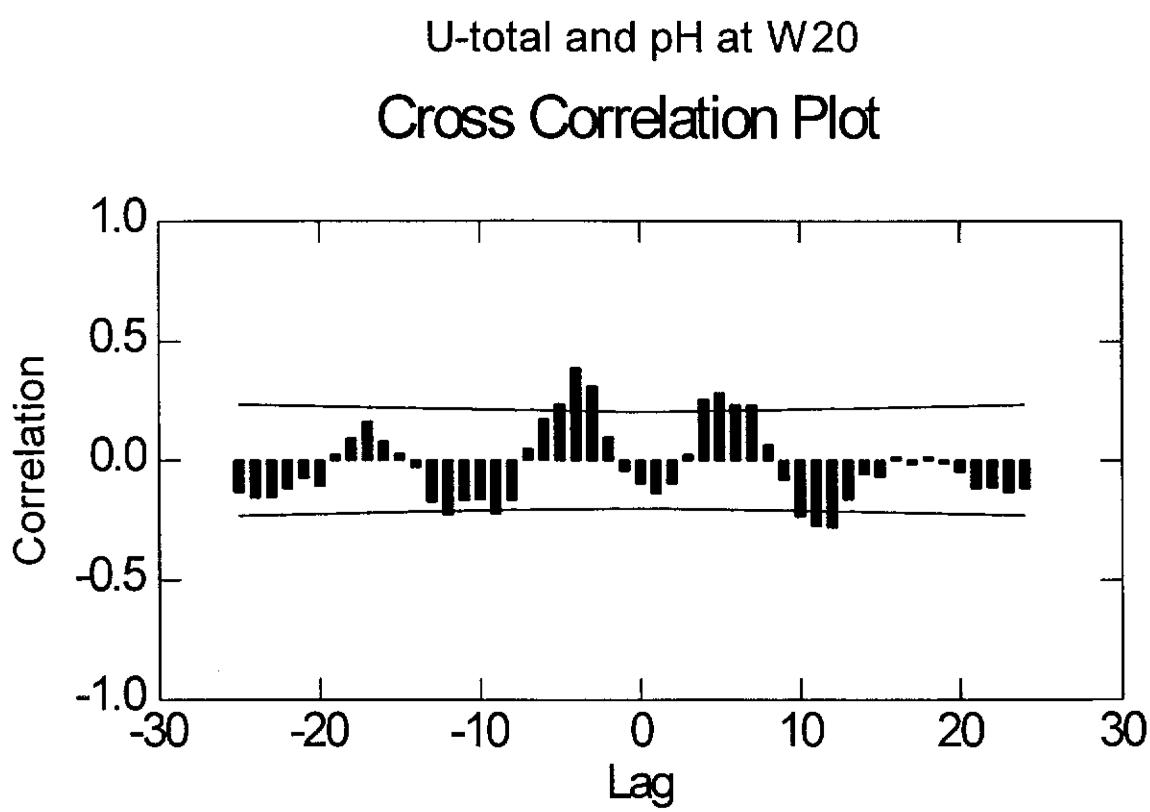
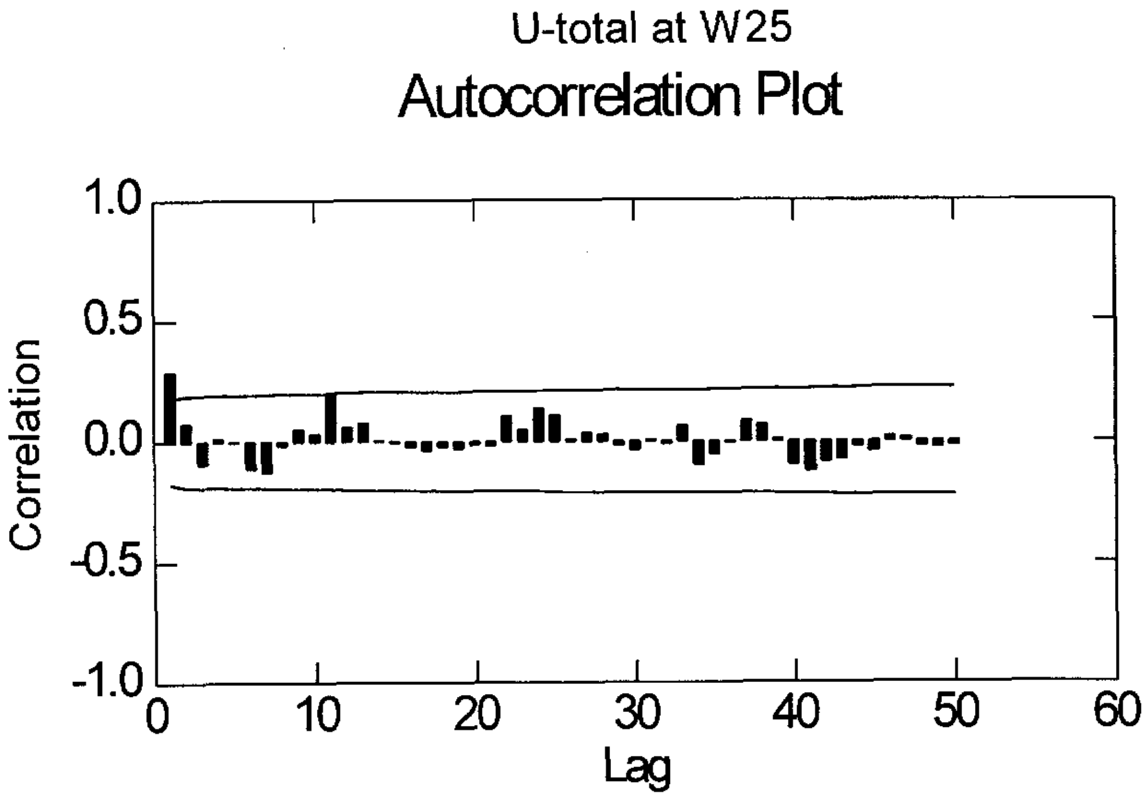


Figure 5.4h. Uranium Auto-Correlation and its Cross-Correlation with pH



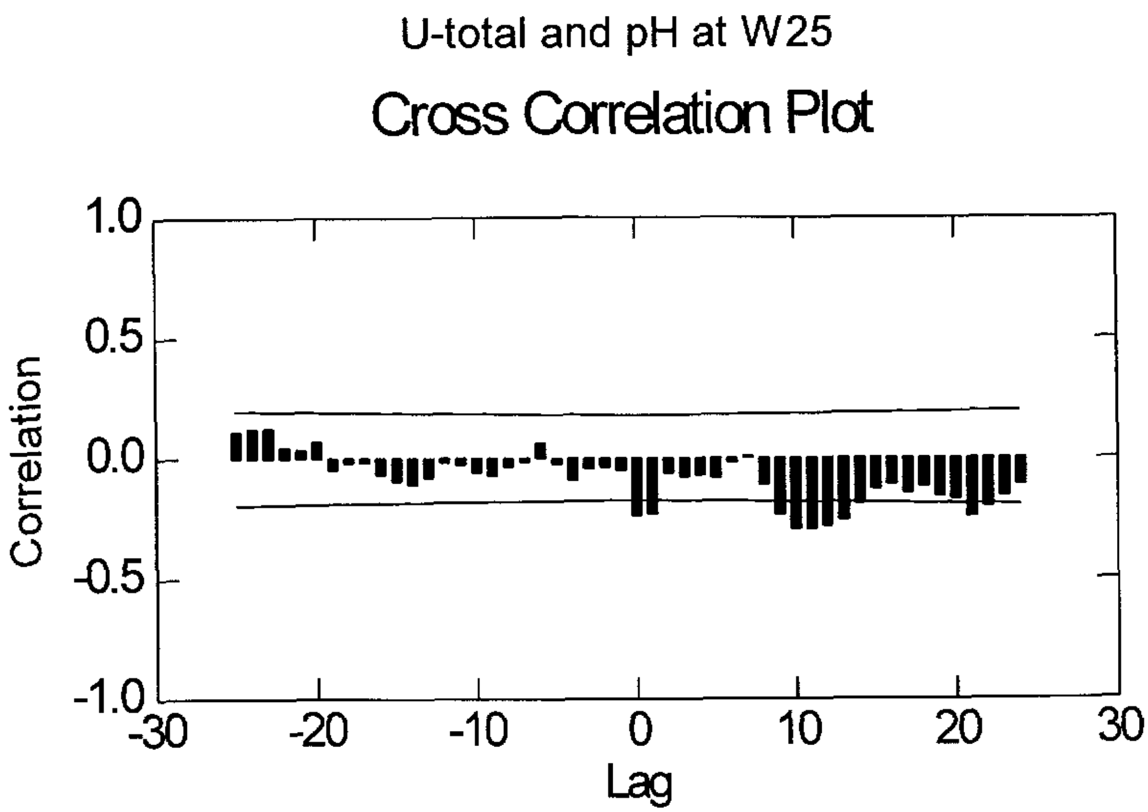
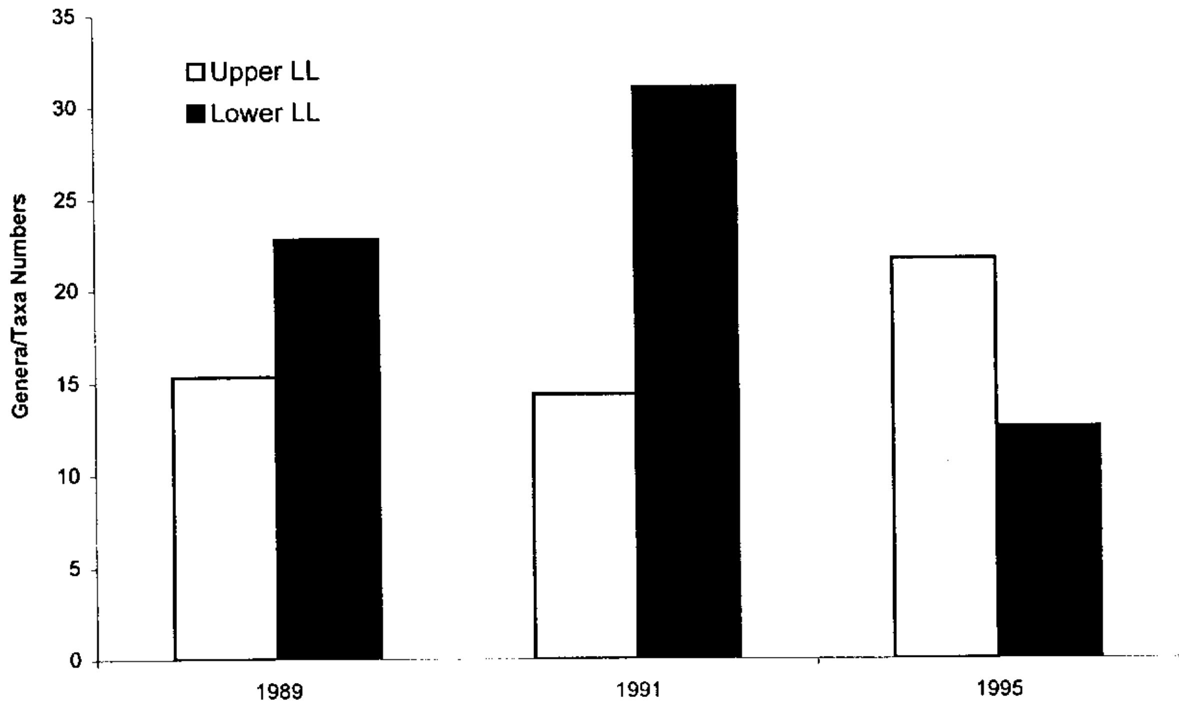


Figure 5.5. Genera/Taxa Richness in Upper and Lower Link Lakes (1989,1991 and 1995)



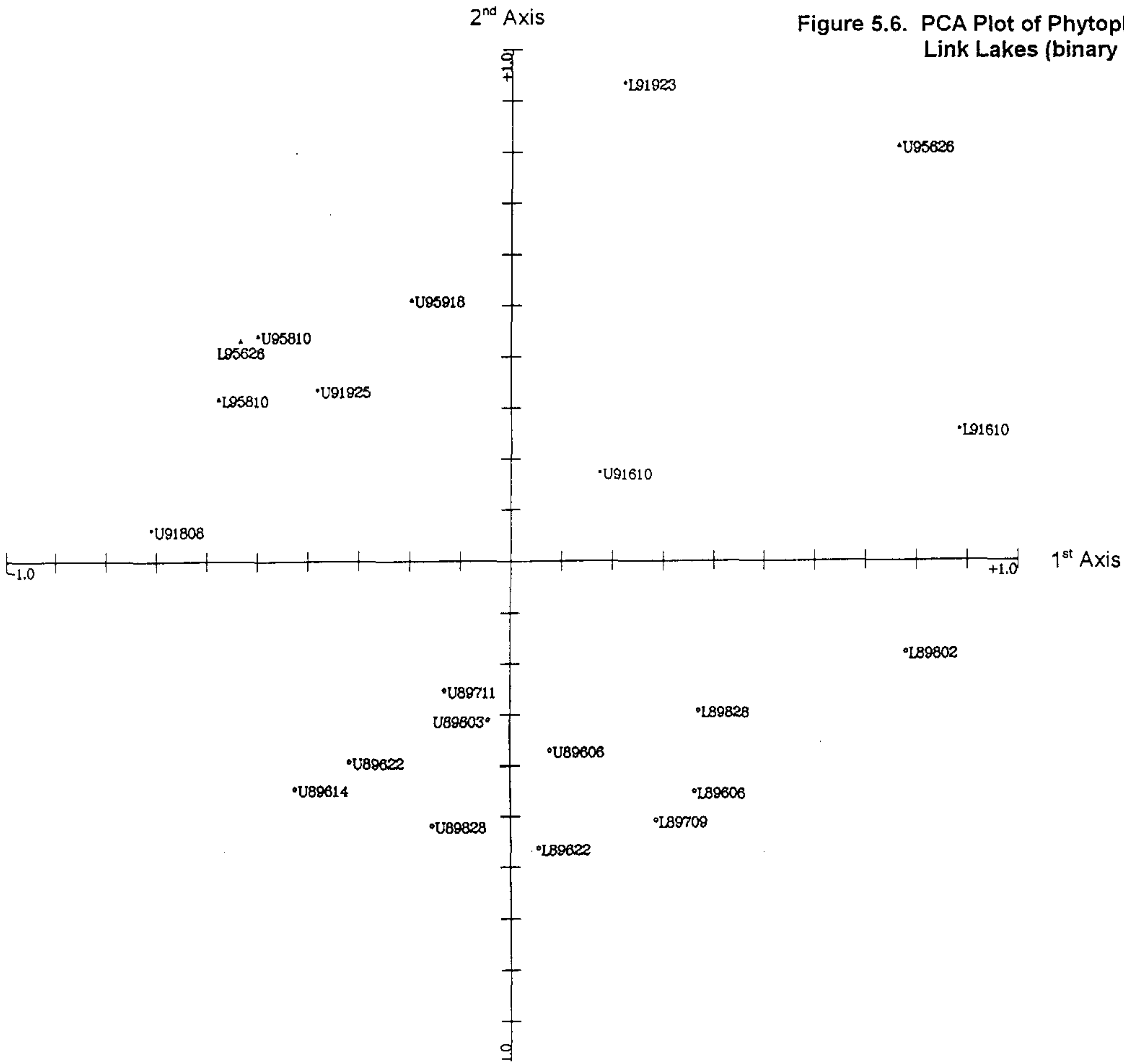
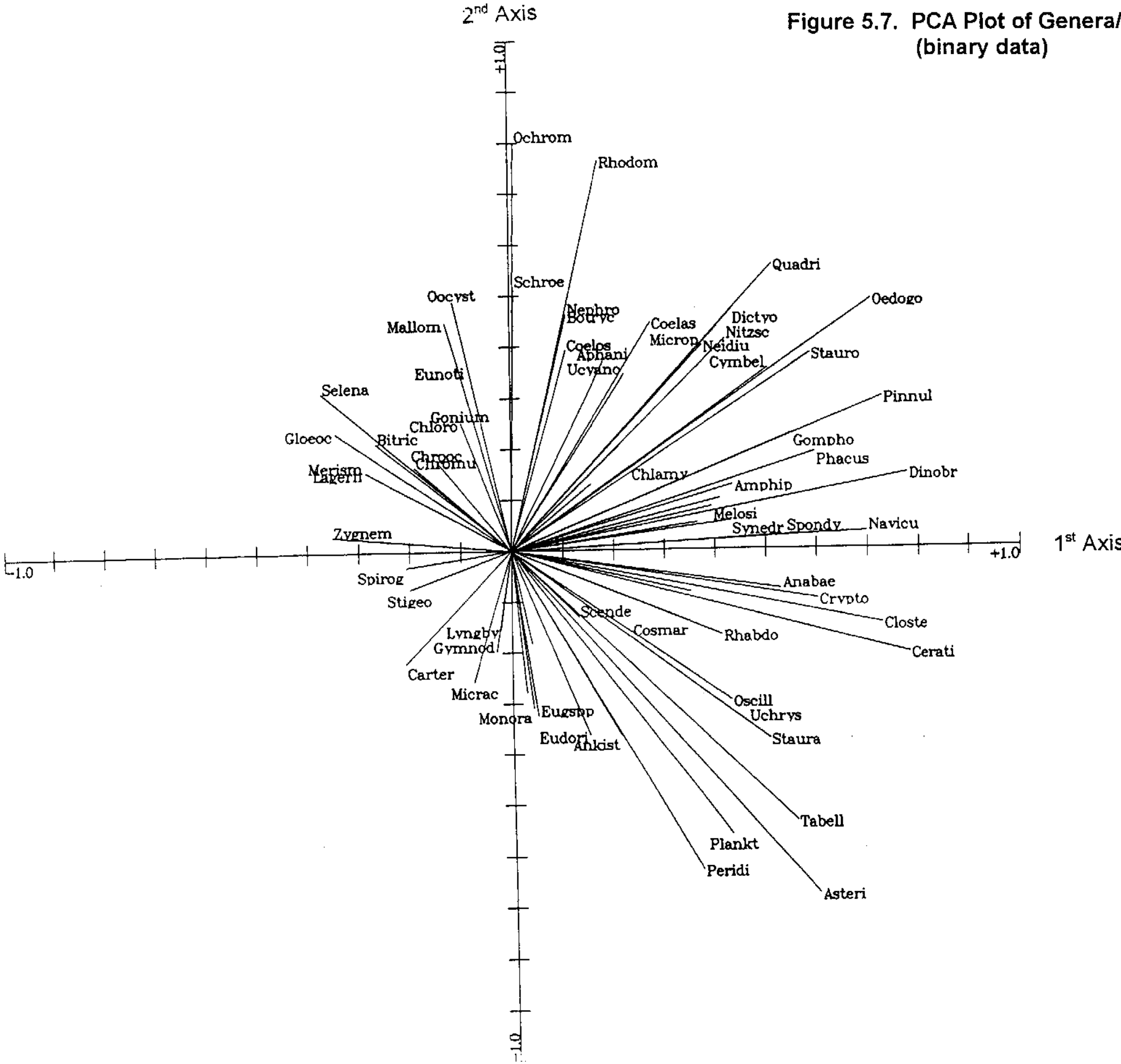


Figure 5.6. PCA Plot of Phytoplankton Samples from Link Lakes (binary data)

F24

Figure 5.7. PCA Plot of Genera/Taxa from Link Lakes (binary data)



PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 6

BUCHANS

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

6.0 Site Description

Buchans is a mining town located in central Newfoundland (48° N, 57° E). Mining for Zn, Cu, Au, Ag and Barite commenced in 1928 and ceased in 1984. The waste management area is currently under the management of ASARCO, and covers several water bodies, including 2 Tailing Ponds, 3 open pits (Oriental East Pit - OEP, Oriental West Pit - OWP, and Lucky Strike Hole), and Buchans River. The OEP and OWP were flooded in 1984 to become artificial lakes, and it was on these two pits that the data analysis concentrated.

The surface area of OEP is 1.95 ha, with a maximum depth of approximately 20m, and the smaller OWP has a surface area of 0.46 ha and a maximum depth of about 7m (Map 5). Water from the Drainage Tunnel, originating partly from Lucky Strike, is pumped into OWP, then OEP, and finally flows out into the biological polishing ponds where, during the summer, Zn is removed (Kalin 1998). Clean and contaminated water also enter both pits, resulting in a more hydraulically dynamic pit system than found in the B-Zone Pit.

The major contaminant in the pits is Zn (>10mg/L). The water pH is close to neutral (6.3-6.6) with high conductivity (1300-3000 µs/cm at OEP, and 600-1000 µs/cm at OWP). These topographic, hydraulic and water quality characteristics differentiate Buchans from the systems described earlier. Boojum Research has conducted extensive research on the limnology, hydrology and water chemistry of these pits.

6.1 Monitoring Program and Data Analysis

The two mining pits at the Buchans site, the Oriental East Pit (OEP) and the Oriental West Pit (OWP), were flooded in August, 1987. There was no surface inflow at that time. Groundwater, which contains elevated Zn and Fe concentrations, discharges into the pits, but clean groundwater also contributes to the inflow. The pH level in the OEP has always been close to neutral, while the OWP water was acidified (approximately 3.5). In 1993, the

OEP was connected with the OWP, increasing its pH to neutral and in 1994, freshwater began to be pumped into OWP, which further influenced the water quality in the pit.

Water quality in the two pits was routinely monitored by the Environmental Section of ARSARCO, Inc. and Boojum Research between 1988 and 1996, normally at monthly intervals. More than 40 elements/parameters were involved, including all major and trace metal elements, ions, nutrients, and pH, conductivity and acidity. A total of 55 and 65 water samples for OEP and OWP, respectively, were retrieved from the database (1988-1996).

ASRACO, Inc. initialised a more focussed monitoring program in 1990 in the OEP, which measured Zn concentration and flow on a weekly basis. A similar program was started for the OWP, but monthly sampling replaced the weekly collection two years later. As a result, 453 records are available for the OEP, compared with 138 for the OWP.

Phytoplankton samples were collected from the two pits in 1991, 1996 and 1998, by Boojum and ASARCO Inc. Of these samples, 17 were collected from the OEP and the OWP (Table 6.1). All 1991 samples were subject to only a qualitative examination, while the ones from 1996 and 1998 were counted. For consistency, the quantitative data were transferred to binary form. As in the previous appendices, a genus level was adopted, except for Euglena, which included two species whose distributions appeared to differ markedly (Olaveson 1999). PCA ordination was applied to the binary data set to define the characteristics of the phytoplankton communities.

6.2 Results and Discussion

6.2.1 Water Quality

The concentrations of the majority of trace elements were consistently low and so were excluded from this data analysis. Table 6.2 summarises 26 water quality variables for the OEP and the OWP over the period of 1988-1996, according to the monthly data.

Most metal elements were also at low concentrations, but with considerably wide standard deviations, indicating within-pit variability. Firstly, some elements differed significantly between the surface and the bottom water. For example, the average Fe concentration was 1.85 mg/L in surface water, but 49.8 mg/L at the bottom of the OEP. Other elements/variables that showed similar depth-variations include Ca, K, Mg, Mn, Na, Zn, Cl, conductivity, and acidity. In the OWP, a vertical variation also existed, but to a lesser extent, indicating less groundwater discharge.

Several elements/variables also exhibited significant change over time, particularly in the cases of pH and Al in the OWP. From 1988 to 1993, the average pH was 3.7, but it increased to 6.3 after being connected with the OEP in 1993. The Al concentration in the OWP dropped from 3.7 mg/L to 0.5 mg/L, and Zn from 35.3 mg/L to 20.7 mg/L. Many others were also impacted by the connection.

As a whole, the concentrations of most elements/ions were higher in the OEP than in the OWP, due to more groundwater discharging into the former. For example, Fe concentration was 18 mg/L in the OEP, compared with 1.4mg/L in the OWP, and average conductivity was 1080 (cm/s), compared with 665 cm/s. Zn, however, was higher in the OWP even before 1993, when pH was low there.

Zn is clearly the major contaminant. Its concentration in the OWP is 33 mg/L, compared to 24 mg/L in the OEP. Other trace metal elements, such as Cu, Cd, As, Co, Ni, Pb and

Cr were at much lower levels, while Fe, Al, and Mn levels, although considerably higher, were not regarded as major restrictions to the biological system. A detailed analysis was conducted on Zn based on the weekly data described earlier.

Zn concentrations decreased significantly and markedly between 1990 and 1998 in the OEP (Pearson's $r = -0.78$, $P<0.01$) and in the OWP (Pearson's $r = -0.7$, $P<0.001$), but in the last five of those years, they have been fairly stable, with a much slower decrease (Figure 6.1). Zn concentrations have remained well above 10 mg/L in most samples, however, and in the early 1990s they were as high as 40 mg/L. This is comparable to the range that was observed in Boomerang Lake, although that lake reached much lower pH levels, which represents a major difference between the two water bodies.

6.2.2 Phytoplankton Community

A total of 44 genera/taxa was recorded in the three sampling years (Table 6.3). Almost half of these taxa occurred just once, but 13 genera/taxa that were present in more than 20% of the samples were regarded as common or frequent. *Scenedesmus* was the dominant species in terms of occurrence frequency (61%), followed by *Achnanthes* (55%), *Stichococcus* (44%), *Chlamydomonas* (39%), *Elakatothrix* (39%), and *Navicula* (39%). *Eunotia*, *Dinobryon*, *Euglena mutabilis*, *Trachelomonas*, *Ulothrix*, and two unidentified taxa were also frequent.

A PCA ordination of the samples in the first two dimensions is shown in Figure 6.2, where the 1st axis explains 28.7% of the total variance and the 2nd axis 15.3%. Together, these two axes account for a large proportion of the variance (44%). From the figure, it is clear that the 1991 samples are located to the left, while the 1996 samples are to the right, indicating a change in community composition. Figure 6.3 shows the change in genera/taxa composition for Buchans Pits from 1991 to 1998. The codes are described in Table 6.3. Figure 6.4 shows the association between the samples and particular genera/taxa. It was also noted, however, that a single 1996 sample is located between the two groups, suggesting that there was no obvious time-trend in the community composition.

Table 6.1. Sample codes for PCA ordination using binary data from Buchans pits.

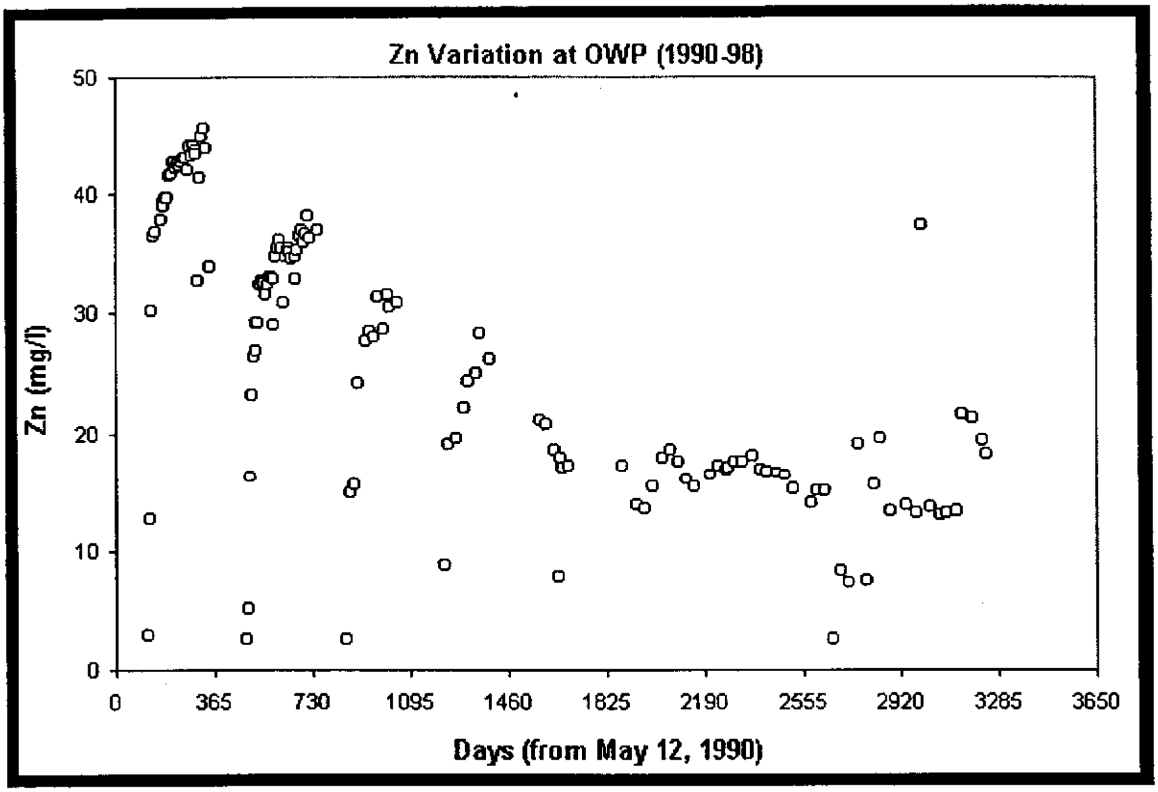
Date	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91	17-Oct-91
Pits	OWP	OWP	OWP	OWP	OEP	OEP	OEP	OEP	OEP
Sampl Loc.	LC3	Curtain	LC2	Centre	LC4	LC1	LC6	Centre	LC5
Codes	W91C3	W91CT	W91C2	W91CE	E91C4	E91C1	E91C6	E91CE	E91C5
Date	3-May-96	9-Jul-96	29-Sep-96	29-Sep-96	3-May-96	10-Jul-96	29-Sep-96	21-Sep-98	
Pits	OWP	OWP	OWP	OWP	OEP	OEP	OEP	OWP	
Sampl Loc.	Center	Center	Center	Center	Center	Center	Center	Center	
Codes	W9605	W9607	W9609A	W9609B	E9605	E9607	E9609	W9809	

Table 6.2. Summary of major water quality variables for OEP and OWP at Buchans (1988-1996)

	OEP						OWP					
	NO.	Max	Min	Mean	StDev	Median	NO.	Max	Min	Mean	StDev	Median
Al (mg/l)	52	12.4	0.01	1.0728	3.1216	0.025	61	7.4	0.025	3.0626	2.0758	3
As (mg/l)	36	0.5	0.01	0.0528	0.1017	0.02	43	0.5	0.01	0.1851	0.2218	0.04
Ca (mg/l)	48	525	150	382.85	94.58	390	54	168	31	86.86	30.33	80.5
Cd (mg/l)	52	0.4	0.003	0.0262	0.0759	0.01	61	0.4	0.01	0.0887	0.0579	0.08
Co (mg/l)	52	0.2	0.006	0.0296	0.0405	0.02	61	0.1	0.005	0.0270	0.0284	0.012
Cr (mg/l)	52	0.08	0.005	0.0137	0.0164	0.01	61	0.03	0.005	0.0086	0.0062	0.005
Cu (mg/l)	52	0.08	0.003	0.0168	0.0170	0.01	61	2.3	0.021	0.7183	0.5125	0.61
Fe (mg/l)	52	88.9	0.005	18.04	28.09	2.5	61	21.1	0.02	1.45	3.02	0.9
K (mg/l)	48	22	0.01	3.95	4.40	3.25	54	19	0.01	2.61	3.35	1.95
Mg (mg/l)	48	53	13.3	38.09	8.57	40.6	54	17	4	10.09	2.90	10
Mn (mg/l)	52	15.4	4.21	10.38	2.90	10.5	61	5.11	0.177	2.29	1.09	2
Mo (mg/l)	52	1	0.01	0.1046	0.2826	0.01	62	0.4	0.01	0.0182	0.0505	0.01
Na (mg/l)	48	170	34.8	109.80	28.70	110.5	54	24.2	1	5.07	5.39	3
Ni (mg/l)	52	1	0.01	0.0717	0.2323	0.01	62	0.27	0.01	0.0210	0.0341	0.01
P (mg/l)	48	7.5	0.01	0.4648	1.1562	0.1	55	7.6	0.01	0.2998	1.0566	0.1
Pb (mg/l)	52	1	0.01	0.0936	0.2302	0.025	61	17	0.025	1.2114	2.1393	1
Zn (mg/l)	52	61	1.74	24.25	12.70	23.5	61	75	10	32.66	13.28	31.6
pH	36	7.52	6.04	6.55	0.38	6.45	52	6.76	2.94	4.27	1.07	3.81
Conductivity	34	3100	1080	1839	546	1715	52	1980	245	666	322	640
Eh	26	514	20	128.35	130.60	62.5	41	729	140	329.61	141.79	293
Acidity (mg/l)	23	510	35	170.95	150.49	96	35	235	32.9	105.63	60.54	94.3
Cl (mg/l)	24	256	46.5	145.88	58.53	142	20	80	0.83	10.00	20.03	1.52
SO_4 (mg/l)	20	2625	220	829.50	522.54	796.5	17	873	102	283.53	182.11	286
NO_2 (mg/l)	9	0.2	0.03	0.104	0.043	0.1	12	0.2	0.0986	0.1164	0.0390	0.1
NO_3 (mg/l)	14	11	0.01	1.02	2.89	0.13	13	12	0.13	1.2489	3.2566	0.2
HCO_3 (mg/l)	16	285	25.93	157.36	62.64	163.5	13	61.9	0.1	11.66	22.00	0.1

Table 6.3. Occurrences of Phytoplankton taxa in Buchans Pits (OWP and OEP)

	Occurrences	Frequency %	Genera/Taxa	Codes
1	11	61.11	Scendesmus	Scende
2	10	55.56	Achnanthes	Achnan
3	8	44.44	Stichococcus	Sticho
4	7	38.89	Chlamydomonas	Chlamy
5	7	38.89	Elakatothrix	Elakat
6	7	38.89	Navicula	Navicu
7	7	38.89	Unidentified Chlorophyceae	U_chlor
8	6	33.33	Eunotia	Eunoti
9	5	27.78	Dinobryon	Dinobr
10	4	22.22	Euglena mutabilis	Euglen
11	4	22.22	Trachelomonas	Trache
12	4	22.22	Ulothrix	Ulothr
13	4	22.22	Unidentified Chrysophyceae	U_chrys
14	3	16.67	Nitzschia	Nitzsch
15	3	16.67	Planktosphaeria	Plankt
16	3	16.67	Pinnularia	Pinnul
17	2	11.11	Glenodinium	Glenod
18	2	11.11	Gomphonema	Gompho
19	2	11.11	Microthamnion	Microt
20	2	11.11	Neidium	Neidiu
21	2	11.11	Rhizosolenia	Rhizos
22	2	11.11	Tabellaria	Tabell
23	2	11.11	Tolypothrix	Tolypo
24	1	5.56	Carteria	Carter
25	1	5.56	Chlorogonium	Chlorog
26	1	5.56	Chroomonas	Chroom
27	1	5.56	Cocconeis	Coccon
28	1	5.56	Cosmarium	Cosmar
29	1	5.56	Epipyxis	Epipyx
30	1	5.56	Euglena sp.	Eugl-1
31	1	5.56	Gloeococcus	Gloeoc
32	1	5.56	Gomphosphaeria	Gomria
33	1	5.56	Gymnodinium	Gymnod
34	1	5.56	Kephryon	Kephry
35	1	5.56	Lepocinclis	Lepoci
36	1	5.56	Micrasterias	Micras
37	1	5.56	Ochromonas	Ochrom
38	1	5.56	Oscillatoria	Oscill
39	1	5.56	Pediastrum	Pedias
40	1	5.56	Synura	Synera
41	1	5.56	Temnogametum	Temnog
42	1	5.56	Unidentified Cryptophyceae	U_crypt
43	1	5.56	Unidentified Cyanobacteria	U_Cyano
44	1	5.56	Unidentified Bacillariophyceae	U_diatom



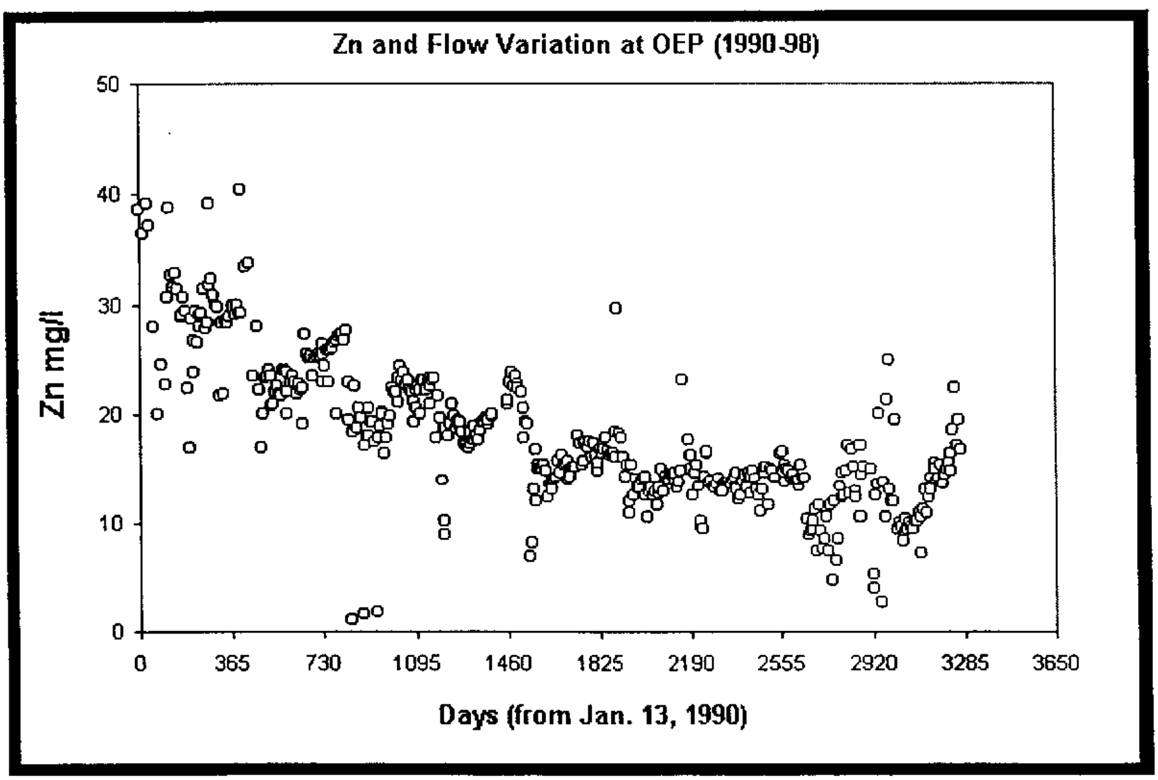


Figure 6.1. Variation in Zn concentration in OEP (upper) and OWP (lower) during The period of 1990-1998.

Figure 6.2. PCA Plot of Phytoplankton Samples from Buchans Pits (1991, 1996 and 1998)

SEE TABLE 6.1 FOR SAMPLE CODES

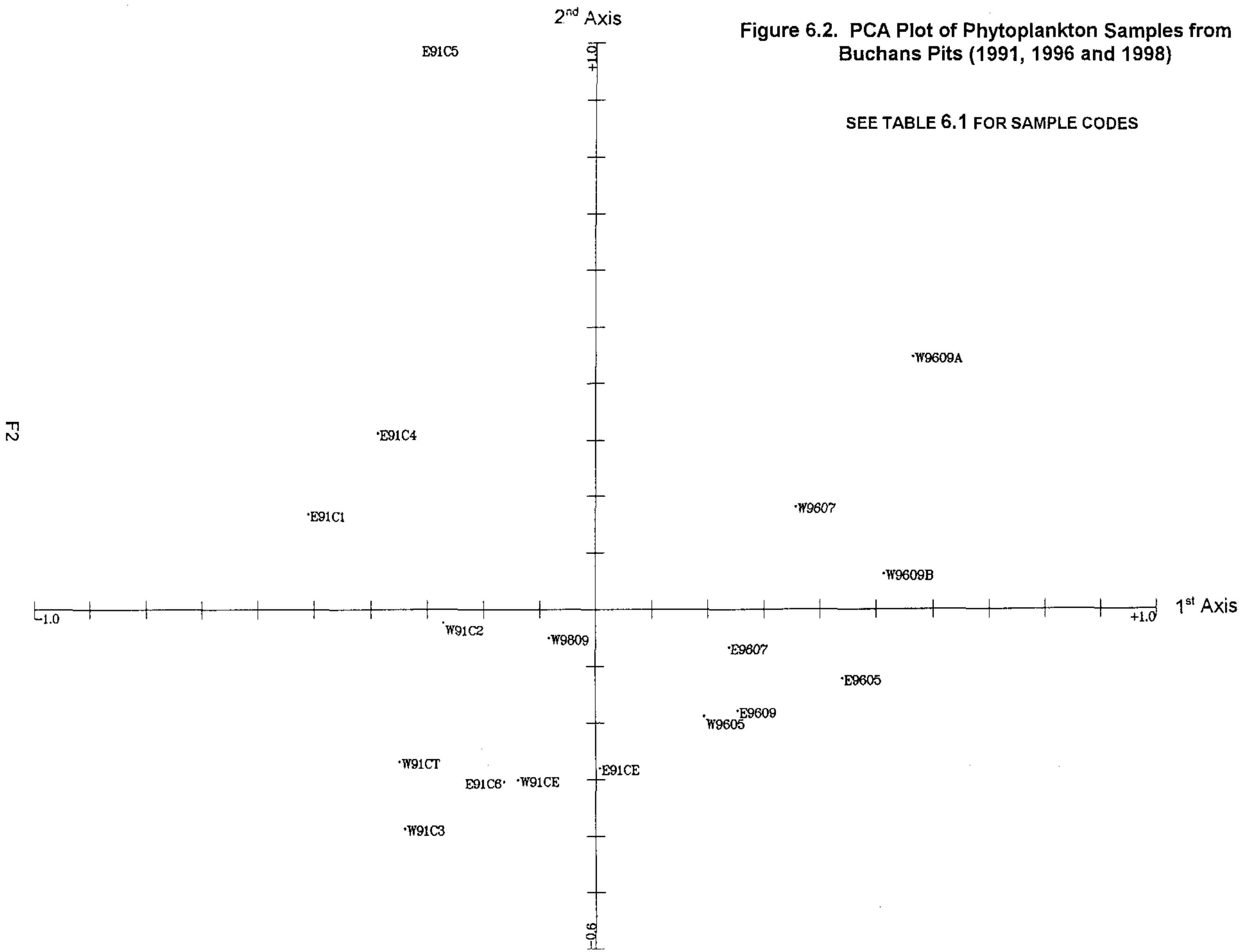


Figure 6.3. PCA Plot of Phytoplankton Taxa (binary data) from Buchans Pits (1991, 1996 and 1998)

SEE TABLE 6.3 FOR SAMPLE CODES

F3

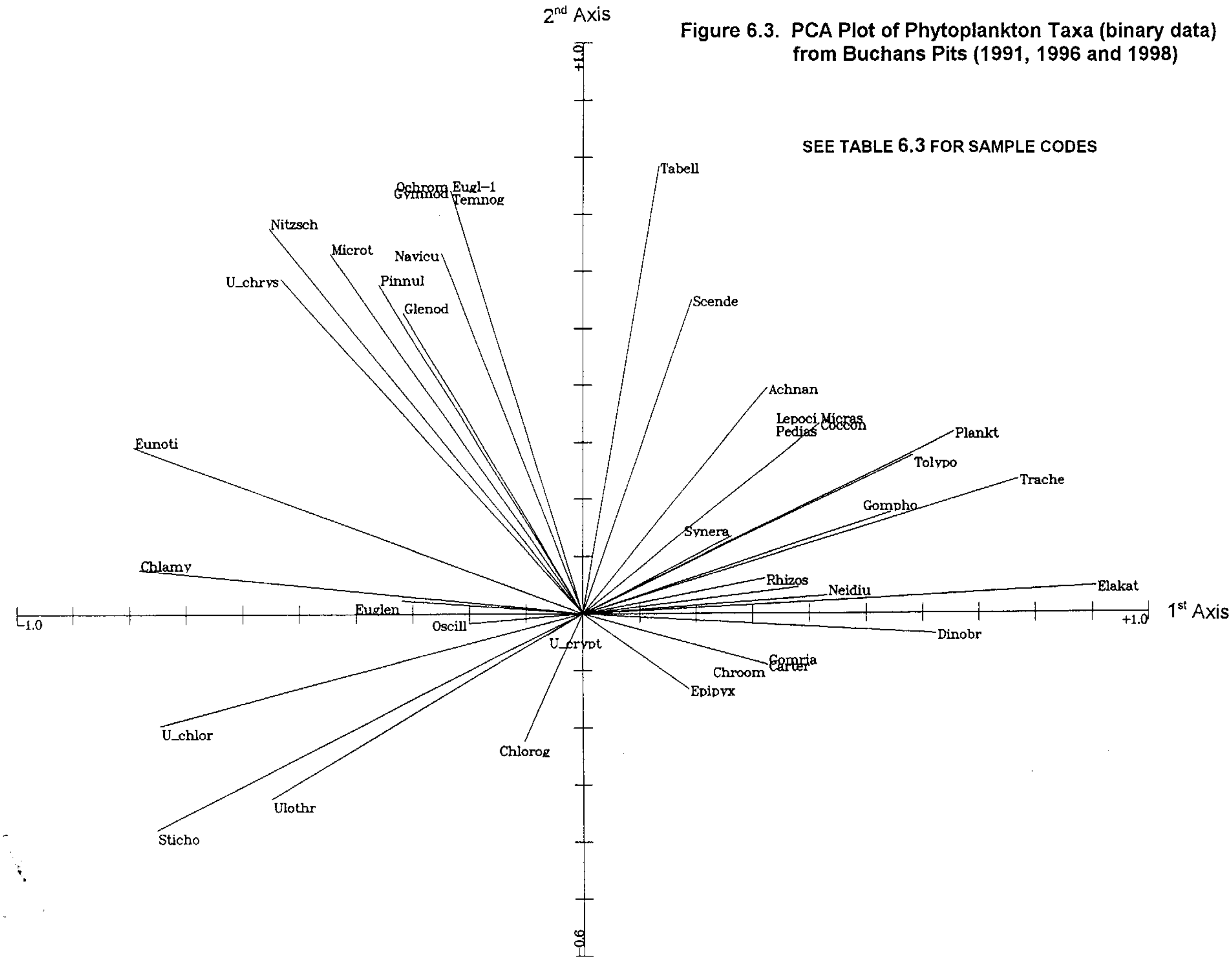
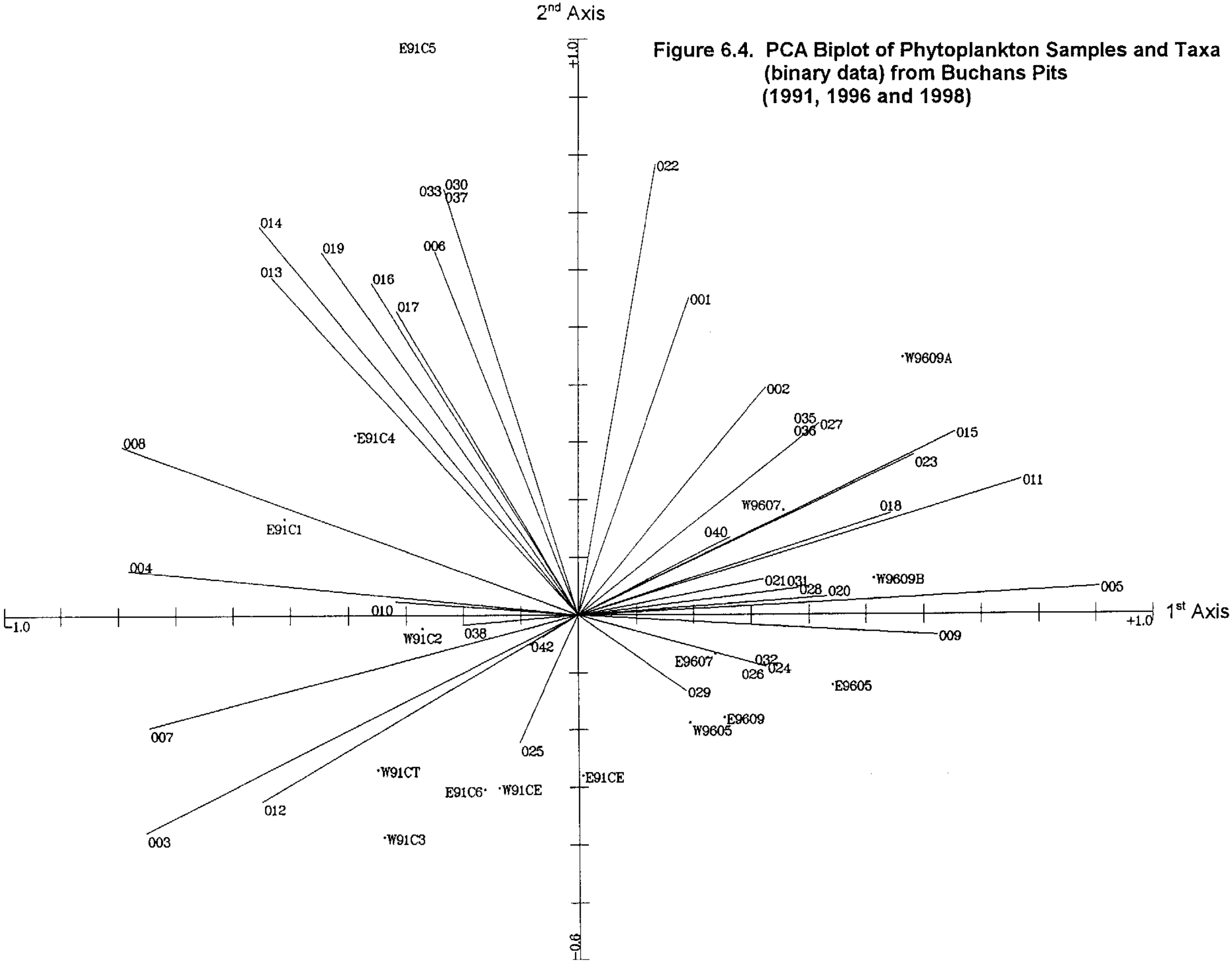


Figure 6.4. PCA Biplot of Phytoplankton Samples and Taxa (binary data) from Buchans Pits (1991, 1996 and 1998)



PHYTOPLANKTON IN MINE WASTE WATER COMMUNITY STRUCTURE, CONTROL FACTORS AND BIOLOGICAL MONITORING

APPENDIX 7

KEY LAKE

BIOTECHNOLOGY FOR MINING

CONTRACT # 23440-8-1016/001/SQ

June, 1999

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

7.0 Site Description

Key Lake is located in Northern Saskatchewan at about 58°N, 105° E. In fact, Key Lake includes many lakes of varying size, as shown on Map 6. The area is underlain by Archean and Aphebian-aged granites and gneisses, with fine soils mostly underlain by sand, so that they drain well. All the lakes are oligotrophic and low in most elements.

Further details of the hydrology, topology and limnology can be found in the Key Lake Mining Corporation Key Lake Project EIS (CAMECO 1979). CAMECO also collected phytoplankton and chemical data for six of the lakes - Horsefly, Little McDonald, McDonald, Zimmer, Douglas and Wilson.

From an ecological perspective, Horsefly Lake should no longer be regarded as a lake, but rather as a wastewater discharge area. It receives groundwater discharge at a rate of 0.1 m^3/sec. Its volume has been reduced from approximately 480,000 m^3 in 1997, to a current level of about 380,000 m^3, due to accumulating iron hydroxide, which precipitates as the ground water emerges. The iron precipitation process eliminates small phytoplankton from water column.

From Horsefly Lake, the groundwater effluent flows into Little McDonald and then McDonald Lake. Water quality parameters in these lakes are generally good, but Ni concentrations are slightly high. This study area provides an opportunity to examine the effect of the metal contaminant at relatively low concentrations.

Our focus is on the phytoplankton communities in these potentially impacted lakes in comparison with those observed at the other sites. The data collected in 1993 and 1994 were analysed by Rosaasen (1997) using several multivariate techniques, and those collected in 1998 by Conor Pacific (1998) using similar methods. There are a number of major changes made in this study:

1) Horsefly Lake was excluded from the analysis, because it is no longer considered as a lake, but a point of discharge, as justified earlier;

2) in this analysis, 1993-94 and 1998 data were examined together using both DCA and CCA, which is expected to provide more details about temporal variation; 3) the results were compared with our observations in the other mine sites.

7.1 Sampling Program

Phytoplankton samples were collected from lakes that are potentially impacted by the groundwater effluent (Little McDonald: 37.3 ha, and McDonald: 326.3 ha) in 1993, 1994 and 1998. Zimmer Lake (2026 ha), used as a reference site, was sampled in 1993, 1994 and 1998, and Wilson Lake and Douglas Lake were added as further reference sites in 1998. In total, the data set involves 6 lakes and 64 samples were collected. All the phytoplankton samples were processed and taxonomically analysed by Algatax. Genus level was adopted for taxonomic consistency. The phytoplankton sampling program was summarised in Table 7.1.

Water samples were collected at the same locations and times except in June, August and September in 1993 and 1994, when water samples were collected once each month, but phytoplankton samples collected twice (Table 7.1). In total, there are 55 water samples available. A total of 26 elements/parameters were involved in the raw data, but 14 elements/variables were retained for data completeness, including all important trace metals (Ni, Zn, Cu, Mg, Pb), Ca, Fe, SO_4, HCO_3, total-P, pH, Hardness and conductivity. A data summary is given below for these elements/variables:

	Ca mg/L	Cu mg/L	Fe mg/L	Hard. mg/L	Mg mg/L	Ni mg/L	P-total mg/L	Pb mg/L	pH	SO_4 mg/L	Cond. µs/cm	Zn mg/L
No	55	55	54	50	55	55	55	46	49	29	48	45
Max	8.00	0.12	0.68	35.54	7.60	0.17	0.28	0.01	8.10	20.00	81.00	0.03
Min	1.00	0.00	0.00	5.00	0.50	0.00	0.01	0.01	6.25	0.20	15.10	0.01
Average	3.19	0.00	0.07	13.63	1.37	0.04	0.05	0.01	7.25	9.50	44.52	0.01
St. Dev.	1.66	0.02	0.10	6.69	1.03	0.05	0.04	0.00	0.49	6.71	20.08	0.00

7.2. Data Analysis

Principal Component Analysis (PCA) was applied to 55 water-quality samples and 12 water quality variables with data standardised to 0 mean and 1 variance. Missing data were replaced with the average at the site. Sample codes are listed in Table 7.1. Then, Detrended Correspondence Analysis (DCA) was performed on 64 phytoplankton samples with 59 genera/taxa recorded. Finally, nine phytoplankton samples were excluded to match the water quality samples, which enables a Canonical Correspondence Analysis (CCA). Genus codes are shown in Table 7.2.

7.3 Results and Discussions

7.3.1 Water Quality

A PCA ordination of 55 water samples on the first two axes is shown in Figure 7.1. The first axis accounts for 41.9% of the total variance with the second axis adding further 14.1%. The water samples well disperses along the first axis, with Little McDonald Lake on the right, followed by McDonald Lake while the three reference lakes on the left. This axis represents a spatial gradient, which is characterised by levels of Ni, Ca, conductivity and hardness (Figure 7.2). Nickel concentration decreased along the gradient. The second axis differentiates the sampling time for Little McDonald Lake, McDonald Lake and Zimmer Lake. For each of the lake, 1993-94 samples are at upper positions and 1998 samples at lower positions. Therefore, this second axis reflects temporal changes. Lead, pH, and Cu are more closely related to this axis.

These results largely agree with Rosaasen (1997), who observed a water quality gradient of Horsefly Lake - Little McDonald Lake - McDonald Lake - Zimmer Lake. The PCA analysis of 1996 data yielded similar results (Conor Pacific 1998).

7.3.2 Phytoplankton Communities

DCA ordination of 64 phytoplankton samples is shown in Figure 7.3. The first axis distinguishes 1993-94 samples, on the left, from 1998 samples on the right. On the second axis, the various lakes well disperse, with Zimmer Lake, Wilson Lake on the top, followed by McDonald Lake and Douglas Lake, and Little McDonald Lake at the bottom. This second axis corresponds to the water quality gradient defined in PCA ordination. However, neither of the two axes accounts for large proportion of the total variance: 11.2% by the first axis and 7.4% by the second axis. Therefore, there is a large percentage of variance remaining unexplained. Furthermore, the inter-years variation is more significant than the change across the different lakes. Conor Pacific (1998) used DCA to analyse the data of two sampling years (1994 and 1998) that include Horsefly Lake, and found that the first axis corresponds to the water quality gradient. The inclusion of Horsefly Lake appears to account for the difference between these two analyses.

CCA was employed to analyse the data set further. Phytoplankton samples were trimmed to 55 in order to match the water quality samples. As with DCA, phytoplankton data were log- transformed before CCA was performed. The sample ordination on the first two axes is shown in Figure 7.4. As in DCA plot (Figure 7.3), the first axis, which accounts for 9.2%

of the total variance separates 1993-94 samples from 1998 samples. The second axis appears to represent the water quality gradient with Little McDonald Lake on the top, followed by McDonald Lake, and Zimmer Lake, Douglas Lake and Wilson Lake on lower positions. On these first two axes, samples also form lake-based clusters. The ordination of genera (Figure 7.5) indicates the community structure changes over time and space. The majority of the genera are compacted around the centre indicating their wide distribution. A number of genera are close the end of each axis, and they are uncommon and associated with a particular sample(s). Figure 7.6 is the CCA triplot for samples (circle), genera (cross) and water quality variables (arrow). Little McDonald Lake and McDonald Lake are associated with higher level of Ni, Ca, conductivity and hardness, higher densities of *Navicula*, *Dictyosphaerium*, and *Pseudokephyrion*. Zimmer Lake samples collected in 1993-94 are associated with lower level of all most all elements and other variables, and the presence or higher densities of several genera, such as *Botrycoccus*, *Nephrocytium*. Wilson Lake, Douglas Lake and Zimmer Lake (1998) are similar in both phytoplankton community structure and water quality. Rosaasen (1997) used CCA to analyse 1993-94 data only. In his study, a CCA ordination revealed a spatial pattern in the phytoplankton community structure that matched the Ni gradient. Phytoplankton samples from Horsefly Lake were associated with higher levels of Ni, Ca, U, and Fe. Ni weighed most heavily on the first CCA axis. Again, the inclusion of Horsefly Lake makes the water quality gradient strong.

In the same study, Two Way Indicator Species Analysis (TWINSPAN) was used to classify and characterise phytoplankton samples. A sensitivity/tolerance list of genera was produced accordingly. *Ankistrodesmus*, *Cryptomonas*, *Dinobryon*, *Chromulia*, *Pseudokephyrion*, and *Bitrichia* were inferred to be tolerant of high Ni concentrations, while *Anabaena*, *Coelosphaerium*, *Rhabdoderma*, *Stephanodiscus*, *Nitzschia*, *Synedra*, *Asterionella*, *Botrycoccus*, *Gyromonas*, *Rhodomonas*, *Ceratium*, and *Gyromitus* were among the more sensitive genera. *Ankistrodesmus*, *Cryptomonas*, and *Dinobryon* are also common in one or more other mine sites, however, *Pseudokephyrion*, and *Bitrichia* were abundant only in Little McDonald Lake in this study. Many of the "sensitive" genera are actually pretty common in the other mine sites, such *Nitzschia* in B-Zone and Link Lakes, *Synedra* and *Asterionella* in Boomerang Lake and Link Lakes.

7.4 Conclusions

A number of conclusions can be drawn from this data analysis. First of all, a water quality gradient characterised by Ni concentration exists along Little McDonald - McDonald Lake - the other reference lakes. Phytoplankton community structure corresponds to the gradient. This observation agrees with two previous reports (Rosaasen 1997, Corner Pacific 1998). However, the variation in each lake over sampling years is greater than the changes across the lakes. Secondly, the water quality gradient can only explain a small proportion of the total variance, leaving a larger percentage of variance unexplained. This implies that the groundwater discharge influenced phytoplankton communities in Little McDonald Lake, and to a lesser extent, McDonald Lake, but the impact is less significant than the temporal variation.

These findings vary from the previous analysis including Horsefly Lake in heat. Rosaasen (1997) using the 1993-94 data, found a strong relationships were evident between the distribution of phytoplankton taxa and the effluent gradient. Ni, Fe and U were the key variables in defining the effluent gradient and the community structure was a sensitive indicator of water quality.

Corner Pacific (1998), which used the 1998 Data found phytoplankton community structure changed slightly, probably as a result of improved effluent treatment, due to reverse osmosis water treatment. The influence of the effluent discharge appeared to cease in McDonald Lake and downstream from there.

Table 7.1. Phytoplankton and Water-quality sampling in Key Lake

	McDonald Lake	
Date	Phytoplankton	Water Quality
31-May-93	M93_5	M93_5
12-Jun-93	M93_6a	M93_6a
26-Jun-93	M93_6b	
10-Jul-93	M93_7	M93_5
07-Aug-93	M93_8a	M93_6a
21-Aug-93	M93_8b	
04-Sep-93	M93_9a	M93_9a
19-Sep-93	M93_9b	
28-May-94	M94_5	M94_5
05-Jul-94	M94_7	M94_7
16-Aug-94	M94_8	M94_8
16-Sep-94	M94_9	M94_9
20-Oct-94	M94_10	M94_10
24-May-98	M98_5	M98_5
30-Jun-98	M98_6	M98_6
25-Jul-98	M98_7	M98_7
24-Aug-98	M98_8	M98_8
20-Sep-98	M98_9	M98_9

	Zimmer Lake	
Date	Phytoplankton	Water Quality
31-May-93	Z93_5	Z93_5
12-Jun-93	Z93_6a	Z93_6a
26-Jun-93	Z93_6b	
10-Jul-93	Z93_7	Z93_7
07-Aug-93	Z93_8a	Z93_8a
21-Aug-93	Z93_8b	
04-Sep-93	Z93_9a	Z93_9a
19-Sep-93	Z93_9b	
28-May-94	Z94_5	Z94_5
05-Jul-94	Z94_7	Z94_7
16-Aug-94	Z94_8	Z94_8
16-Sep-94	Z94_9	Z94_9
20-Oct-94	Z94_10	Z94_10
24-May-98	Z98_5	Z98_5
30-Jun-98	Z98_6	Z98_6
25-Jul-98	Z98_7	Z98_7
24-Aug-98	Z98_8	Z98_8
20-Sep-98	Z98_9	Z98_9

Little McDonald		
Date	Phytoplankton	Water Quality
31-May-93	L93_5	L93_5
12-Jun-93	L93_6a	L93_6a
26-Jun-93	L93_6b	
10-Jul-93	L93_7	L93_7
07-Aug-93	L93_8a	L93_8a
21-Aug-93	L93_8b	
04-Sep-93	L93_9a	L93_9a
19-Sep-93	L93_9b	
28-May-94	L94_5	L94_5
05-Jul-94	L94_7	L94_7
16-Aug-94	L94_8	L94_8
16-Sep-94	L94_9	L94_9
20-Oct-94	L94_10	L94_10
24-May-98	L98_5	L98_5
30-Jun-98	L98_6	L98_6
25-Jul-98	L98_7	L98_7
24-Aug-98	L98_8	L98_8
20-Sep-98	L98_9	L98_9

	Douglas	
Date	Phytoplankton	Water Quality
24-May-98	D98_5	D98_5
28-Jun-98	D98_6	D98_6
25-Jul-98	D98_7	D98_7
23-Aug-98	D98_8	D98_8
19-Sep-98	D98_9	D98_9

	Wilson	
Date	Phytoplankton	Water Quality
23-May-98	W98_5	W98_5
30-Jun-98	W98_6	W98_6
26-Jul-98	W98_7	W98_7
25-Aug-98	W98_8	W98_8
19-Sep-98	W98_9	W98_9

Table 7.2. Genus codes for DCA and CCA ordinations

	Genus	Code		Genus	Code
1	Anabaena	Anabae	31	Mallomonas	Mallom
2	Ankistrodesmus	Ankist	32	Melosira	Melosi
3	Arthrodesmus	Arthro	33	Merismopedia	Merism
4	Arthrospira	Artira	34	Monoraphidium	Monora
5	Asterionella	Asteri	35	Navicula	Navicu
6	Bitrichia	Bitric	36	Nephrocytium	Nephro
7	Botrycoccus	Botryc	37	Nitzschia	Nitzsc
8	Ceratium	Cerati	38	Ochromonas	Ochrom
9	Chlamydomonas	Chlamy	39	Oosystis	Oosyst
10	Chromulina	Chromu	40	Oscillatoria	Oscill
11	Chrysolykos	Chryso	41	Peridinium	Peridi
12	Chrysosphaerella	Chrylla	42	Pinnularia	Pinnul
13	Coelosphaerium	Coelos	43	Planktosphaeria	Plankto
14	Cryptomonas	Crypto	44	Pseudokephyrion	Pseudo
15	Cyclotella	Cyclot	45	Quadrigula	Quadri
16	Cymbella	Cymbel	46	Rhabdoderma	Rhabdo
17	Dictyosphaerium	Dictyo	47	Rhizosolenia	Rhizos
18	Dinobryon	Binobr	48	Rhodomonas	Rhodom
19	Epipyxis	Epipyx	49	Scendesmus	Scende
20	Euastrum	Euastr	50	Spondylosium	Spondy
21	Euglena	Euglea	51	Stephanodiscus	Stepha
22	Fragilaria	Fragil	52	Surirella	Surire
23	Frustulia	Frustu	53	Synedra	Synedr
24	Glenodinium	Glenod	54	Synura	Synura
25	Gloeocapsa	Gloeoc	55	Tabellaria	Tabell
26	Gomphosphaeria	Gompho	56	Unid-Crypt	Ucrypt
27	Gymnodinium	Gymnod	57	unid-Chrys	Uchrys
28	Gyromitus	Gyromi	58	Unid-Cyano	Ucyano
29	Kephryon	Kephry	59	Unid-Chlor	Uchlor
30	Kirschneriella	Kirsch			

Figure 7.1: PCA Plot of 55 Water Samples Based on 11 Water Quality Variables on the First Two Axes (Refer to Table 7.1 for Sample Codes)

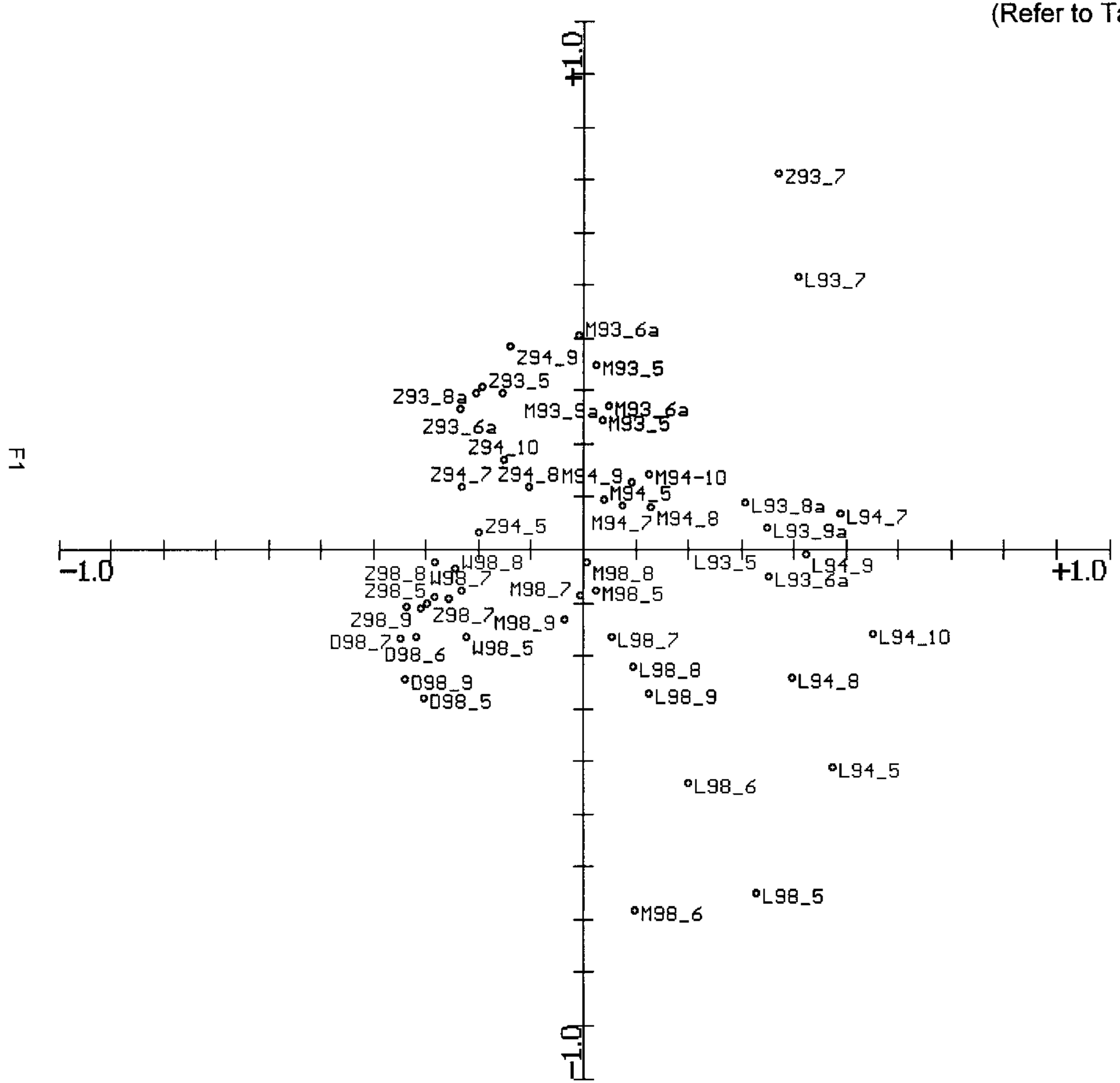


Figure 7.2: PCA Bi-plot of Water Samples and Water Quality Variables on the First Two Axes (Refer to Table 7.1 for Sample Codes)

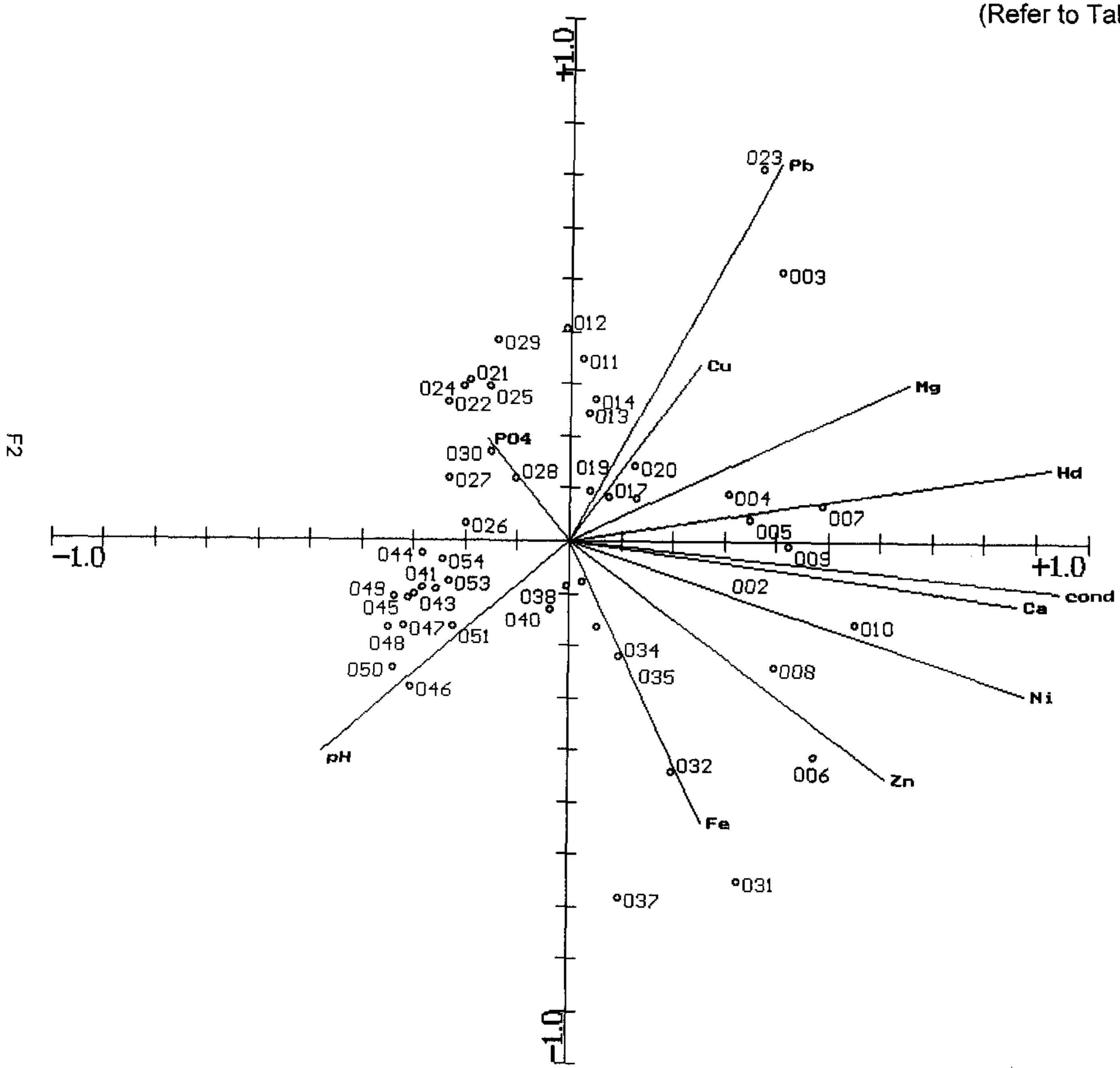


Figure 7.3: DCA Plot of 64 Phytoplankton Samples using 59 Genera on the First Two Axes (Refer to Table 7.1 for Sample Codes)

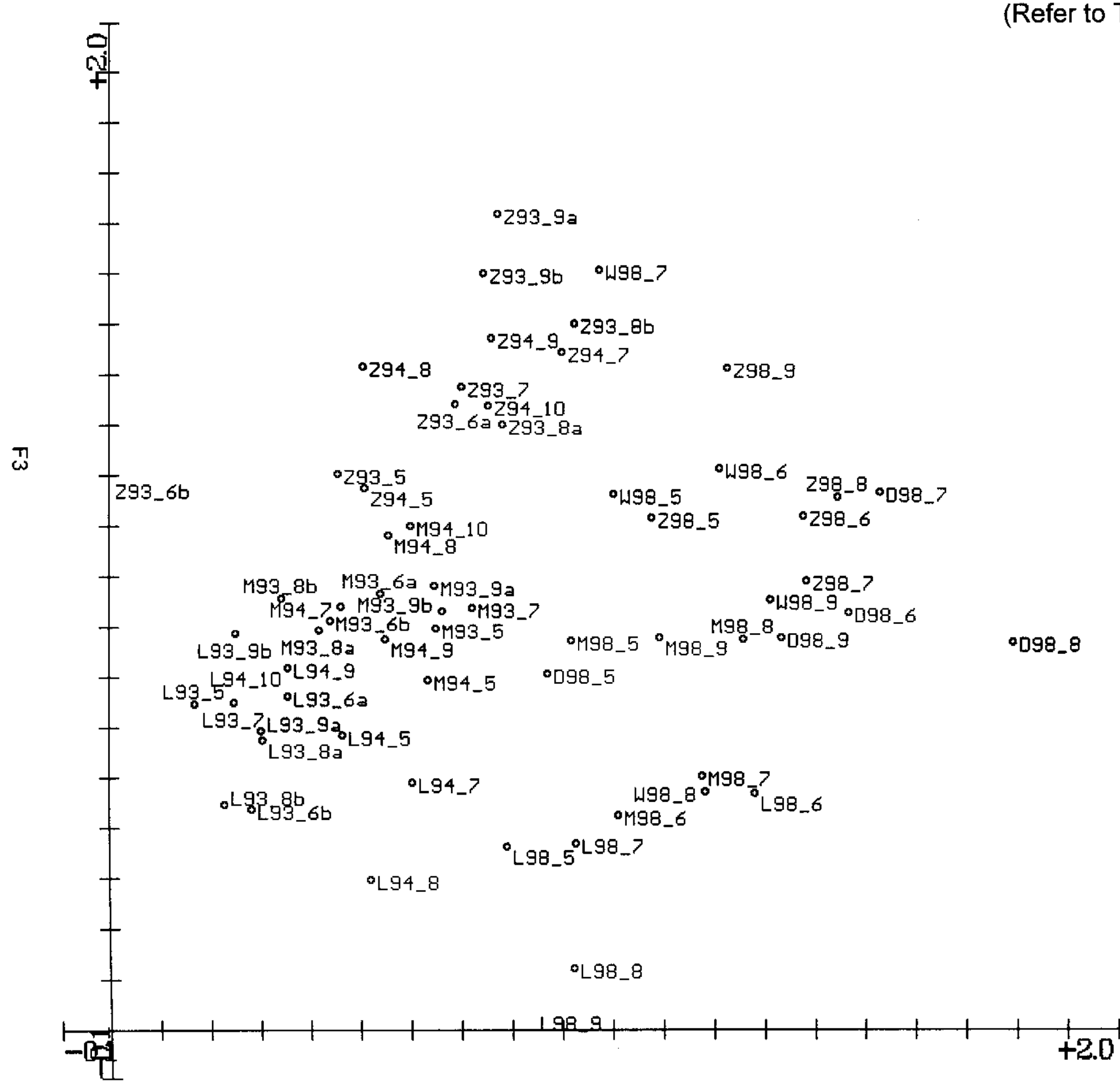


Figure 7.4: CCA Plot of 55 Samples on the First Two Axes (Refer to Table 7.1 for Sample Codes)

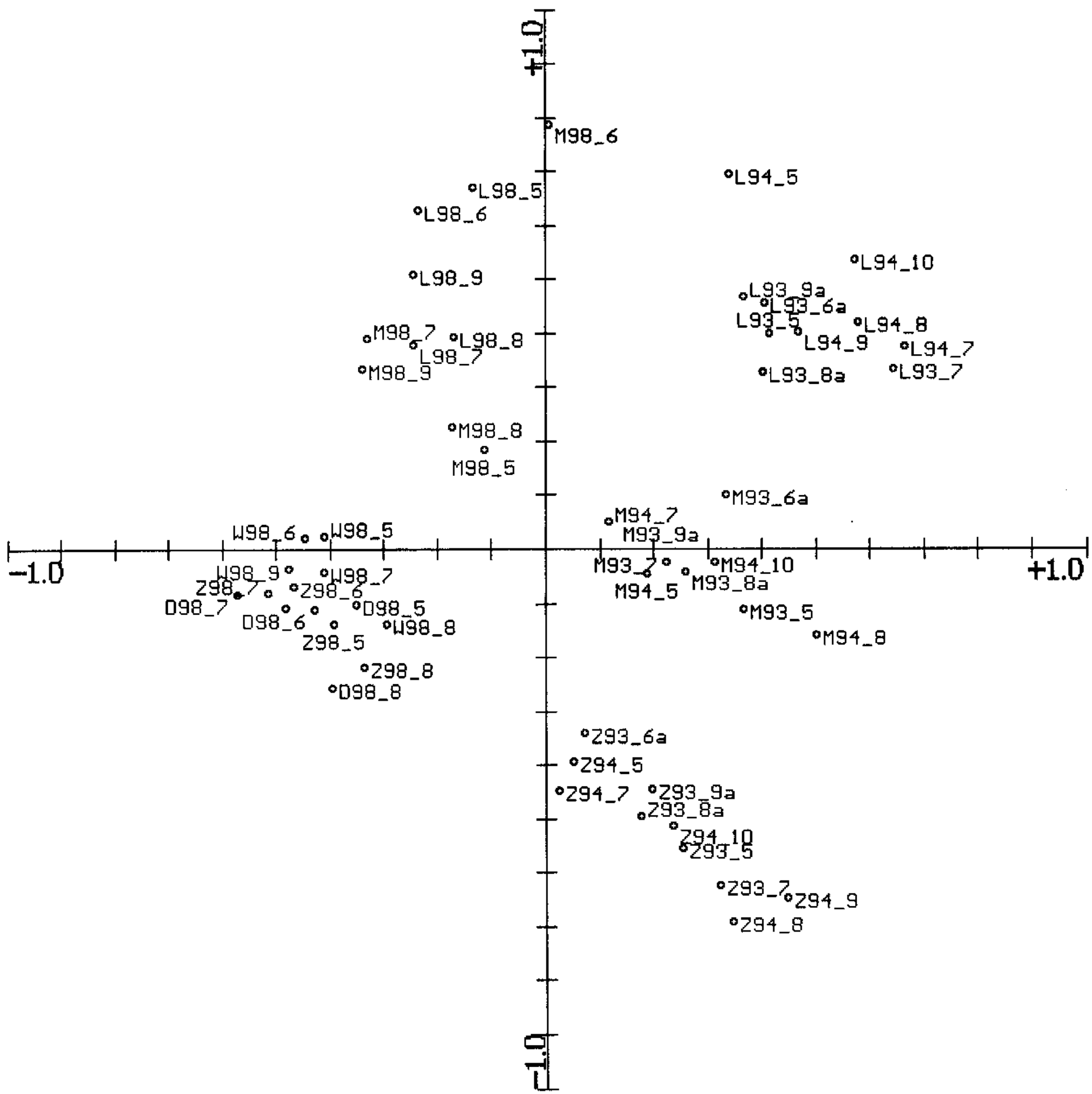
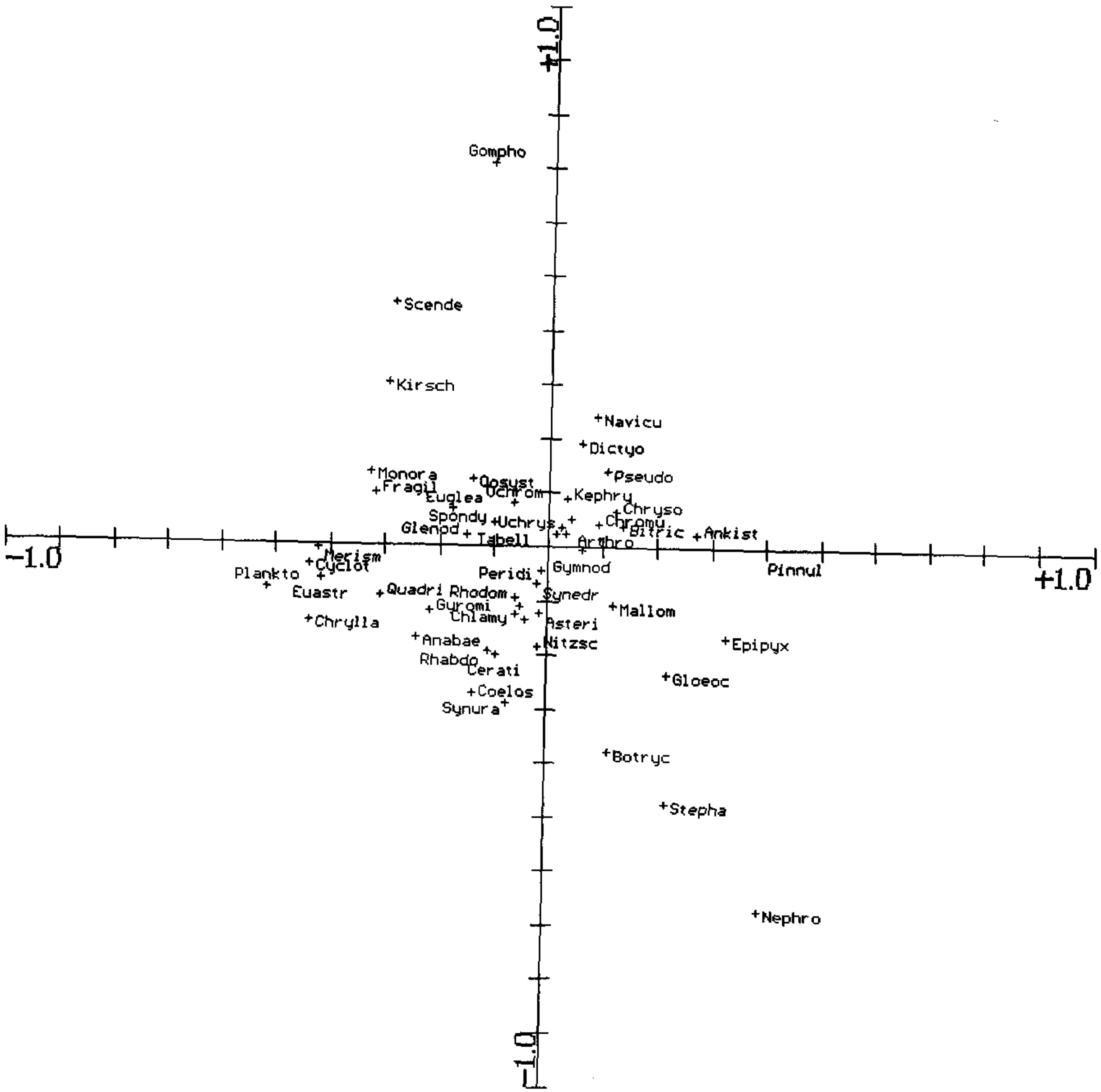


Figure 7.5: CCA Plot of 59 Genera on the First Two Axes (Refer to Table 7.2 for Genus Codes)



F6

Figure 7.6: CCA Plot of Samples, Water Quality Variables and Genera on the First Two Axes (Refer to Tables 7.1-7.2 for Coding)