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THE EFFECTS OF BLEACHED KRAFT MILL EFFLUENT ON AQUATIC MACROPHYTE COMMUNITIES IN A LAKE SYSTEM ON THE NORTHERN SHORE OF LAKE SUPERIOR

BY MICHAEL COOTE ©

MARCH 1996

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ABSTRACT

This research focuses on assessing the major effects that bleached kraft mill effluent (BKME) has on aquatic macrophyte communities of inland lakes. Research included three areas, the first involved a survey of water and sediment quality and vegetation distribution, diversity and biomass in three lakes, within the Terrace Bay study area. A comparison of trophic parameters was made between the three lakes, which differ in their exposure to BKME.

The impacts of BKME on lake limnology, included a reduction in available light and dissolved oxygen. The water temperature, conductivity and alkalinity of exposed lakes were elevated while loading of receiving waters and sediments with organic and inorganic nutrients occured. The result is a reduction in total biomass and species diversity and changes in species composition, to favour emergent aquatic macrophytes over submersed and floating leaf species.

The effect of depth on the growth of aquatic macrophytes within a lake that forms part of the effluent stream from the pulp paper mill was investigated. All 8 major aquatic macrophyte species were able to survive transplanting and grow within the exposed lake water and sediment, when propagated in water levels above the average secchi depth of 12 cm.

An investigation was conducted of the levels of congeners of polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) in the sediment and aquatic vegetation of the two lakes exposed to BKME. Both these organochlorine groups have historically been measured in BKME and widely reported as anthropogenic xenotoxins.

The levels of PCDD/PCDF were found to be elevated in all aquatic macrophytes with higher levels being found in submersed species than emergents.

The PCDD/PCDF homologue profiles were similar for the lake sediments and the aquatic vegetation, with total levels in the three lakes, reflecting the exposure history to BKME.

ACKNOWLEDGEMENTS

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Special thanks go to Jim Murphy and the Thunder Bay Ministry of the Environment and Energy, for their support in sample analysis and to Ken Cullis of the Thunder Bay Remedial Action Programme, for their financial support and in supplying equipment required in the surveys of the lakes.

I would like to thank my wife and family for their support and encouragement during these studies.

Grateful acknowledgement also goes to the Kimberly-Clarke Canada Inc. mill at Terrace Bay, for allowing access to the lakes within the study area.

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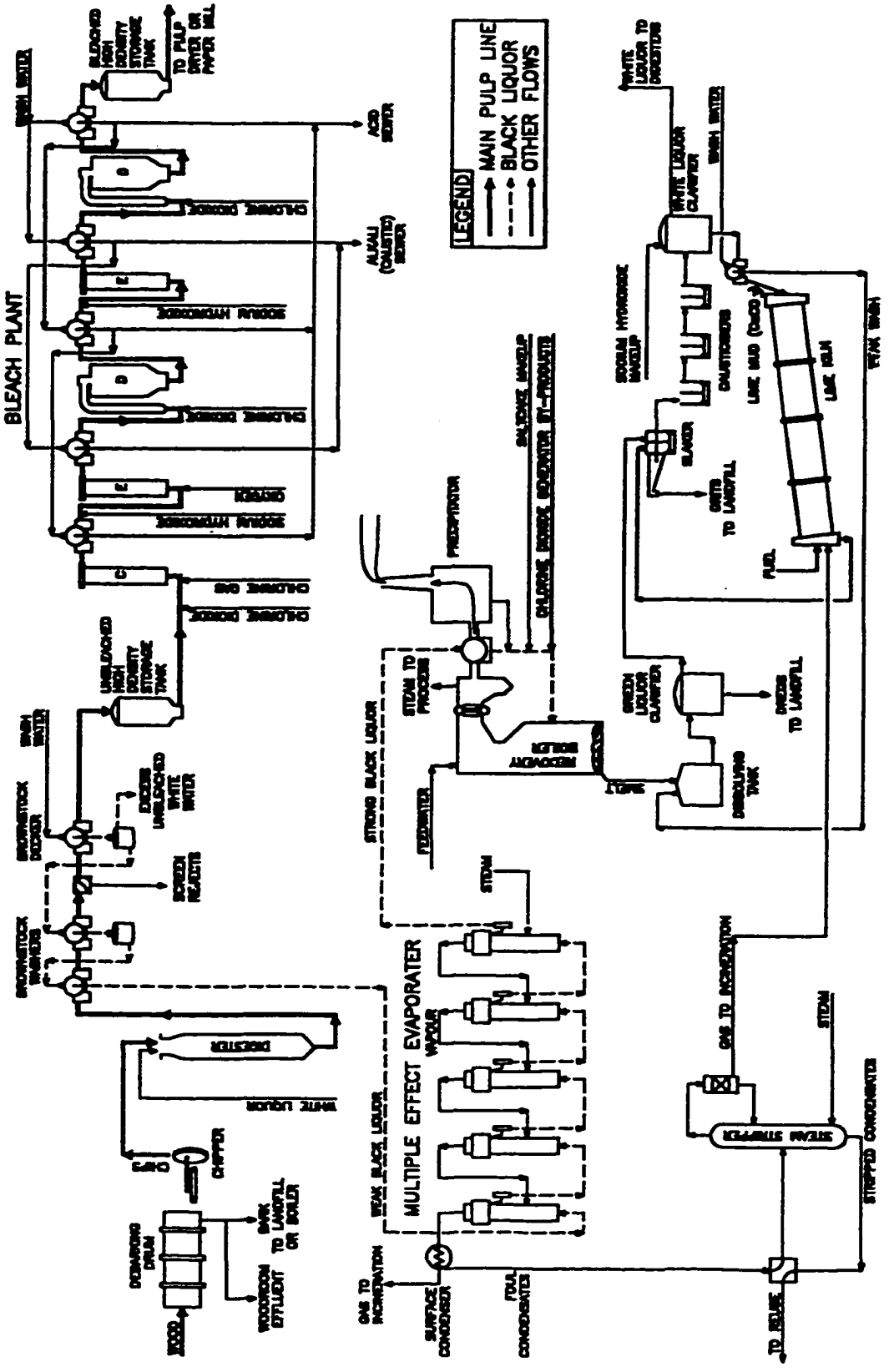
1. GENERAL INTRODUCTION

The effluent resulting from the bleaching stage of the Kraft pulp paper process, is referred to as bleached kraft mill effluent (BKME). In Ontario, Canada the bleaching stage occurs immediately after the washing/screening operation (Fig. 1.1), referred to as the Kraft process (MISA 1992).

The conventional bleaching process is the source of approximately 50% of the BOD, all of the organochlorines, most of the colour (lignin-derived components) and most of the measured toxicity in BKME (MISA 1992). Two sewer outlets remove the effluent from the bleaching process, which accounts for approximately 25% of the total effluent release from the mill. One sewer outlet carries the effluent from the sodium hydroxide dissolution stage and the other carries the effluent from the chlorine/chlorine dioxide bleaching stage (Fig. 1.1). The two effluent sources undergo variable primary treatment, mixing and pH adjustment and often secondary treatment, before release into the effluent stream.

Investigation of the environmental effects of BKME has largely been directed towards the xenotoxic contents. This is justified in light of their extreme persistence and bioconcentration in the lipid components of biota (Corbet *et al.* 1988; Safe 1990).

Figure 1.1 Typical kraft mill flowsheet showing the sewer outlets from the bleaching stage. (MISA 1992).



Xenotoxics produced in the mill include some of the most toxic anthropogenic chemicals identified, including 2,3,7,8-Tetrachlorodibenzo-p-dioxin. The toxic effects of this chemical in mammals, include the impairment of immune responses (Alsharif and Stohs 1992; Lundberg *et al.* 1992), carcinogenesis and teratogenicity (Flodstrom and Ahlborg 1992; Huff 1992), and reproductive toxicity (Moore *et al.* 1992).

Contamination from this congener and others in the group of polychlorinated dioxins and furans has been widely reported in receiving waters and associated ecosystems surrounding pulp and paper mills (Andersson *et al.* 1988; Rappe *et al.* 1987,1990,1991), including at the present study site of Jackfish Bay (Sherman *et al.* 1990).

1.1 THE EFFECTS OF BKME ON THE PHYTOBENTHIC COMMUNITIES

There have been numerous published studies on the toxicities and bioaccumulation by aquatic biota, of the major chlorinated hydrocarbons that have historically been shown to be contained within BKME (Poland and Knutson 1982; De Vault 1989; Opperhuizen and Sijm 1989; Whittle *et al.* 1992). However, there are only limited studies on the wider environmental impacts of BKME.

Poole *et al.* (1978) reviewed the impact of BKME on the aquatic environment, while Pearson (1980), reviewed changes to the marine environment. Both of these studies concentrated on the impact of anoxic conditions created by BKME.

Later studies identified the toxic effects of BKME on phytobenthic communities (Lehtinen *et al.* 1988; Rosemarin *et al.* 1990; Balk *et al.* 1993). A review of the environmental impacts of BKME on the Baltic Sea has been published by Sodergren (1993).

1.1.1 The Effects of BKME on Vegetation

Published studies of the effects of BKME on vegetation, have dealt with uptake mechanisms (Facchetti and Balasso 1986; Reischl *et al.* 1989 and Bacci *et al.* 1992), uptake pathways (Muller *et al.* 1993; Schroll and Scheunert 1993 and Schroll *et al.* 1994) and contaminant levels in terrestrial vegetation (Hulster and Marschner 1993 and Wuthe et. al. 1993).

The only published studies of the effects of BKME on vegetation within receiving waters have concerned the marine phytobenthic algal populations, surrounding paper mills on the Baltic sea (Rosemarin *et al.* 1986; Lehtien *et al.* 1988; Kautsky 1988; Rosemarin *et al.* 1990 and Kautsky 1992). These marine studies have found that plant communities usually demonstrate a greater response to the effluent than invertebrate communities (Sodergren 1993).

Responses observed included: reduced species diversity and total biomass, changes to species composition, distribution, increased biomass of a number of opportunistic annual species and morphological changes in the brown algae *Fucus vesiculous*

(Kautsky 1992). Observed changes to marine plant communities in the study area, were linked to the presence of substances that have been shown to be toxic to plants, notably chlorate (ClO_3^-) (Lindvall 1984; Rosemarin *et al.* 1986; Lehtinen *et al.* 1988) and increased turbidity and eutrophication (Kautsky *et al.* 1988; Kautsky 1992).

1.1.2 Toxic Response

Disappearance of the brown algae *Fucus vesiculosus*, from large regions adjacent to a pulp mill at Monsteras, on the Baltic sea, was correlated to the levels of chlorate effluent (Lindvall 1984). Reduced biomass levels of *F. vesiculosus*, was often accompanied by morphological changes including, dwarfed growth of branches in dense clusters on the thallus (Sodergren 1993).

The toxicities to submerged aquatic macrophytes of some of the heavy metals that have been shown to be present in BKME (OMOE 1991c) were reviewed by Stanley (1974) and more recently by Guilizzoni (1991). The threshold toxicity of most of the metal salts was reported to be 2 $mg.l^{-1}$, although a 50% inhibition of root weight in *Myriophyllum spicatum*, was noted at 0.25 $mg.l^{-1}$ (Stanley 1974). The usefulness of such values has been questioned due to the isolation of the test species and chemical (Lovett Doust *et al.* 1994). In reality the affected species living within a polluted environment, is subject to a diverse array of chemicals and the perceived adverse impact induced as a result of

synergistic interactions from a mixture of chemicals. Similarly the nutrient status of the sediment and water column may significantly alter the the toxicity response of test species (Lovett Doust *et al.* 1994).

Toxic responses include hastened senescence and reduction in rates of photosynthesis and respiration, with the resultant reduction in shoot weight and length (Guilizzoni 1991). Both inter-species and intra-species variation in toxic response, is to be expected in stands of aquatic macrophytes, due to varying physical and chemical environmental variables, as well as individual "tolerance phenomena" of species to toxic levels of contaminants (Guilizzoni 1991).

1.1.3 Response to Reduced Irradiance

Several studies have shown the key role that light attenuation has on controlling the distribution of submerged aquatic macrophytes (Spence 1982; Canfield *et al.* 1985; Howard-Williams *et al.* 1986b; Sand-Jensen 1989).

Aquatic macrophyte diversity, as well as their distribution and density, can all be affected by reduced irradiance and the variation among species that exists in mechanisms allowing plants to adapt to changed light conditions (Spence 1982).

Canfield *et al.* (1985), developed an empirical model to predict the maximum depth of colonization by aquatic macrophytes, using secchi disc measurements.

In the Baltic sea studies (Kautsky *et al.* 1988; Kautsky 1992), marine phytobenthic algal populations were found to be limited to the surface, close to the BKME source. Further out towards the sea, the transparency of the water increased, nutrient levels decreased and the biomasses of the algal species became more evenly distributed.

1.1.4 Other Factors Affecting Aquatic Macrophyte Growth

In an extensive review of the factors that effect aquatic macrophyte growth, Harper (1992), concluded that, besides light, the most important factors are nutrient supply and water temperature. Early research centred on factors affecting the growth of non-macrophytes, with the development of models for algal growth under non-limiting conditions. These models describe biomass increase as a function of the starting biomass (Ahlgren 1988). The same study expressed the maximum specific growth rate, as a function of the external or internal concentration of a single nutrient.

Nutrient levels and the chemical water properties, including pH and alkalinity of the water that the plants are growing in, have been shown to effect aquatic macrophyte growth in natural and created wetlands (Lathwell *et al.* 1969; Lathwell *et al.* 1973; Ozimek 1978; Lee and Stewart 1981).

Reader (1978) reviewed the effects of elevated loading of most sediment organic and inorganic nutrient fractions, including total nitrogen, in promoting primary production in northern

wetlands. The three major nutrients most often found to be limiting in aquatic macrophyte productivity in northern bog-marshes were potassium, phosphorus and nitrogen. The outcome of these nutrient enrichment experiments was highly influenced by the substrate pH and water levels, which also affected levels of anoxia and availability of the nutrients.

Nutrient overloading in aquatic wetlands has been one of the most well documented influences of human activity on aquatic macrophyte growth. Studies of impacts include effects of municipal and industrial sewage on species composition, distribution and floristic changes in plant communities (Eloranta 1970; Uotila 1971, Ozimek 1978) as well as plant morphology and productivity (Ozimek and Sikorska 1975). Sloey *et al.*(1978) reviewed the impact of nutrient overloading, with special reference to wetland management. Major findings included: species diversity often decreased (Ozimek 1978), while productivity of certain species, including small floating plants such as duckweed (*Lemna perpusilla*) and duck-meat (*Spirodela oligorhiza*) increased.

Biochemical reactions within a cell are temperature dependent, showing an exponential increase with temperature up to a maximum, which varies between 25 - 40°C depending on cell type and species (Harper 1992). Research using algal growth models (Welch 1980) indicates that the rate of increase in maximal photosynthetic rates is approxiamately 2, per 10°C increase in temperature.

Other research indicates water depth and fluctuations in depth, may both affect total biomass in aquatic macrophytes (Lathwell *et al.* 1973). In Canada, these factors are more likely to be artifacts of the available littoral surface area within the photic zone and the resultant increase in turbidity, due to depth fluctuations leading to a reduction in available light (Cook and Powers 1958; Howard-Williams *et al.* 1995).

Competition from phytoplankton for available light, following eutrophication, has been shown to affect the growth of submerged aquatic macrophytes (Jones *et al.* 1993; Zimmerman *et al.* 1991; Zimmerman *et al.* 1994). Blooms of epiphytic algae, following eutrophication, have also been shown to be responsible for limiting growth of marine macrophytes (Cambridge and McComb 1984).

Anthropogenic disturbance of wetland systems can have a myriad of effects on aquatic macrophyte distribution and density, although the often dramatic changes relate mostly to deterioration of light availability, nutrient availability and status of the sediment and water temperature (Harper 1992).

BKME has been shown to have impacted the phytobenthic fauna in the receiving waters of Baltic Sea mills (Kautsky 1992), Canadian mills (Munkittrick *et al.* 1992, Muller and Haliburton 1990, Whittle *et al.* 1993) and in the current study area of Jackfish Bay, on the north shore of Lake Superior (Sherman *et al.* 1990). Many of these studies have also shown increased toxic loads of sediment layers, that serve as a sink and reservoir and

possible secondary source of exposure (Rappe *et al.* 1987, Balk *et al.* 1993, Paasivirta *et al.* 1993). BKME has also been shown to affect marine algal populations (Lehtinen *et al.* 1988, Kautsky 1992), but there have been no published reports of specific impacts on aquatic macrophyte populations.

This study was conducted to quantify the visible impacts of BKME that included colour and odor, on the water and sediment quality within three lakes that vary in their exposure to the effluent. This study also relates these physical/chemical impacts, to the productivity and diversity of aquatic macrophyte populations within the three lakes.

1.2 SITE DESCRIPTION

Jackfish Bay was identified in 1985 by the Iternational Joint Commission (IJC) as one of 42 areas of concern (AOC) in the Great Lakes Basin (RAP 1991). Jackfish Bay is located on the north shore of Lake Superior, approximately 250 km northeast of Thunder Bay (Fig. 1.2). The study area includes three separate lakes (A,B and C), situated on the Blackbird Creek system, which carries the wastewater discharge from the Kimberley-Clark Canada Inc. pulp paper mill at Terrace Bay.

Jackfish Bay, was classified as an area of concern by the IJC, based on site impairment related to conventional pollutants, heavy metals, toxic organic contaminants, contaminated sediments, fish consumption advisories and impacted biota, due to an isolated industrial point source and in-place pollutants (toxic sinks) (RAP 1991).

The Kimberley-Clark pulp paper mill began using the bleached kraft process outlined in figure 1.1, in 1972 and now processes two thirds softwood and one third hardwood. This operation currently produces wastewater flow rates of 107,753 m^3/day (OMOEE 1996).

The alkaline sewer from the sodium hydroxide dissolution stage of the bleach plant, undergoes primary treatment before being mixed with the effluent from the acid sewer. The combined effluent is pH adjusted to neutral using lime mud, before undergoing secondary treatment in the aerated stabilization basin. The treated effluent is discharged into an effluent canal leading to Blackbird Creek. The effluent usually comprises 90% of stream flow and travels for 14 km, bypassing Lake A and flowing through Lake C, before entering Jackfish Bay, Lake Superior (RAP 1991).

Lakes A and C were both originally between 6 and 7 meters, (RAP 1991), but following extensive settling of paper pulp, both lakes A and C have been substantially filled in. Lake A is now the most shallow of the three lakes with a maximum recorded depth

of 1.55 m. The maximum recorded depth of Lake B was 6.2 m, while Lake C had a recorded maximum depth of 6.4 m.

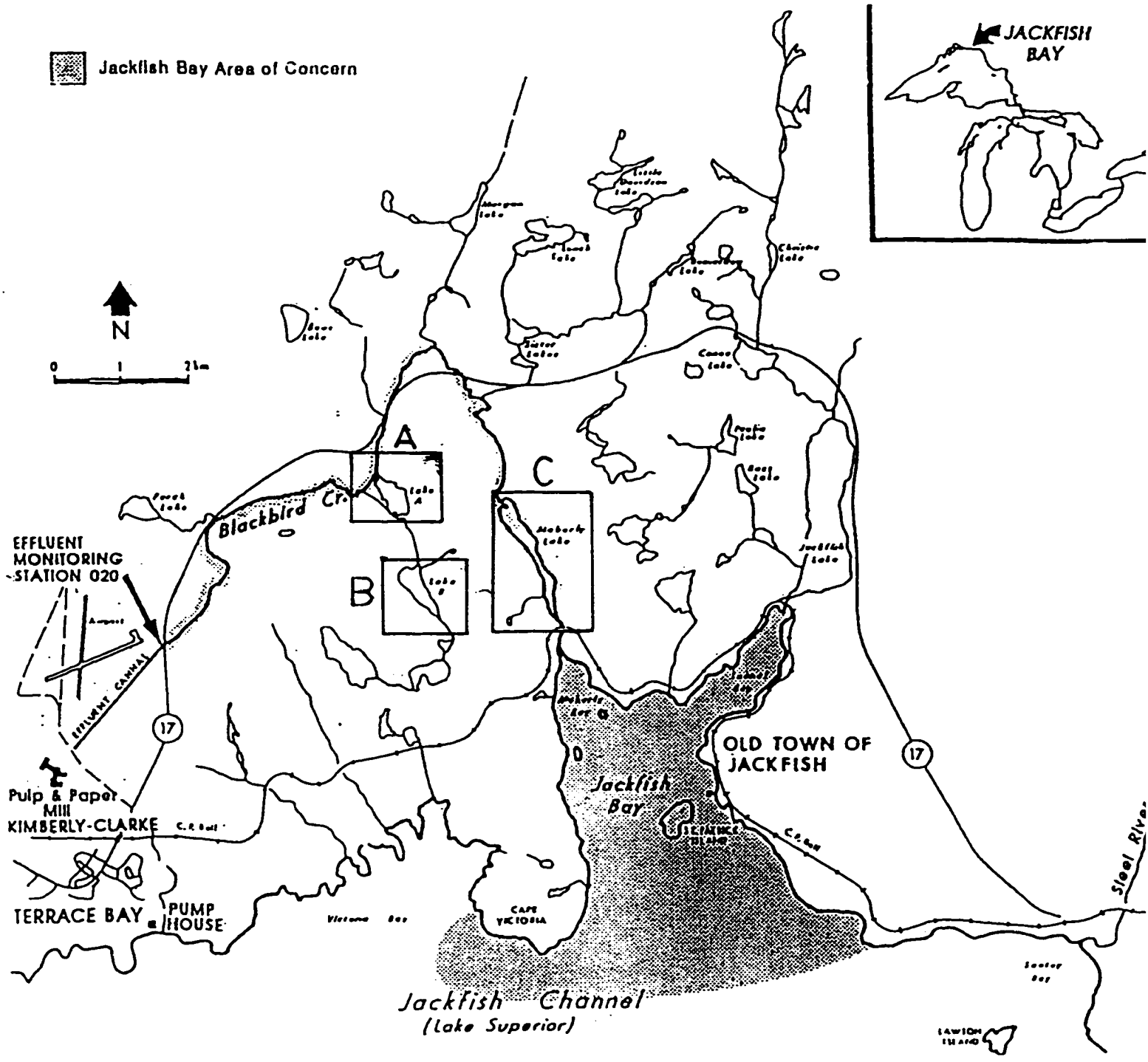
The lakes vary in their exposure to the BKME. Lake A was included in the effluent stream from the opening of the mill in 1948. After the lake became substantially filled in with solid pulp effluent and dredging became less economically viable, it was largely bypassed in the early 1980's by the construction of a barrier, which reduced the entry of BKME into the lake to periods of local flooding, and, maximal BKME volume release. In July of 1993, the barrier across Lake A was breached during local flooding and since then, effluent has been flowing into Lake A. Lake B is upstream from Lakes A and C and has never been a part of the effluent stream. For this reason it has been treated as a control, for comparison of water, sediment and vegetation surveys. Lake C currently remains in the effluent stream.

The priority pollutants contained in the BKME from the Kimberley-Clark mill at Terrace Bay are given in appendix 1, taken from the 1990 (RAP 1991), 1994 and 1995 (OMOEE 1996) effluent monitoring seasons.

Improved wastewater treatment systems at the Kimberley-Clark pulp paper mill have been in place since 1990, and have reduced BOD loading and suspended solids as well as the Adsorbable Organic Halides (AOX), in the effluent since 1990 (appendix 1). This has reduced many of the priority pollutants shown in appendix 1, as well as the level of colour (MISA 1992). However, given the loading of sediment layers (RAP 1991) and the half-

lives of some of the priority pollutants (Muir et. al. 1985b, Miller and Zepp 1987), it is possible that many of these continue to contribute to the biotic burdens that have been detected (RAP 1991).

Figure 1.2 Map of the Jackfish Bay study area, with the 3 lakes highlighted, Lakes A, B, and C.



2. THE EFFECTS OF BLEACHED KRAFT MILL EFFLUENT ON THE DIVERSITY, DISTRIBUTION AND DENSITY OF AQUATIC MACROPHYTES

2.1 ABSTRACT

A survey of the aquatic macrophyte density and diversity was conducted within three lakes (Lakes A,B and C). The three lakes are connected by a common creek, but have historically varied in exposure to Bleached Kraft Mill Effluent (BKME), from a pulp and paper mill in Canada's northern shore of Lake Superior (Fig.1.2).

Lake A was bypassed from the effluent stream in the early 1980's, while Lake B had never been a part of the effluent stream and Lake C currently remains in the effluent stream.

Discriminant analysis using multiple, independent water quality variables, gave 100% separation of the three lakes in the study area for a subset of 36 quadrats. When the full sample of 135 quadrats were included in the discriminant analysis, with just the sediment and vegetation quality data and *in-situ* collected chemical and physical data, 98% accuracy of classification of the three lakes was observed.

Secchi depth was the most significant discriminating variable for the three lakes in all models tested, with colour of water, depth and sediment total nitrogen also being significant.

The plot of the discriminant functions against the total

biomass for the three lakes, gave three clearly separate groups, indicating the statistically distinct effects of exposure to BKME.

Entry of BKME into Lake C was associated with complete loss of aquatic vegetation. Creation of a barrier, reducing BKME entry into Lake A, after 38 years of use as a settling pond, allowed water conditions to improve to the point where colonization of 13 species of aquatic macrophytes occurred.

BKME was diluted across Lake A, which allowed comparative inter- and intra-lake studies of BKME effects to be carried out. BKME reduced total biomass and species diversity within Lake A. In sectors where light availability improved above a minimum level, the nutrient loaded conditions encouraged opportunistic aquatic macrophyte species to grow. Colonies of *Typha latifolia* in Lake A, had greater biomasses than any vegetation samples from the control lake, Lake B.

2.2 INTRODUCTION

Published studies of the biological impacts of BKME on the environment have largely centred on the affects of the adsorbable organic halides (AOX) fractions (Clement *et al.* 1989a,b; Sherman *et al.* 1990; Whittemore *et al.* 1990; Hrutfiord and Negri 1992; Paasivirta *et al.* 1993).

The chemical stability and lipophilicity of these compounds and their resistance to degradation from acids, bases, heat and hydrolysis (Corbet *et al.* 1988), results in their persistence in the environment (Miller and Zepp 1987; Rappe *et al.* 1987) and concentration in the food chain (Crossland *et al.* 1987; Rappe 1992; Theelen and Liem 1994).

The polychlorinated dibenzo-p-dioxins (PCDD's) and polychlorinated dibenzofurans (PCDF's) have received most attention, due to their highly toxic nature in animal testing experiments (Kociba and Schwetz 1982; Poland and Knutson 1982; Birnbaum 1993), and their measured contamination of biota in the receiving waters and sediment, surrounding paper mills using traditional paper bleaching processes (De Vault *et al.* 1989; Rappe 1989; Whittle *et al.* 1992,1993; Balk *et al.* 1993).

Terrestrial vegetation that has been shown to absorb significant levels of the xenotoxic components of BKME, include carrots (Schroll and Scheunert 1993; Schroll *et al.* 1994), lettuce, potato plants and hay (Hulster and Marschner 1993), maize and bean plants (Facchetti *et al.* 1986; Sacchi *et al.*

1986), apples and pears (Muller *et al.* 1993), azalea plants (Bacci *et al.* 1992), conifer needles (Reischl *et al.* 1989), rubber plant, bitter orange, tomatoes and green pepper (Kerler and Schonherr (1988) and barley plants (Briggs *et al.* 1982). None of these studies on terrestrial vegetation have included observations of plant toxicity.

Components of BKME, such as chlorate (ClO_3^-), have been shown to reduce nitrate uptake and net photosynthesis, leading to eradication of the marine algal species, *Fucus vesiculosus*, as far as 1.5 km from the effluent source and reduced growth up to 4 km from the source (Rosemarin *et al.* 1986,1990).

Changes to species composition in favour of opportunistic annual species, reduced species diversity and morphological changes to marine algal populations surrounding paper pulp mills in the Baltic Sea have been reported (Kautsky 1992). The colour and turbidity of BKME, have also been linked to changes in density and distribution of marine algal species in the receiving water of mills in the Baltic Sea (Kautsky *et al.* 1988).

There have been no published studies on the impacts of BKME on aquatic macrophyte populations and this study was established to quantify observed changes to aquatic wetlands exposed to BKME. The effects of BKME on the distribution, density and diversity of aquatic macrophytes were assessed in inland aquatic wetlands. The process of natural regeneration of aquatic wetlands, following cessation of exposure to BKME, was also examined.

2.3 METHODS

The study was conducted over a two year period, including the growing seasons of May-September 1993 and 1994.

2.3.1 Field Procedures

Water and sediment quality, as well as plant distribution and biomass, surveys were carried out on all three lakes. Samples of water, sediment and vegetation, were collected from fifty (20 m x 20 m), randomly selected quadrats, from the littoral zones less than three meters in depth. These regions were assumed to be potentially viable for aquatic macrophyte growth.

Sampling of Lake A was carried out over the entire lake, as the greatest depth did not exceed 1.5 m.

Sediment samples were collected from the top 20 cm of the sediment layer from within each quadrat. The samples were collected with an Eckman dredge, mounted on a bar. Samples were placed in plastic bags and transported on ice to the laboratory, for nutrient analysis.

Secchi depth and absolute depth, were also determined within each quadrat.

Vegetation samples, 0.082 m^2 in area, were collected from each quadrat in mid September, from Lakes A and B, using an

aquatic macrophyte sampler (Marshall and Lee 1994). The area of each sample represented half the circular swath of the aquatic sampler (0.164 m^2) and the volume of vegetation, that was transplanted to buckets, for subsequent growth in both greenhouse and *in-situ* experiments in Lake C. This area was chosen as it represented the maximum biomass of the most prolific emergent species, that could be transplanted into the 10 litre plastic buckets used for propagation trials.

Plants were sheared off by the aquatic sampler at the point where stems emerged from the sediment and placed into plastic bags. The bags were placed on ice for transport to the laboratory.

The expense of water quality analysis limited the number of water column samples to 18 per season. For this reason, water quality data was collected for only 36 quadrats over the three lakes. Water samples were collected 20 cm below the surface, in the summer of 1993 and 1994, from six quadrats for each lake. These samples were placed into plastic sample jars and transported on ice to the Ontario Ministry of the Environment and Energy (OMOEE) laboratory in Thunder Bay, for analysis.

Physical properties of water temperature, colour, turbidity, as well as dissolved oxygen and pH, were measured within each quadrat.

2.3.2 Laboratory Procedures

In the laboratory, plant samples were washed, sorted into species and identified, before being placed into pre-weighed paper bags. The bags containing the plant samples, were oven dried for 48 hours, at 80°C for biomass determination.

Species richness for each quadrat was recorded as the total number of species recorded within the quadrat. Mean species diversity for each lake, represents the Shannon diversity index (Brower *et al.* 1990).

All sediment samples were analyzed in their wet state for loss on ignition, pH, and extractable phosphorus, nitrogen, iron, manganese, zinc, copper, calcium, magnesium and potassium using the standard procedures outlined in Lee (1986), with the exception that total nitrogen was analyzed using the Kjeldahl Digestion method (American Public Health Association 1971).

All sediment nutrient values were calculated as a mass per volume (g/1 m x 1 m x 20 cm sediment depth).

2.3.2 Data Analysis

Due to the nature of the sample collection, two major data sets were generated for statistical analysis.

I. The first data set, referred to as "water quality" is presented in section 2.4.1. This set includes the water quality data, physical and chemical parameters of the water column that could be measured in-situ, sediment quality data, and vegetation data for 36 quadrats, from all three lakes in the study area (Fig. 1.2.1).

II. The second data set, referred to as "sediment quality" is presented in section 2.4.2. This set includes the sediment quality data, physical and chemical parameters of the water column that could be measured *in-situ* and vegetation data, for 156 quadrats from all three lakes.

Statistical analysis was carried out using a networked, Windows SPSS 6.1 version.

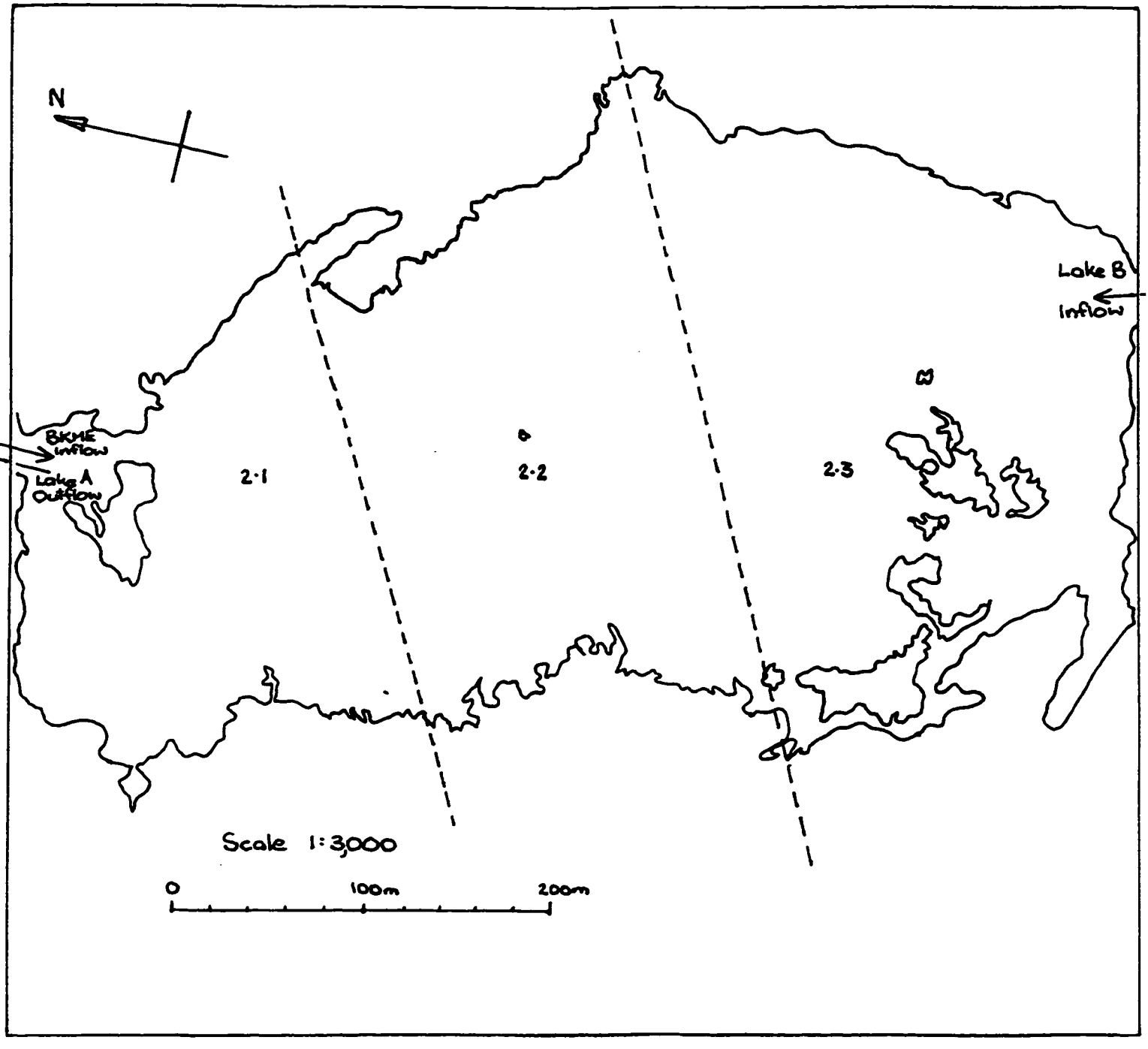
1. Multiple regression, using "biomass" as the dependent variable, was carried out against the independent variables listed in Tables 2.4.2 and 2.4.3.

2. Discriminant analysis was carried out using a lake value 1-3, for separation. As with the regression analysis, the vegetation parameters of biomass and diversity, were excluded from the discriminant analysis. Classified groups were defined by lake value. This method was used so that the vegetation parameters could be correlated to the discriminant functions.

3. The discriminant functions obtained, were then plotted against biomass and species diversity values.

4. Due to the observed, graduated influx of BKME into Lake A, and the amount of variation in both the dependent variable (BIOMASS) and the independent variables (Tables 2.4.2., 2.4.3), for this lake, both data sets were further divided, by sectioning the data collected from Lake A, into three groups gained from three approximately equal areas of the lake. The 3 Lake A sectors, are located at a graduated distance from the source of the BKME into Lake A (Fig. 2.3.1). The statistical analysis was carried out using the same methods as described above, but with 5 lake values, instead of 3.

Figure 2.3.1 Map of Lake A, showing the 3 sections used for the statistical analysis of the "5 lake group" data set.



2.4 RESULTS

Lake C was totally devoid of both submersed and emergent macrophytes. Exposed islands within Lake C contained some pioneer terrestrial grasses, while terrestrial vegetation dominated by *Alnus incana* and *Salix discolor*, grew within a few centimetres of the high water mark on the lake perimeter.

Following the washing away of the barrier that prevented the effluent stream (Blackbird Creek), from mixing with Lake A, darkly stained effluent was observed within the lake. There was a graduated staining within Lake A, that was heaviest at the northern end of the lake (Fig.2.3.1), where both the effluent entered and the lake drained back into Blackbird Creek. At the southern end, where Lake B drained into Lake A, the staining was less.

2.4.1 Water Quality Data Set

There was a significant graduation ($P < 0.05$) in secchi depth and the other parameters that relate to light attenuation (colour and turbidity), between the three lakes ($P < 0.05$), for this data set (Fig. 2.4.2).

There was a similar trend in increasing water temperature, colour, conductivity and total alkalinity, as well as the organic nutrients total phosphorus, total nitrogen, total nitrates, total ammonium and the inorganic nutrients Ca, Cl, Na and Mg between the three lakes, in the following ascending order: Lake B < Lake

A < Lake C (Table 2.4.1). The inverse trend of decreasing dissolved oxygen and secchi depth occurred between the three lakes in the same order as given above (Table 2.4.1).

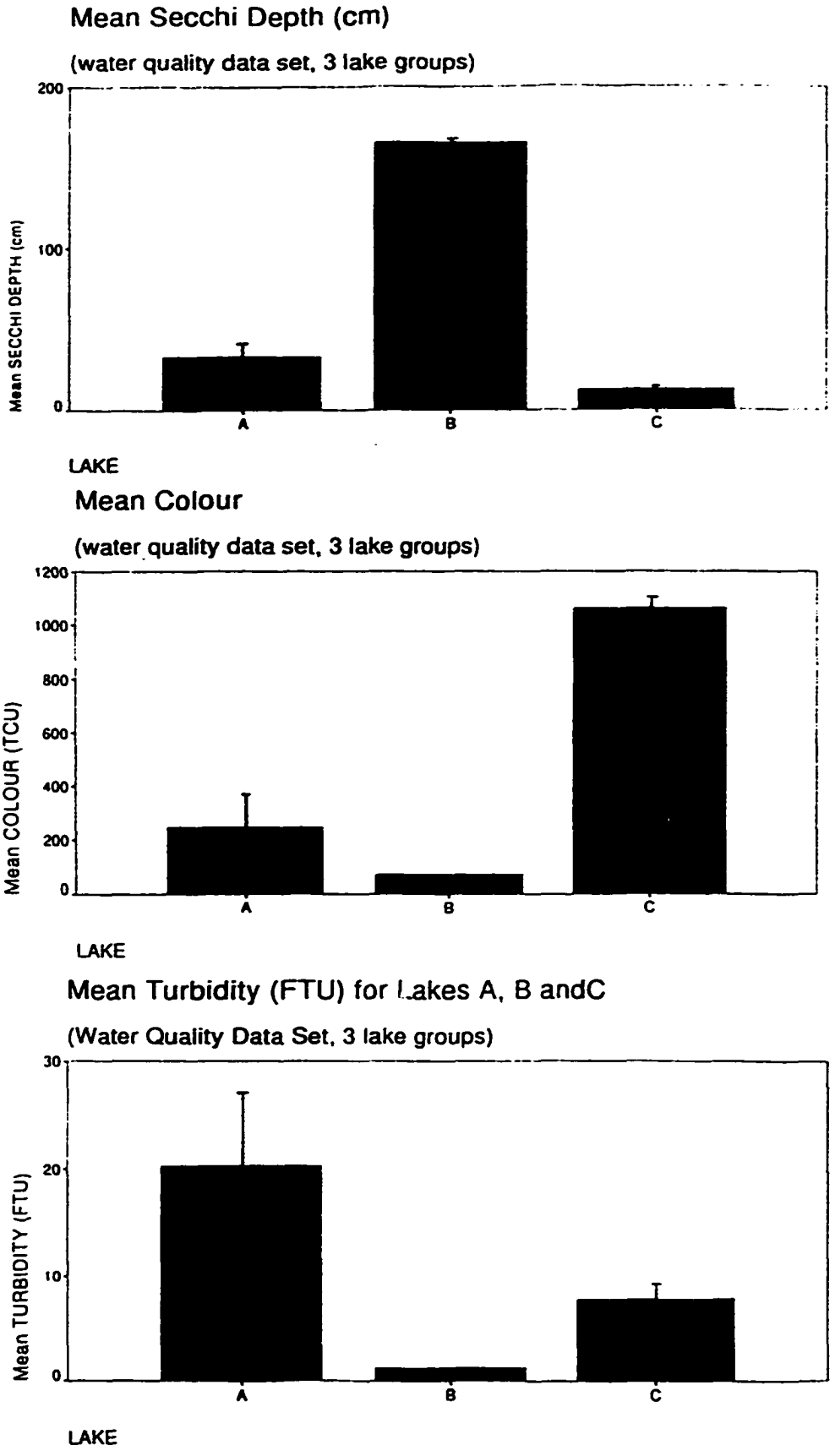
Colour and turbidity were both elevated in lakes A and C, compared to the control lake, Lake B (Fig. 2.4.2).

Similar increasing trends, existed for both total biomass and species diversity, with Lake C being zero, Lake A being intermediate and Lake B being highest (Fig. 2.4.1a,b).

Table 2.4.1 Water Quality Data that includes the physical and chemical parameters measured *in-situ*, and chemical nutrient levels for Lakes A, B and C.

Mean Parameter +- SE	Lake A	Lake B	Lake C
Mean Water Temperature °C	20.24 +- 0.55	18.21 +- 0.22	23.82 +- 0.39
Mean Water pH	7.15 +- 0.05	7.20 +- 0.03	7.45 +- 0.06
Mean Dissolved O_2 (mg/L)	3.91 +- 0.26	9.16 +- 0.03	3.28 +- 0.29
Mean Sample Depth (cm)	55.82 +- 7.89	199.24 +- 35.55	141.03 +- 30.34
Mean Secchi Depth (cm)	33.24 +- 2.01	166.17 +- 0.97	12.8 +- 0.3
Mean Colour (TCU)	249.25 +- 47.1	72.91 +- 0.50	1063.33 +- 15.9
Mean Turbidity. (FTU)	20.28 +- 4.61	1.33 +- 0.03	7.78 +- 0.92
Mean Conductivity (uMho/cm)	416.25 +- 51.42	54.92 +- 0.13	1306.67 +- 34.68
Mean Total Alkalinity (mg/L)	103.25 +- 7.02	22.0 +- 0.0	172.17 +- 4.50
Mean Ca (mg/L)	34.79 +- 2.09	8.1 +- 0.1	58.08 +- 2.09
Mean Cl (mg/L)	56.59 +- 11.29	2.63 +- 2.37	260.0 +- 6.28
Mean Na (mg/L)	53.74 +- 10.75	1.0 +- 0.0	231.67 +- 2.97
Mean Mg (mg/L)	4.74 +- 0.28	1.9 +- 0.0	5.1 +- 0.02
Mean Total N (mg/L)	1.27 +- 0.2	0.43 +- 0.01	1.81 +- 0.07
Mean Total P (mg/L)	0.13 +- 0.03	0.01 +- 0.0	0.31 +- 0.01
Mean K (mg/L)	2.25 +- 0.36	0.29 +- 0.01	8.3 +- 0.09
Mean Total NH_4^{+1} (mg/L)	0.12 +- 0.02	0.02 +- 0.002	0.45 +- 0.04
Mean Total NO_3^{-1}	0.11 +- 0.03	0.01 +- 0.0	1.23 +- 0.04

Figure 2.4.2 Mean secchi depth (cm), colour (TCU) and turbidity (FTU) levels with standard error, for water quality data set (3 lake groups).



Mean sediment total phosphorus and nitrogen, were both elevated in Lake C compared to lakes A and B, while calcium was elevated in both Lakes A and C (Table 2.4.2).

Lake B, the control lake, had a higher biomass and species diversity than Lake A (Table 2.4.3).

Division of the water quality data from Lake A into 3 sectors, giving 5 lake groups, allowed an examination of the source of variation that existed for all of the water/sediment quality parameters listed in table (2.4.1) and both the vegetation parameters, "biomass" and "diversity".

The plots of the mean total biomass and species diversity for the 5 lake groups, indicated a similar trend for both parameters across the three sectors of Lake A (Fig. 2.4.3). The lowest levels for both parameters, were found in sector 2.1, which was closest to the point of entry, of BKME into the lake (Fig 2.3.1). The graduation followed the distance from the BKME source, with the highest levels being recorded for sector 2.3.

The highest biomass for any of the 5 sectors from the three lakes, was recorded for sector 2.3 in Lake A.

Secchi depth increased across Lake A, which was reflected in decreased water colour and turbidity values. These two parameters followed an inverse relationship across Lake A, to secchi depth, with the lowest values recorded in sector 2.1, closest to the BKME source (Fig. 1.2.1) and the highest values recorded in sector 2.3 (Fig. 2.4.4).

Lake water in both Lakes A and C, had elevated

concentrations for all inorganic and organic nutrients measured, compared with the control lake, Lake B (Table 2.4.1). Most significant loadings occurred for the inorganic elements, sodium (x 230) and chloride (x 100) and for the organic fractions total phosphorus (x 300) and total nitrates (x 120), compared to Lake B.

Sediment analysis indicated the sediment in Lake C had elevated concentrations of the inorganic fractions, potassium (x 2) and calcium (x 2) and the organic fractions, total phosphorus (x 2) and total nitrogen (x 6), compared to the control lake, Lake B (Table 2.4.2).

Regression Analysis

Multiple regression analysis using "biomass" as the dependent variable to determine single independent variable effects on biomass within the three lakes, highlighted "secchi depth" as having the only significant effect on biomass. The multiple regression coefficient for secchi depth R, is 0.684, with an adjusted R^2 value of 0.450 and significance of T of 0.0001.

Figure 2.4.3a,b Total mean biomass (dry wt./m^2) with standard error and species diversity (Shannon diversity index), for the water quality data set (5 lake groups).

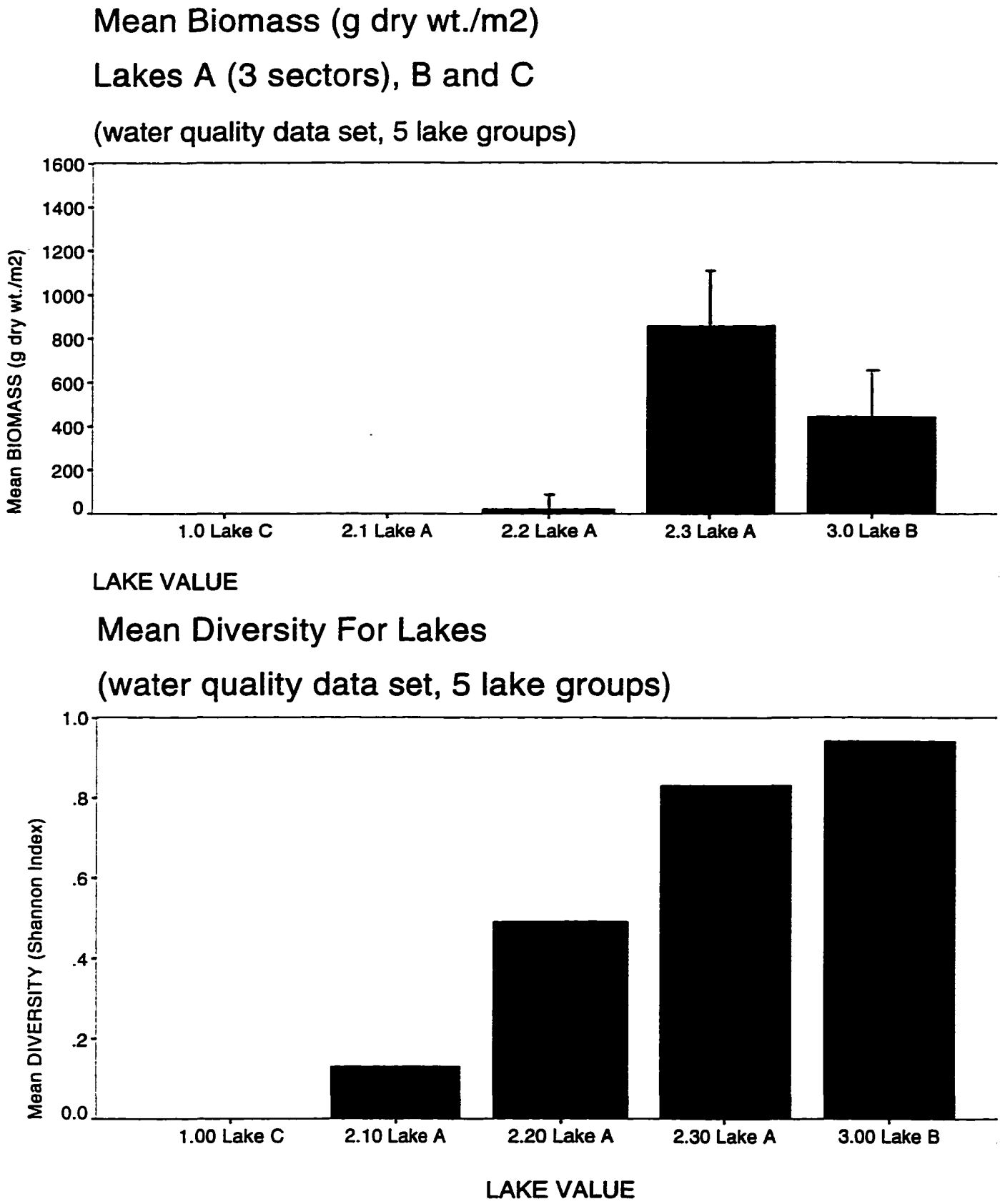


Table 2.4.2 Sediment quality data that includes the physical parameters and chemical nutrient levels for Lakes A, B and C.

Parameter +- SE	Lake A	Lake B	Lake C
Loss on Ignition (g/m^3)	36.67 +- 2.29	32.12 +- 3.3	34.8 +- 2.45
Bulk Density (g/m^3)	0.37 +- 0.02	0.37 +- 0.03	0.42 +- 0.03
Fe (g/m^3)	14.37 +- 3.44	94.11 +- 10.19	40.37 +- 5.37
K (g/m^3)	3.78 +- 0.34	3.13 +- 0.24	6.67 +- 0.25
Mg (g/m^3)	14.44 +- 0.6	22.92 +- 1.29	22.76 +- 0.63
Mn (g/m^3)	2.23 +- 0.24	8.38 +- 0.96	4.91 +- 0.23
Zn (g/m^3)	0.22 +- 0.07	0.43 +- 0.03	0.55 +- 0.21
Cu (g/m^3)	0.02 +- 0.001	0.07 +- 0.005	0.02 +- 0.001
Ca (g/m^3)	304.35 +- 8.11	220.09 +- 11.81	323.16 +- 9.02
Total N (g/m^3)	2.61 +- 0.31	1.68 +- 0.11	10.4 +- 1.08
Total P (g/m^3)	1.01 +- 0.15	1.52 +- 0.1	3.54 +- 0.16

Table 2.4.3 Aquatic macrophyte data for Lakes A, B and C. Mean biomass for each lake was calculated as the mean biomass of half of the sample collected by the aquatic sampler from all of the quadrats (g dry wt./ m^2). Mean species diversity was calculated using the Shannon diversity indice, H' (Brower *et al* 1990).

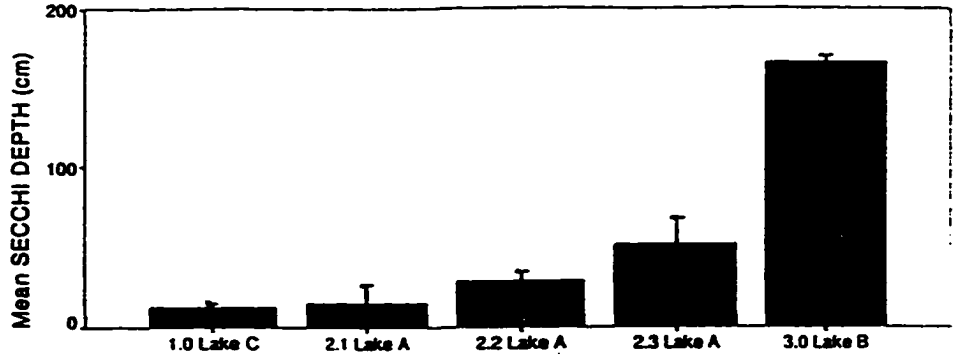
Mean Parameter +- SE	Lake A	Lake B	Lake C
Mean Biomass (g dry wt./ m^2)	315.46 +- 236.42	442.56 +- 125.22	0.0
Mean Species Diversity	0.87	0.96	0.0

Figure 2.4.4 Mean secchi depth, colour and turbidity with standard error, for water quality data set (5 lake groups).

Mean Secchi Depth (cm)

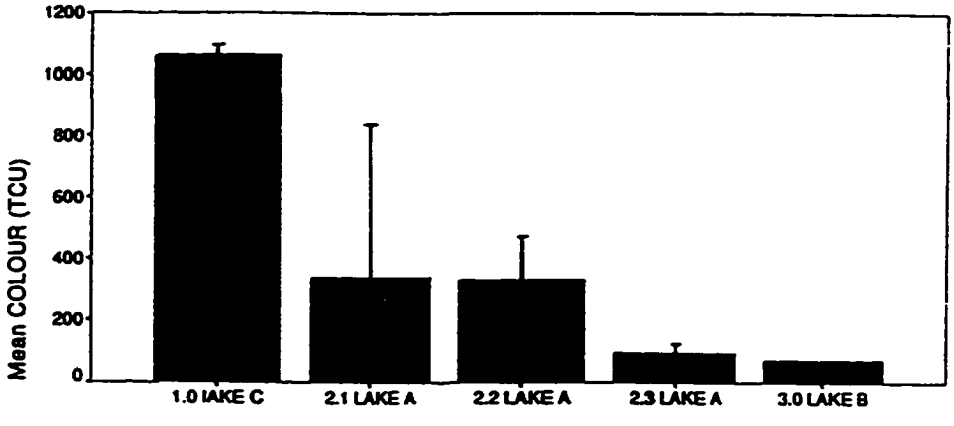
Lakes A, B and C

(Water Quality Quadrats, 5 Lake Groups)



Mean Colour (TCU) for Lakes A, B, and C

(Water Quality Data Set, 5 lake groups)



Mean Turbidity (FTU) for Lakes A, B and C.

(water quality data set, 5 lake groups)

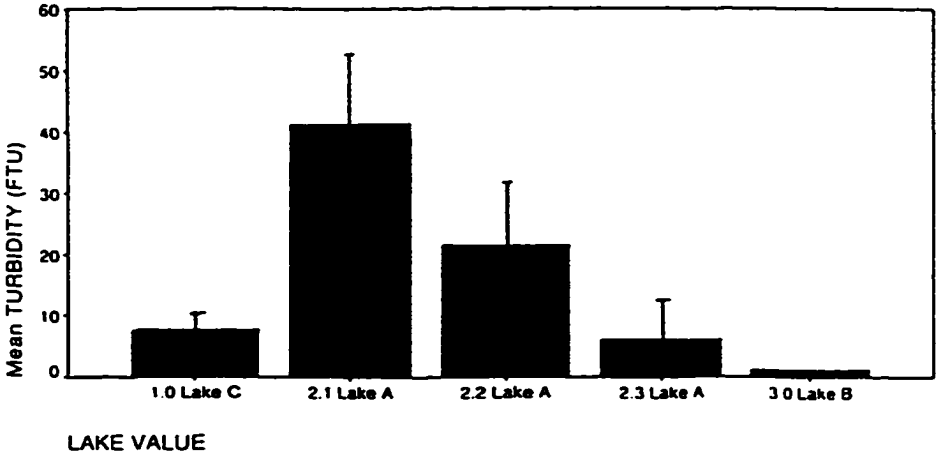


Table 2.4.4a,b. Discriminant functions for the water quality data set that includes the amount of variation explained by each of the two functions (3 and 5 lake group sets). The 3 lake group data set has 2 discriminant functions explaining 100 % of the variation, while the 5 lake group has 4 discriminant functions explaining 100 % of the variation.

2.4.4a (3 Lake Group).

Fn.	Eigen Value	% Var.	Cum.% Var.	Con. Corr.	After Fnc.	WilksLambda	Chi-sq.	df	Sig.
					0	.000003	298.58	20	.0000
1	1051.8	77.1	77.12	.9995	1	.003194	135.04	9	.0000
2	312.07	22.9	100.00	.9984					

2.4.4b (5 Lake Group)

Fn.	Eigen Value	% Var.	Cum. %	Can. Corr	After Fn.	Wilks Lambda	Chi-sq.	df	Sig.
					0	.00000	352.38	44	.0000
1	1086.3	62.10	62.10	.9995	1	.00012	198.56	30	.0000
2	656.45	37.52	99.62	.9992	2	.07909	55.818	18	.0000
3	5.7864	0.33	99.95	.9234	3	.53672	13.690	8	.0902
4	0.8632	0.05	100.00	.6806					

Table 2.4.5 Discriminant function coefficients for water quality data set (3 lake groups). The unstandardized coefficients are the actual values of the variables composing the discriminant functions; the absolute values of the standardized coefficients indicate the relative contribution of each variable to the discriminant functions. The minimum P <0.05, maximum P<0.06.

Parameter	Standardized canonical discriminant function coefficients		Unstandardized canonical discriminant function coefficients	
	Function 1	Function 2	Function 1	Function 2
Cl	0.21455	5.18353	0.0069683	0.1683579
K	-0.23949	-3.1109	-0.264045	-3.429844
Mg	-2.00993	-0.00531	-9.449956	-0.024945
Na	5.04936	-3.14266	0.1877480	-0.116852
Secchi depth	1.94152	0.97791	0.2227968	0.1122188
Total Alkalinity	-3.10395	-0.93923	-0.187273	-0.056667
Total $NH4^+$	0.00981	2.24363	0.0960297	21.960055
Total $NO3^-$	-0.52737	3.8902	-5.227879	38.563797
Turbidity	1.93665	0.22437	0.1702771	0.0197277

Discriminant Analysis

The discriminant functions for both the three lake group and the five lake group, gave a 100% accuracy in group classification (Table 3.5a,b). Function 1 explained 77% of the variation between the classified groups, while function 2 described the remaining 23%.

Function 1, discriminated along the x axis in Figure 2.4.5, classifying the control lake, Lake B, far to the positive end of the scale from lakes A and C, which are shown to be more closely related. Function 2 discriminated between the three lake groups along the y axis, giving evenly spaced, but clearly separated classification groups for the lakes.

There was little difference in the distribution of the classified lake groups, between the 3 and 5 lake group data sets (Fig. 2.4.5a,b). The 3 sectors of Lake A, 2.1, 2.2 and 2.3, in Figure 2.4.5b, were all classified within the spread of the points classified for the lake group 2 (Lake A) , in Figure 2.4.5a.

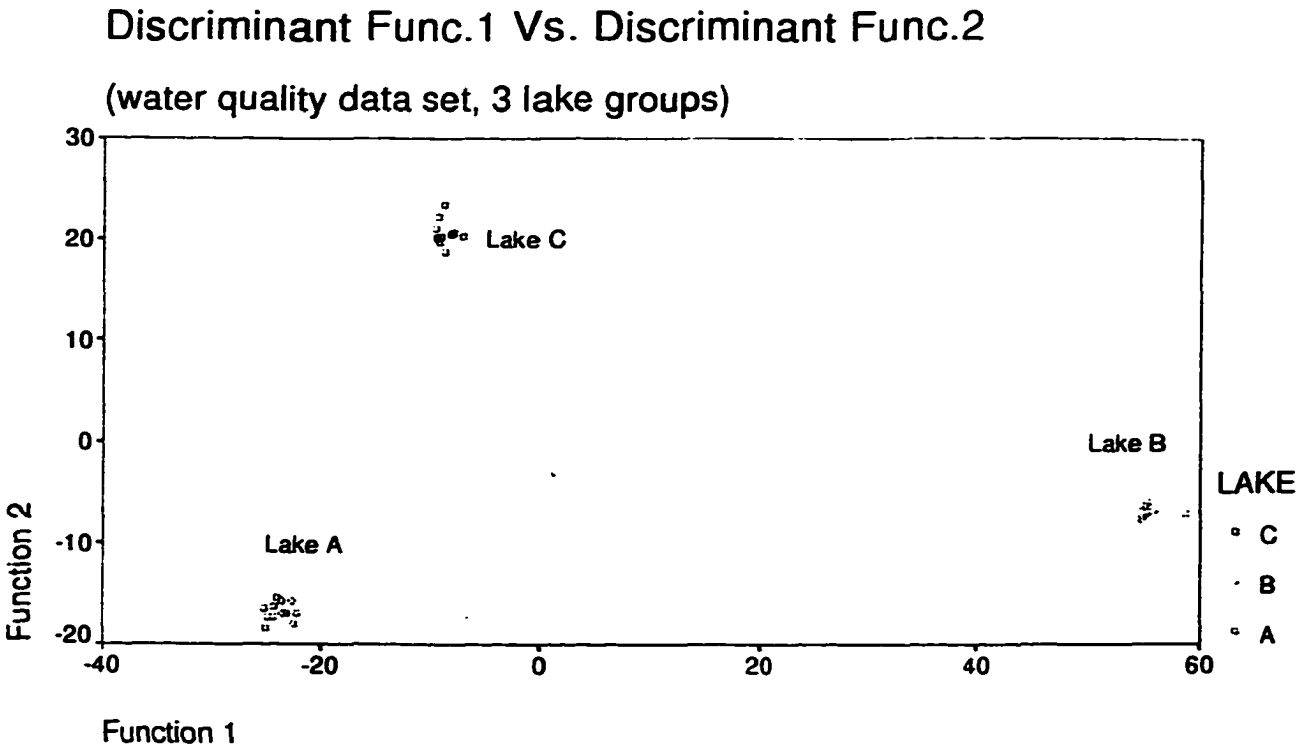
The parameters included in the canonical discriminant functions and their relative contribution to the accuracy of the classification into lake groups, is shown in Table 2.4.5 for the water quality data set.

The concentration of sodium and magnesium in the lake water and total alkalinity, were the most discriminating chemical parameters, while secchi depth and turbidity were the most discriminating physical parameters between the three lakes (Table

2.4.5).

The plots of the two discriminant functions from the 3 lake group set, verses the total biomass of the three lakes (Fig.2.4.6a,b), indicates the variation of the biomass sampling in the three lakes. Lake A has the largest amount of variation in biomass.

Figure 2.4.5a,b. Plot of the canonical discriminant functions for the water quality data set, indicating the spread of the classified lake groups (3 and 5 lake group sets).



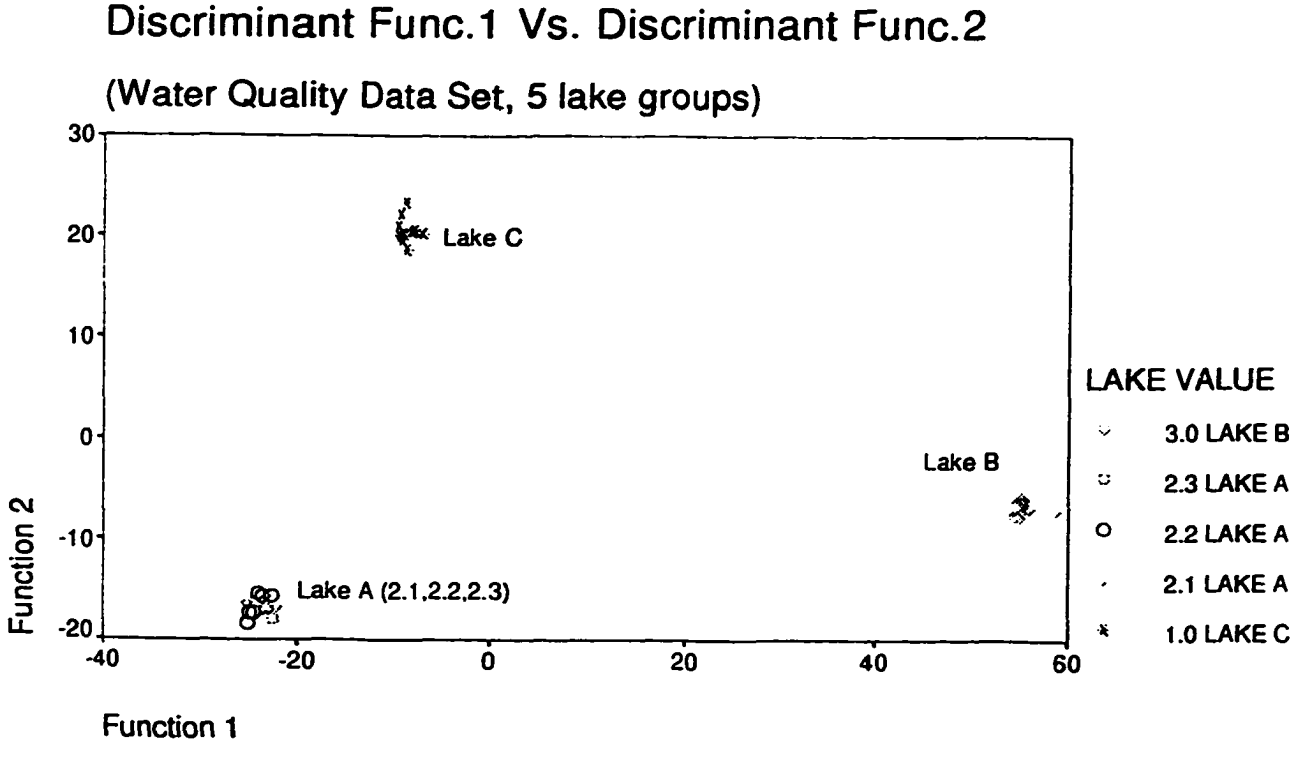
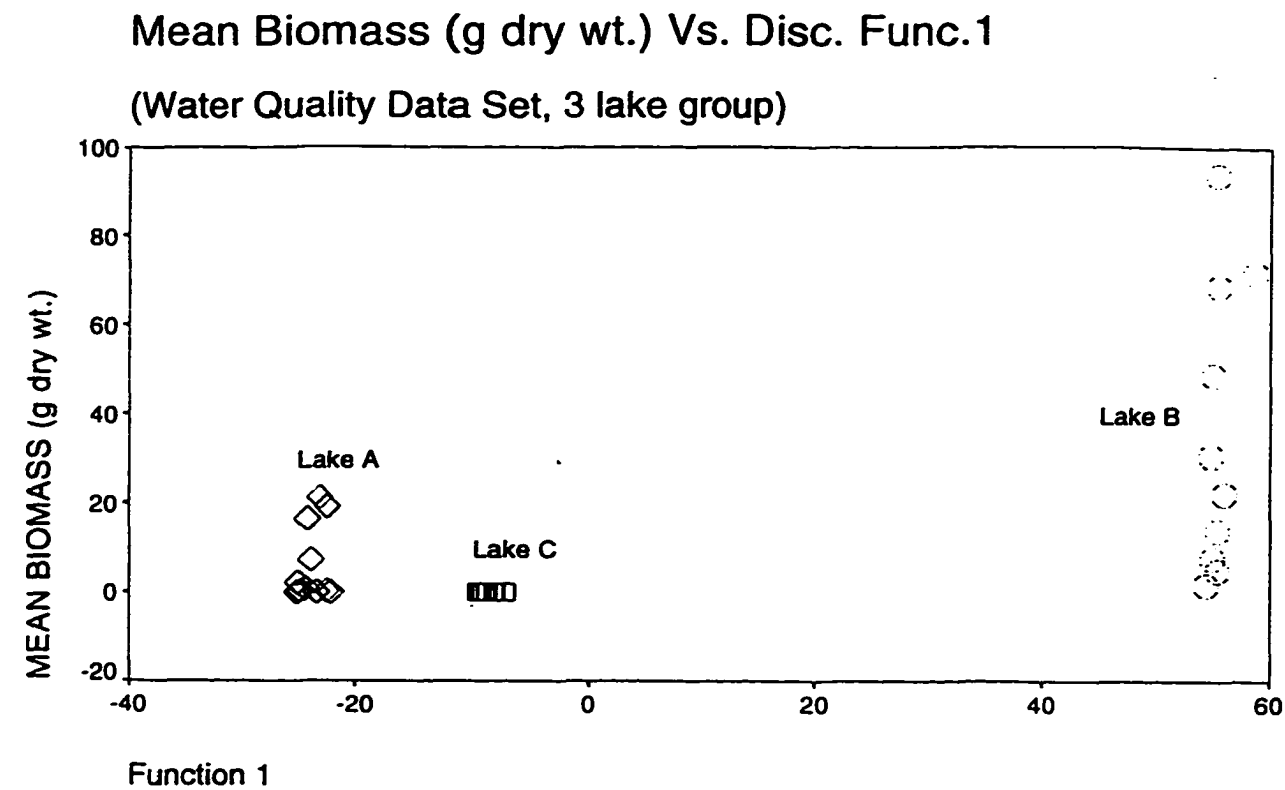
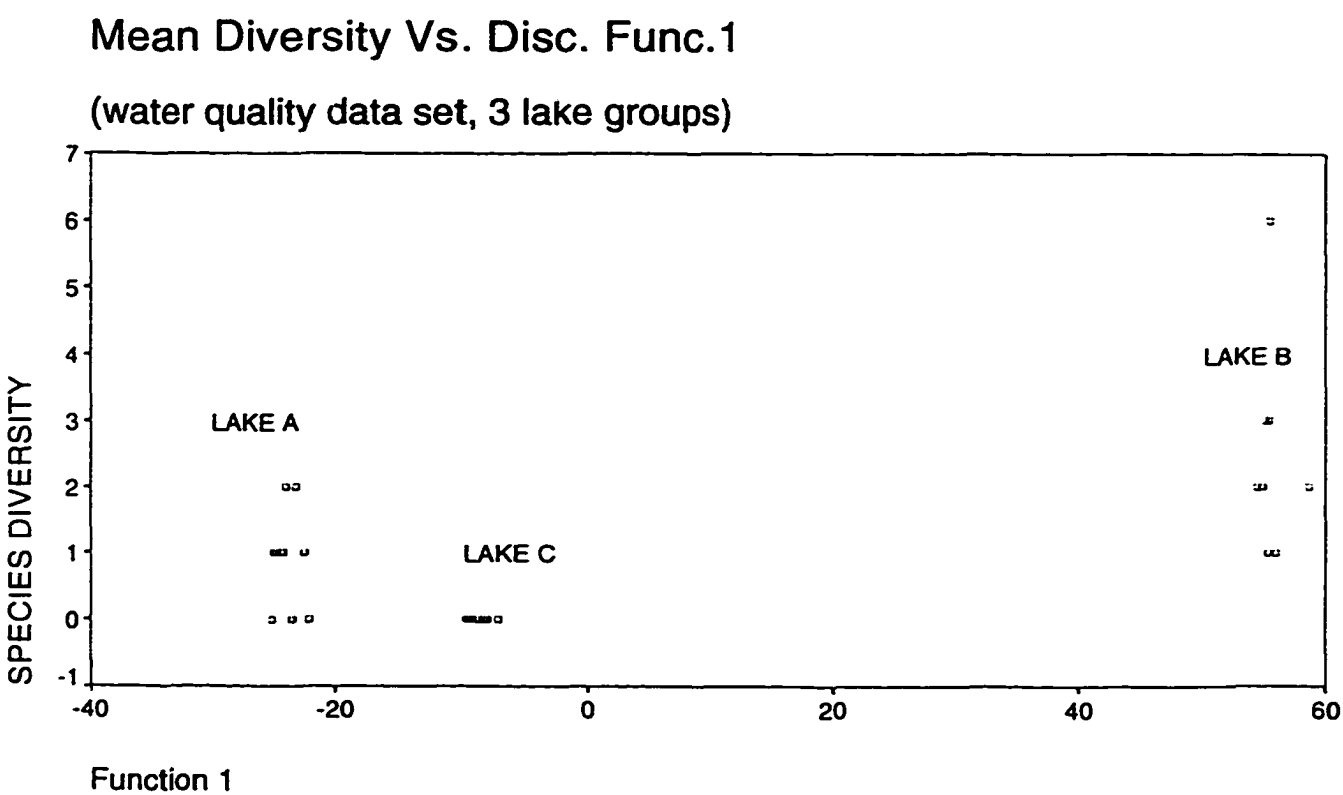


Figure 2.4.6a,b Plots of total mean biomass and species diversity Vs. discriminant functions 1. for Lakes A, B and C, showing the spread of the biomass data within each classified lake group (water quality data set).





2.4.2 Sediment Data Set

Similar trends existed in the limnological parameters measured for the three lakes for the sediment data set (Table 2.4.6), as for the water quality data set (Table 2.4.1). There was a graduation between the three lakes, for the vegetation parameters, biomass and diversity (Fig. 2.4.7), secchi depth (Fig. 2.4.8) and dissolved oxygen content, with Lake C being the lowest, Lake A intermediate and Lake B being the highest. The inverse trend existed for water temperature and the concentrations of calcium and the organic fraction total nitrogen (Fig. 2.4.9), with Lake C being the highest and Lake B being the lowest.

With the Lake A biomass, undivided into 3 sectors, the mean total biomass of both lakes A and B, was significantly higher than Lake C, while there was not a significant difference in mean biomass between Lake A and Lake B at $P < 0.05$.

As in the water quality data set, dividing Lake A biomass data into three sectors permitted a more accurate definition of the variation within the lake. The largest variation in biomass came from the sector of Lake A that was furthest from the source of BKME into the lake, sector 2.3 (Fig. 2.2.1).

Each sector was significantly different from the other two, while the sector closest to the source of BKME, 2.1, was not significantly different from the mean biomass of Lake C. The sector furthest from the BKME source, 2.3, was not significantly different from the control lake, Lake B at $P = 0.05$.

The results indicating the light conditions for the three lakes in the sediment data set followed the same trends (Fig. 2.4.8) as for the water quality data set (Fig.2.4.4). The mean secchi depth increased across Lake A, was lowest in Lake C and highest in Lake B.

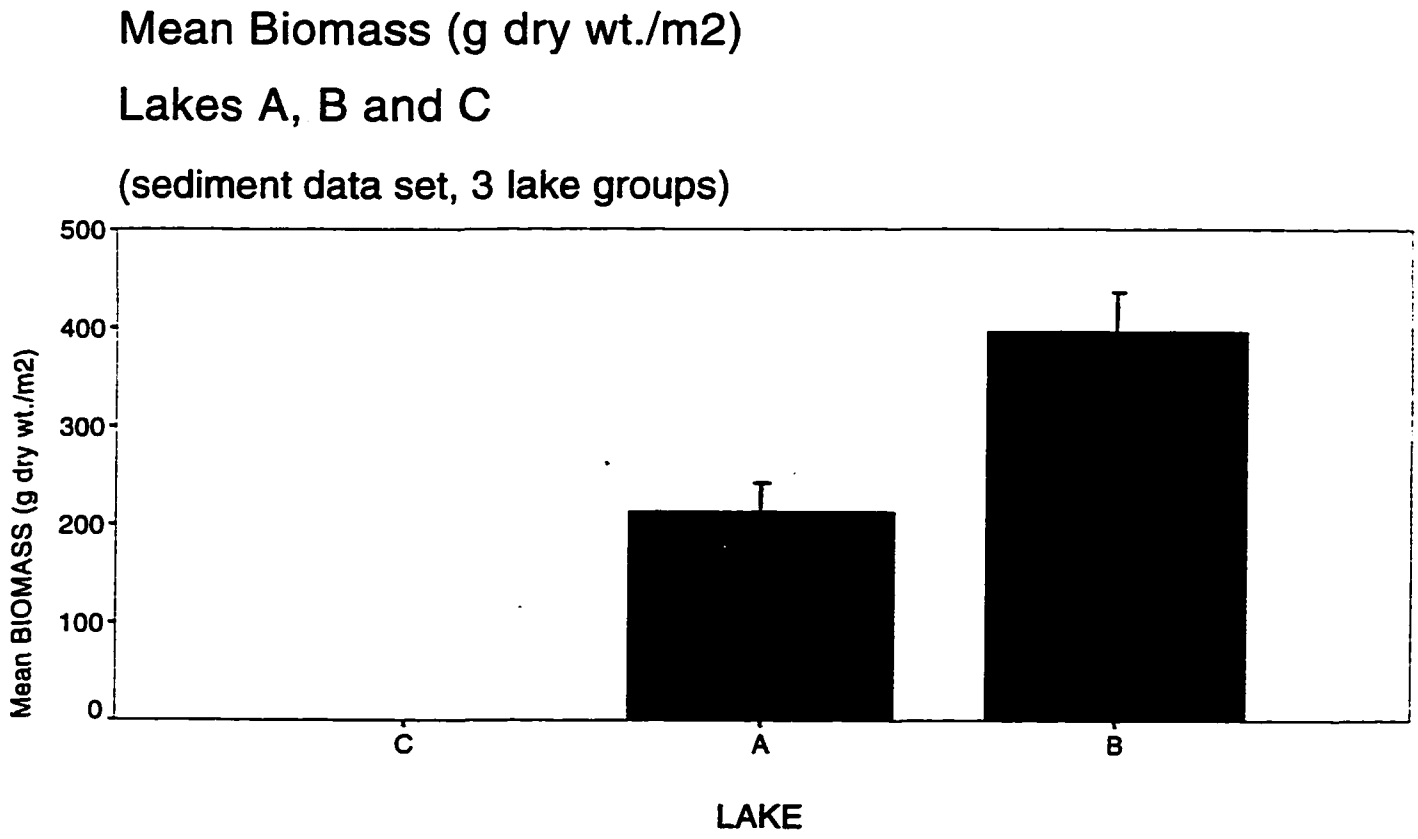
Several differences existed in the species diversity and composition of aquatic macrophytes between the three lakes (Table 2.4.7). Thirteen species were identified in Lake A, nine of which, were emergent species and five were unique to this lake. Sixteen species were identified in Lake B, 7 of which, were emergent species and eight were unique.

Typha latifolia was noted as a fast growing emergent macrophyte, found only in Lake A. This species grew prolifically in floating islands and in dense, anchored colonies, close to the shore line. There was a graduation in species diversity across Lake A (Fig. 2.4.11). Two species occurred in the sector closest to the leakage of BKME, seven species in the middle sector and eleven species in the sector furthest from the leakage.

Table 2.4.6 Mean vegetation, water quality and sediment quality parameters +- SE, measured in Lakes A, B and C (135 quadrats). Mean species diversity was calculated using the Shannon diversity index (Brower *et al.* 1990) for each lake.

Parameter	Lake A +- SE	Lake B +- SE	Lake C +- SE
Mean Biomass (g dry wt./ m^2)	213.13 +- 74.62	396.39 +- 56.57	0
Mean Spp. Diversity	0.96	0.99	0
Mean Bulk Density (g\m^3)	0.365 +- 0.02	0.366 +- 0.028	0.421 +- 0.025
Mean Sample Depth (cm)	59.82 +- 2.89	113.24 +- 9.55	97.03 +- 14.34
Mean Secchi Depth (cm)	34.24 +- 2.00	177.17 +- 0.97	12.80 +- 0.30
Mean Water Temperature (°C)	20.08 +- 0.28	18.52 +- 0.08	24.39 +- 0.28
Mean Loss on Ignition (g/m^3)	36.67 +- 2.29	32.122 +- 3.304	34.798 +- 2.445
Mean pH	6.624 +- 0.02	6.072 +- 0.04	6.692 +- 0.04
Mean Sediment Ca (g/m^3)	304.35 +- 8.105	220.094 +- 11.813	323.159 +- 9.024
Mean Sediment Cu (g/m^3)	0.019 +- 0.001	0.066 +- 0.005	0.017 +- 0.002
Mean Sediment Fe (g/m^3)	14.372 +- 3.438	94.106 +- 10.193	40.37 +- 5.373
Mean Sediment K (g/m^3)	14.372 +- 3.438	3.13 +- 0.239	6.665 +- 0.253
Mean Sediment Mg (g/m^3)	14.443 +- 0.595	22.922 +- 1.29	22.764 +- 0.628
Mean Sediment Mn (g/m^3)	2.233 +- 0.236	8.38 +- 0.96	4.906 +- 0.233
Mean Sediment Tot.N (g/m^3)	2.612 +- 0.309	1.676 +- 0.111	10.395 +- 1.083
Mean Total P (g/m^3)	1.012 +- 0.15	1.523 +- 0.1	1.012 +- 0.15
Mean Sediment Zn (g/m^3)	0.221 +- 0.066	0.433 +- 0.033	0.553 +- 0.221
Mean Dissolved O_2 (mg/L)	3.99 +- 0.15	9.08 +- 0.02	3.96 +- 0.15

Figure 2.4.7a,b Mean total biomass with standard error, for Lakes A, B and C for 3 and 5 Lake Groups (135 quadrats). Mean biomass calculated as dry mass/ m^2. The 5 lake group includes Lakes B and C as well as the 3 sectors of Lake A.



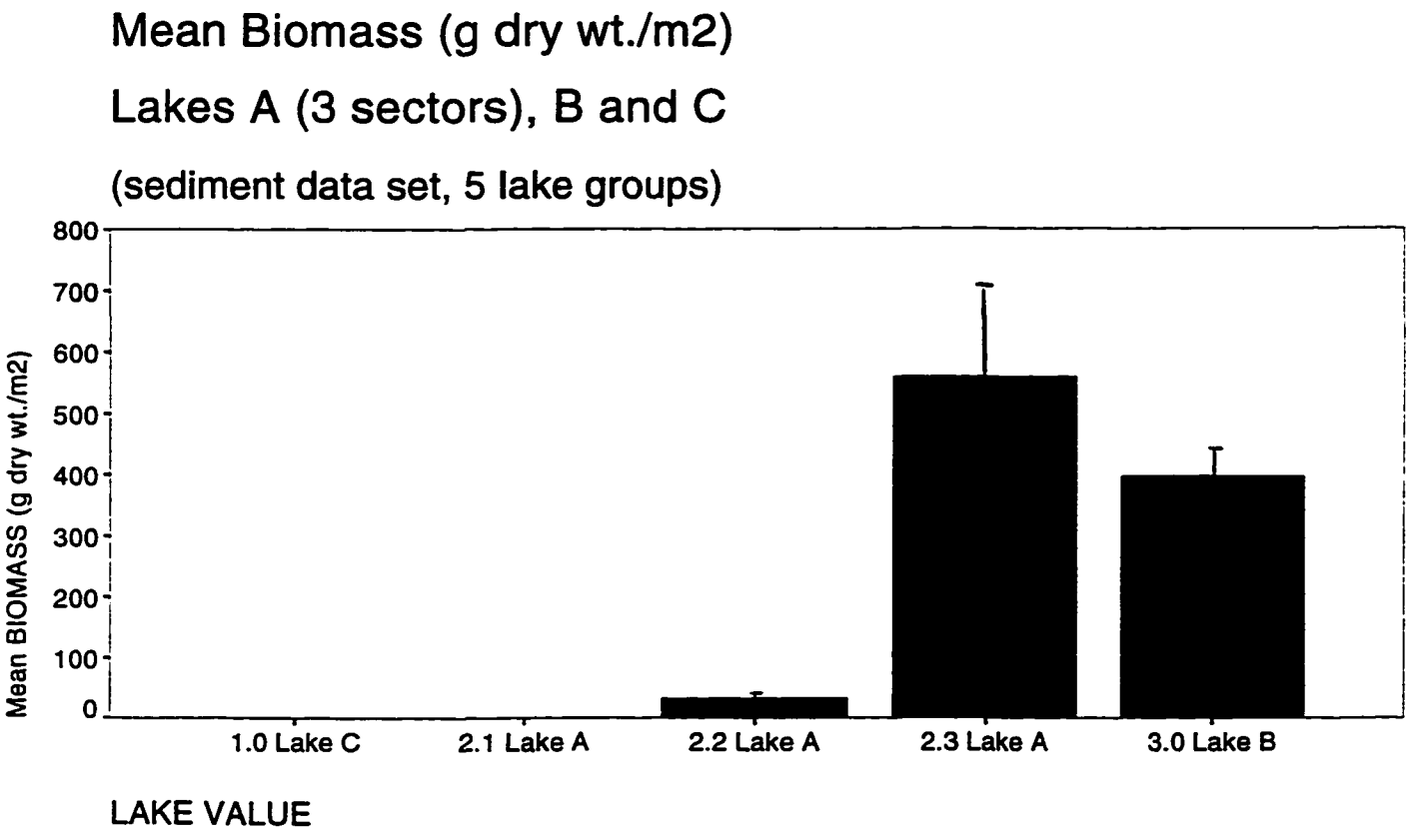
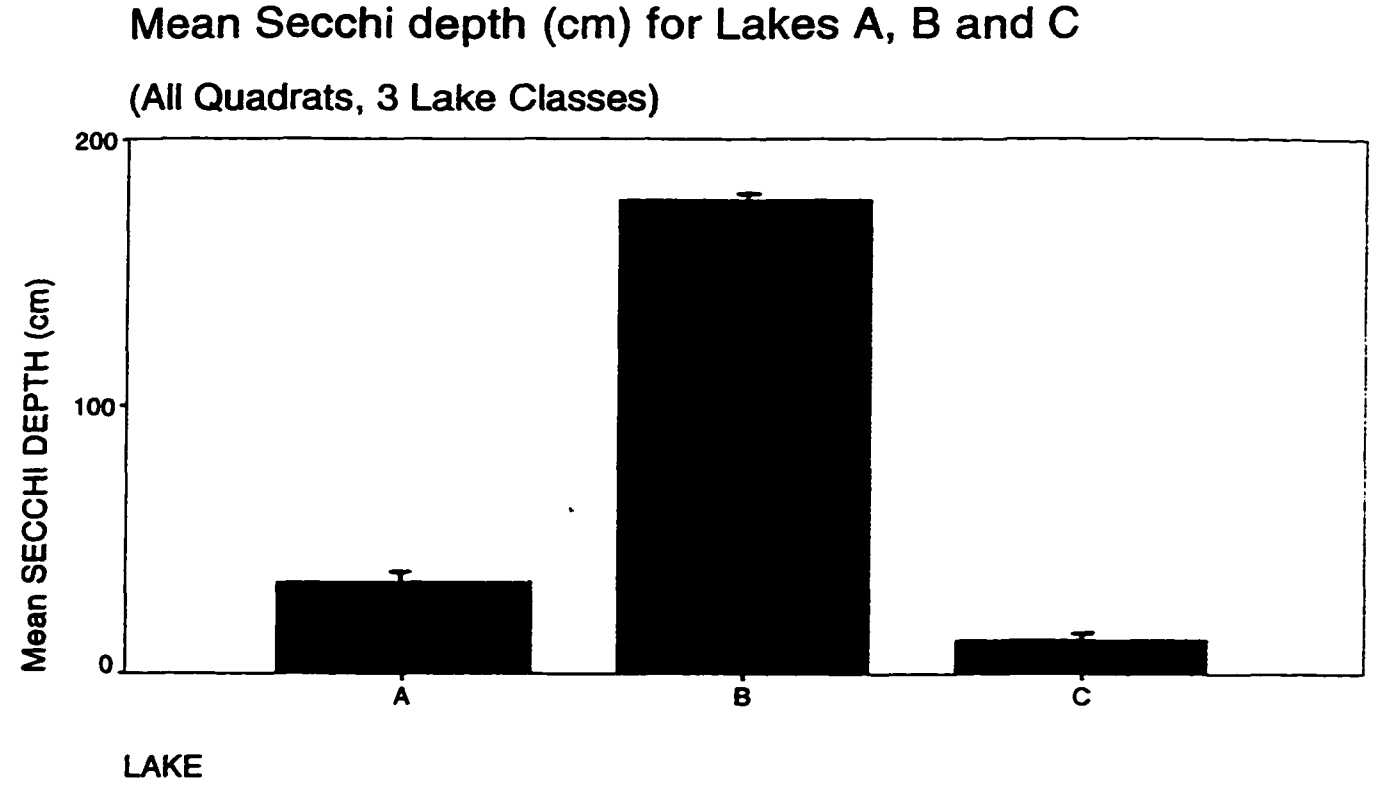


Figure 2.4.8a,b. Mean secchi depth with standard error, for Lakes A, B and C for 3 and 5 Lake Groups (All Quadrates). The 5 lake group includes Lakes B and C and the 3 sectors of Lake A.



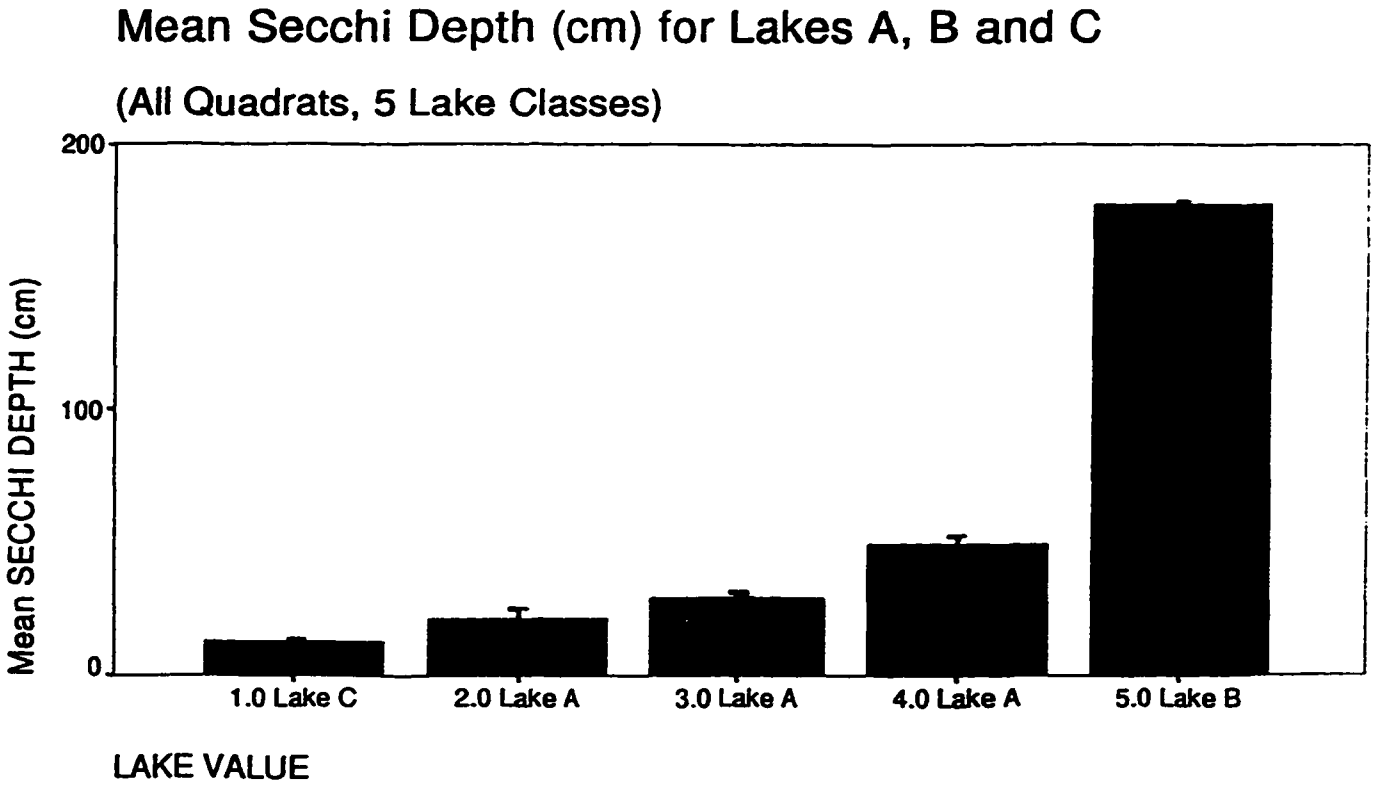


Figure 2.4.9 Sediment total nitrogen (sed.TN) with standard error, for Lakes A, B and C (All Quadrates, 5 Lake Group, consisting of Lakes B and C and the 3 sectors of Lake A).

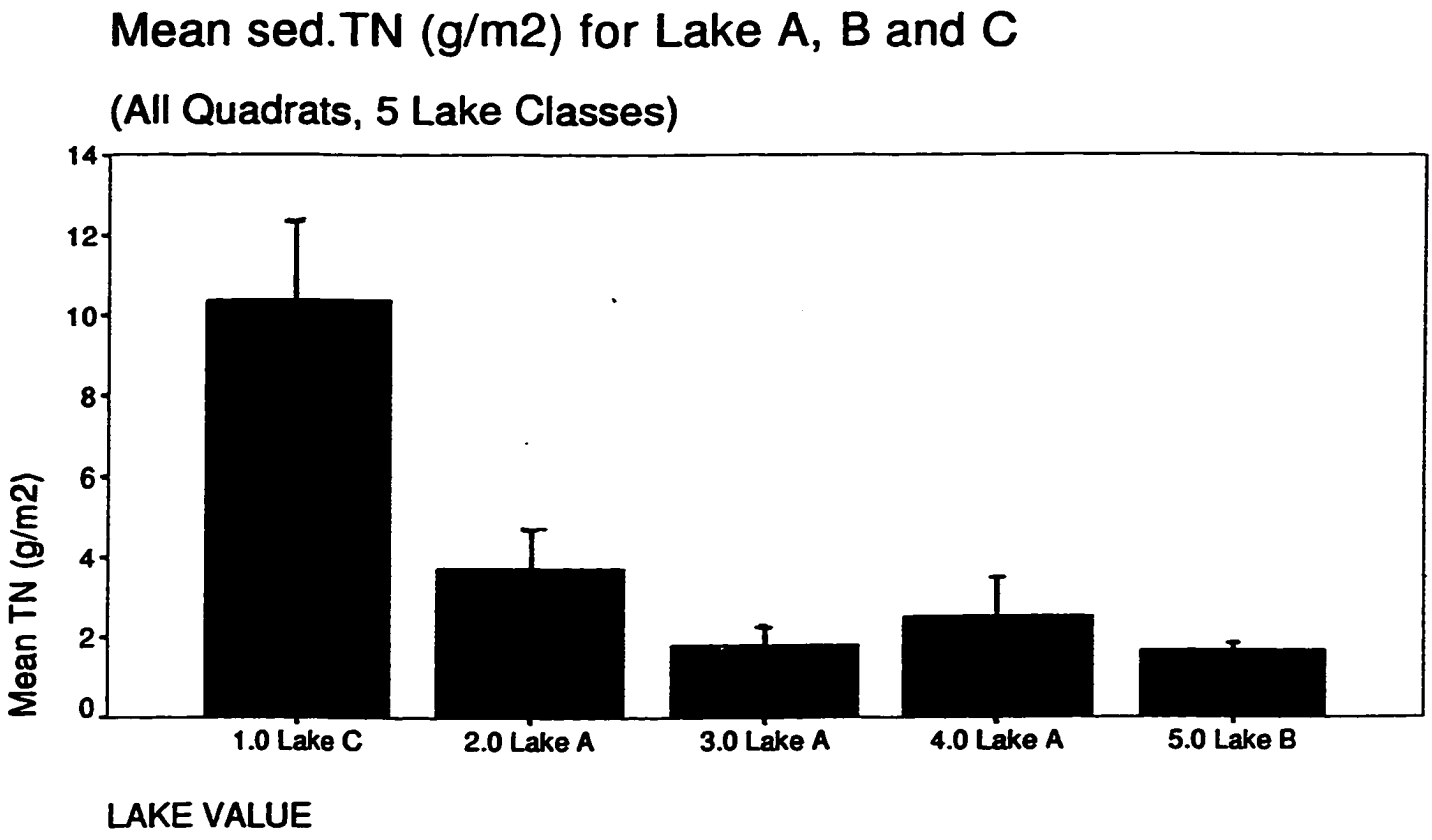


Table 2.4.7 Aquatic macrophyte species composition and biomass (g dry wt./m^2) for the 2 lakes, A and B where aquatic macrophytes were found. The mean biomass is the mean of all plant samples taken from 50 quadrats across both lakes A and B.

SPECIES	MACROPHYTE TYPE	LAKE A MEAN BIOMASS (g dry wt./ m^2)	LAKE B MEAN BIOMASS (g dry wt./ m^2)
Calamagrostis canadensis	emergent	23.17	195.12
Calamagrostis purpurasce	emergent	not present	74.39
Carex aquatilis	emergent	2081.71	657.32
Carex lacustris	emergent	1901.22	580.49
Drepanocladus sp.	submersed	not present	41.46
Eleocharis acicularis	submersed	not present	36.59
Eleocharis smallii	emergent	441.46	501.22
Equisetum fluviatile	terrestrial	13.41	7.32
Glyceria borealis	floating leaf	not present	60.98
Nuphar advena	floating leaf	not present	268.29
Polygonum amphibium	floating leaf	not present	17.07
Potamogeton alpinus	floating leaf	not present	162.20
Potamogeton pusillus	submersed	118.29	not present
Potamogeton natans	floating leaf	50	not present
Sagittaria cuneata	submersed	32.93	not present
Scirpus acutus	emergent	113.41	90.24
Scirpus validus	emergent	46.34	not present
Sparganium emersum	floating leaf	9.76	101.22
Sparganium fluctuans	floating leaf	not present	15.85
Typha latifolia	emergent	2039.02	not present
Utricularia vulgaris	submersed	110.98	97.56
TOTAL SPECIES COMPOSITION		9 Emergent 2 Floating Leaf 2 Submersed	7 Emergent 6 Floating leaf 3 submersed
TOTAL SPECIES		13	16

Discriminant Analysis

Discriminant analysis of the sediment data set (Table 2.4.6), gave a similar high level of accuracy in classification of the lake groups, as did the water quality data set. The data points were separated for the 3 lake groups, with an accuracy of 98%, while division of Lake A into 3 sectors, slightly reduced the accuracy of classification to 96%.

The graduation in both dependent variables (biomass and diversity) and many of the independent variables measured, across Lake A, was mirrored in the distribution of the classified points from the first 2 discriminant functions. These 2 functions were responsible for explaining 99.64% of the variation of the classified groups. The clear similarity between the first 4 lake groups (Lakes A and C), that was indicated in the 3 lake group distribution (Fig. 2.4.10a), was present. The clear discrimination of Lake B from the other lake groups was also evident.

The parameters included in the canonical discriminant functions and their relative contribution to the accuracy of the classification into lake groups, is shown in Table 2.4.9 for the sediment data set.

As with the water quality data set, the secchi depth was the most significant physical parameter, distinquishing between the three lakes (Table 2.4.9). The relative significance of the standardized discriminant function coefficient, indicated that function 1 was primarily an indication of secchi depth.

Figure 2.4.10 Mean species diversity with standard error, (sediment quality data set, 5 lake groups). Mean species diversity was calculated as the mean number of species from each quadrat within each lake.

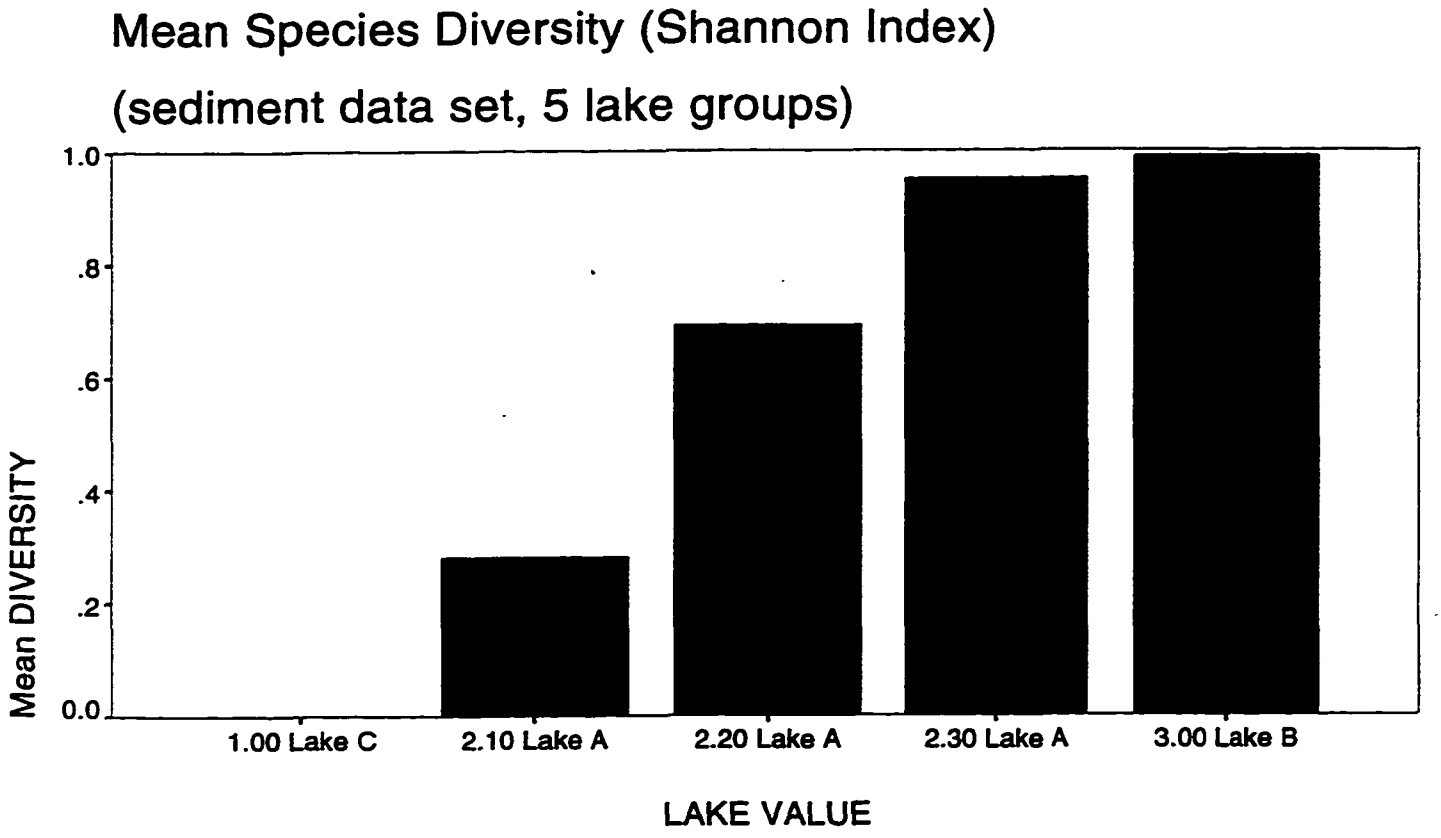


Table 2.4.8a,b. Discriminant functions for the sediment quality data set (135 quadrats, 3 and 5 lake groups).

2.4.8a) 3 lake group

Fn	Eigen Value	% Var	Cum %	Canon Corr	After Fn	Wilks Lambda	Chi-Sq	df	Sig
					0	0.00166	755.10	18	.00
1	113.54	96.4	96.4	.9956	1	1.19048	195.67	8	.00
2	4.25	3.6	100.0	.8997					

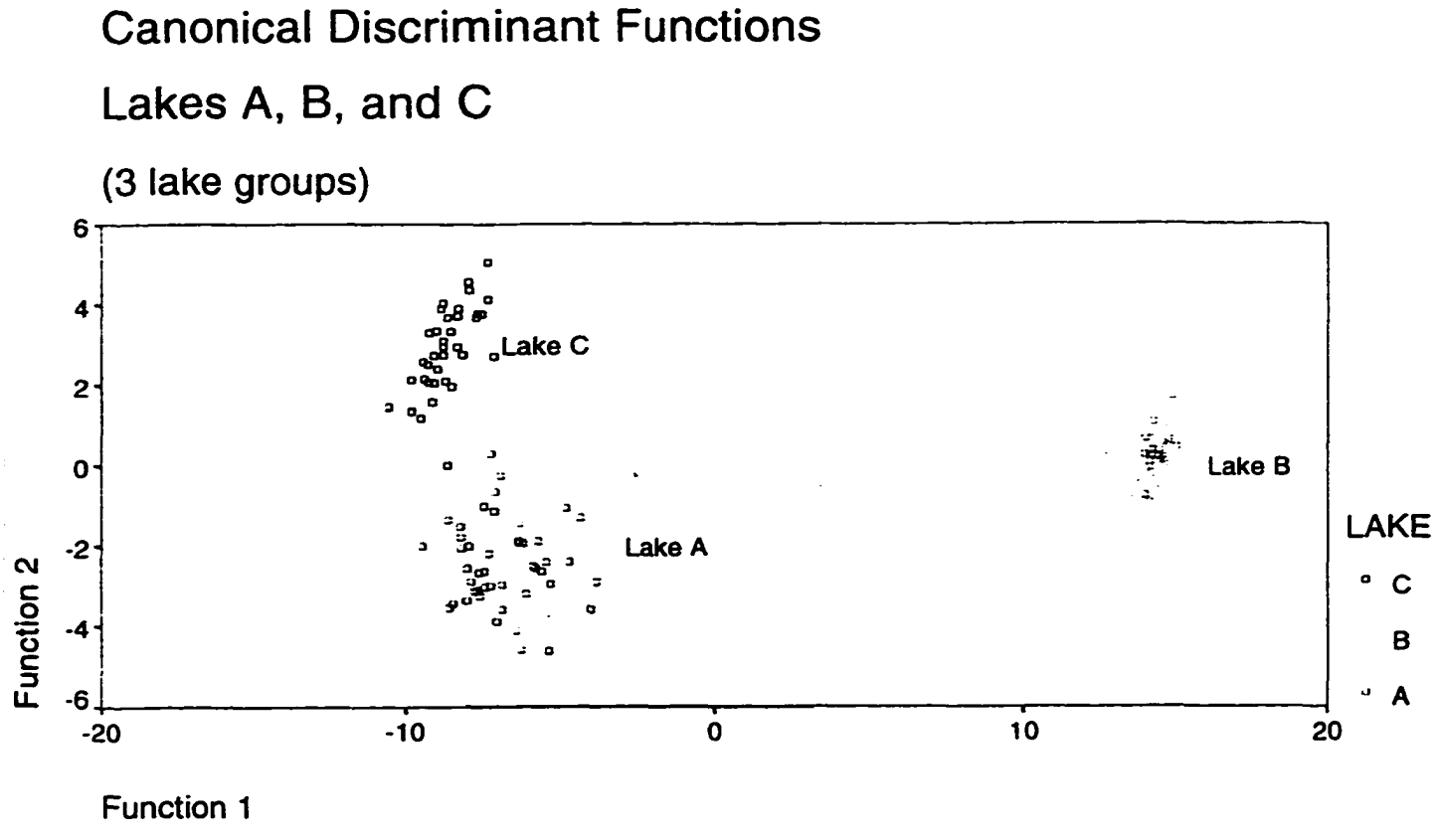
2.4.8b) 5 lake group

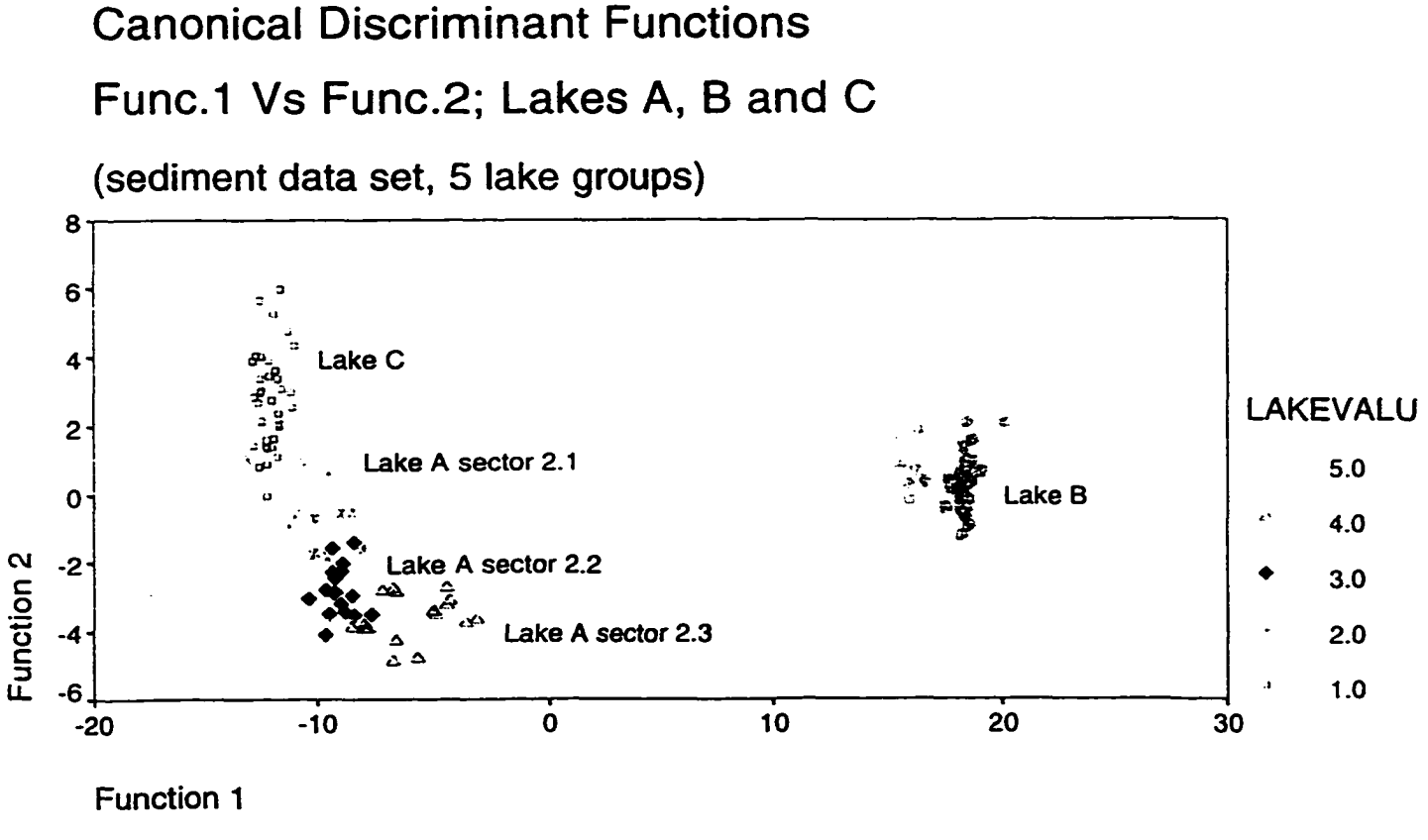
Fn	Eigen Value	% Var	Cum %	Canon Corr	Afte Fn	Wilks Lambda	Chi-Sq	df	Sig
					0	.00041	913.05	60	.00
1	197.9091	96.90	96.90	.9975	1	.08119	293.78	42	.00
2	5.4658	2.68	99.57	.9194	2	.52496	75.4	26	.00
3	0.8325	0.41	99.98	.6740	3	.96199	4.53	12	.97
4	0.0395	0.02	100.00	.1950					

Table 2.4.9 Discriminant function coefficients for sediment data set (3 lake groups). The unstandardized coefficients are the actual values of the variables composing the discriminant functions; the absolute values of the standardized coefficients indicate the relative contribution of each variable to the discriminant functions. A minimum value was used of $P<0.05$ and a maximum value of $P<0.06$.

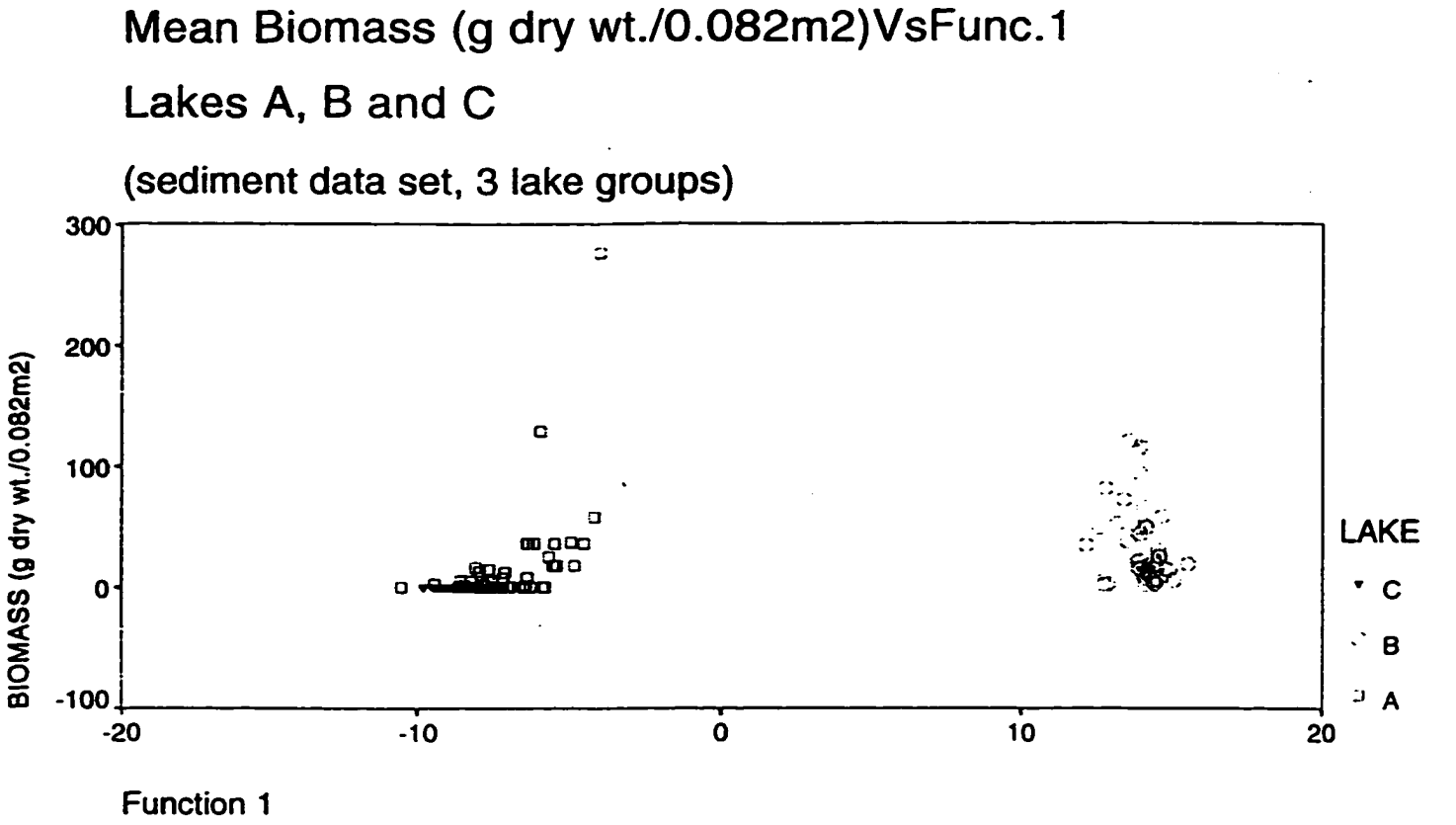
Parameter	Standardized canonical discriminant function coefficient		Unstandardized canonical discriminant function coefficients	
	Functn. 1	Functn. 2	Functn. 1	Functn. 2
Bulk Density	0.11653	-0.09259	0.704609	-0.55986
Loss on Ignition	-0.0021	0.28588	-1.13612	0.015495
pH	-0.15986	0.11582	-0.75094	0.54405
Dissolved Oxygen	0.52352	-0.32383	0.662666	-0.4099
Secchi Depth	1.10064	0.30004	0.113527	0.030948
Temperature	0.18776	0.98556	0.125863	0.660674
Sediment Cu	0.09791	0.03697	4.242183	1.601769
Sediment Ca	-0.06648	-0.24067	-9.95406	-0.0036
Sediment Mg	0.28412	0.38864	0.0458	0.062649
Sediment Fe	-0.15821	0.10393	-3.30715	0.002173
Sediment Mn	0.398	0.02236	0.096031	-0.00539
Sediment Total N	-0.00686	0.04504	-0.00181	0.011849
Sediment Total P	-0.14423	0.67451	-0.17537	0.82016
Sediment Zn	-0.01019	0.23506	-0.01313	0.30272
Sediment K	-0.33927	0.01618	-0.17457	0.00833

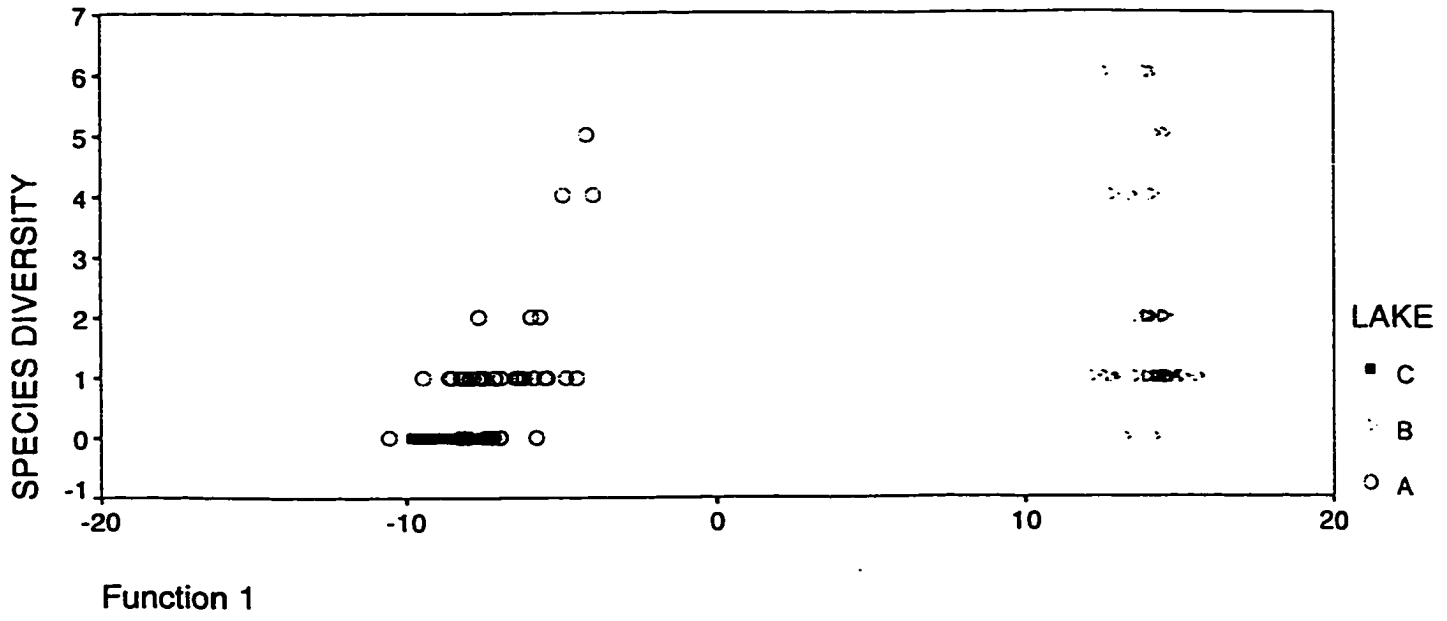
Figure 2.4.11a,b. Distribution of the canonical discriminant functions (sediment quality data set, 3 and 5 lake groups).





Figures 2.4.12a,b. Plots of "biomass" and "species diversity" against discriminant function 1, for the sediment data set, with 3 lake groups.





2.5 DISCUSSION

The importance of light availability for the growth of aquatic macrophytes has been shown to be crucial (Chambers and Kalff 1985, Kautsky 1992, Zimmerman *et al.* 1994). Secchi depth as it relates to the attenuation of light in the water column (Walker 1982), is an indicator of the depth to which light is available for plant photosynthetic reactions.

There was a consistent and significant graduation in secchi depths ($P < 0.05$), between the three lakes in both the water quality data set (Fig. 2.4.2) and sediment data set (Fig. 2.4.8), that was consistent with the exposure of the lakes to BKME. In both data sets, Lake C had the lowest secchi depth followed by Lake A, and finally, Lake B had the highest, indicating the effect that BKME, with its high colour (Table 2.4.1) due to dissolved lignin-derived components and inorganic fractions (RAP 1991), has on light attenuation.

The effect of BKME on aquatic macrophyte growth was graphically displayed in the complete removal of all aquatic vegetation within Lake C. The effects of BKME on aquatic macrophyte biomass and diversity were objectively assessed in Lake A, where the BKME is diluted across the lake, these results have been compared to both the control lake and Lake C.

2.5.1 Water Quality Data Set

The multiple regression analysis (MRA) of the water quality

data set indicated that secchi depth was the only measured parameter from table (2.4.2), that was significantly related to the biomass of aquatic macrophytes (multiple R = 0.68). The result indicated that secchi depth accounted for 68 % of the variation of the biomass and the most useful indicator of the direct impact of BKME on aquatic macrophyte biomass.

Secchi depth as it relates to water transparency and light attenuation and availability (Walker 1982), has been shown to have a primary role in influencing the distribution, composition and density of aquatic macrophyte populations (Canfield *et al.* 1985; Chambers and Kalff 1985; Kautsky 1988, 1992).

The narrow range of water/sediment quality parameters, indicated by the MRA to be related to biomass, is likely due to the large variability in the biomass (Fig. 2.4.1), that was not reflected by a similar variation in measured parameters. The unexplained variation of biomass, in regions with similar levels of the other measured parameters often occurred because of the inherent variation in biomass between aquatic macrophyte species that grew within a sampling region (Fig. 3.4.2).

Future sampling, concentrating on the biomass of dominant species only, may help more clearly indicate the relationship between water/sediment quality parameters and biomass, that have been shown in other studies: alkalinity and concentrations of related ions (Moyle 1944; Lee and Stewart 1981; Mitsch W.J. 1986); turbidity and eutrophication (Cook and Powers 1958; Dane 1959; Ozimek 1978; Cambridge and McComb 1984; Harper 1992;

Sodergren 1993; Shimoda 1993; Zimmerman *et al.* 1994).

The means of the biomass for the three lakes (Fig. 2.4.1a), indicate a trend of decreasing productivity with the presence of the BKME within the three lakes. As with the trend in secchi depth, biomass decreased in the three lakes in the order: Lakes B, A and C (Table 2.4.1).

The trend for mean species diversity between the three lakes (Fig. 2.4.1b), was similar to that for mean biomass, and indicated a close relationship between the two vegetative parameters. BKME appears to negatively effect both the biomass and diversity of aquatic macrophyte species at high concentrations.

Both water colour and turbidity also show trends that suggest the contributory nature of these parameters to the secchi depth results (fig 2.4.2).

Concentrations of the potassium ion fraction, as well as phosphorus and nitrate concentrations, were elevated in lakes A and C (Table 2.4.1), compared to concentrations reported in the literature for northern wetlands (Heinselman 1970; Klopatek 1978; Richardson *et al.* 1978).

All three of these nutrients have been found to be major limiting nutrients in aquatic macrophyte growth (potassium [Goodman and Perkins 1968], phosphorus [Tamm 1954] and nitrogen [Gore 1963]), particularly for emergent macrophytes which rely almost entirely on sediments for nutrient uptake.

BKME was also responsible for increasing mean conductivity

and alkalinity, as well as the associated inorganic ion concentrations of calcium, magnesium, sodium and chloride in the water column (Table 2.4.1). Although these levels are either inside, or just outside maximum recorded levels for undisturbed northern wetlands (Klopatek 1978), alkalinity is known to influence the distribution of commercial stands of wild rice (Moyle 1944; Lee and Stewart 1981) and extreme and sudden fluctuations in conductivity can reduce species diversity and the total biomass of aquatic macrophytes within freshwater wetlands (Stone *et al.* 1978).

Sodium ion concentration was the most important discriminating parameter in the water quality data set and together with the other inorganic ions Cl^-, K^-, Mg^{2+} chosen by the discriminant programme to discriminate among the three lakes indicates that the levels of these ions and their contribution to conductivity and alkalinity are important parameters for indicating the impact of BKME on lake limnology and possibly aquatic macrophyte biomass. Although the multiple regression analysis of all the measured limnnological parameters against the independent, "biomass", did not include any of these parameters as significantly related to biomass, it may be this is a result of too few samples for the number of independent variables. (Tabachnick and Fidell 1989). The mean conductivity for Lake C of 1307 +- 35 uMho/cm, converted to 784 mg/L total dissolved solids and was classified as "marginal salinity", based on the salt content (E.P.A. 1976). Two factors were important in assessing

the potential for conductivity or salinity, to impact on the biomass of aquatic macrophytes : 1. conductivity was significantly elevated in Lake C compared to the control, lake B at $P<0.01$, but was within the range of values recorded for undisturbed northern lakes (Klopatek 1978); 2. Conductivity was neither selected in the multiple regression analysis against "biomass", or, in the discriminant analysis, although component ion concentrations were. The high conductivity may be limiting the growth of aquatic macrophytes in the two lakes exposed to BKME, but the growth of all eight species chosen for trials in Lake C, indicates that the conductivity of BKME, is unlikely to be the major limiting factor preventing natural recolonization of Lake C.

Alkalinity likely effects transformation reactions and availability of limiting nutrients, particularly phosphorus (Sloey *et al.* 1978). BKME was also associated with raising mean water temperatures and lowering the mean dissolved oxygen levels (Table 2.4.1).

Very little work has been published on the effect of water temperature on productivity of aquatic macrophytes, although Gorham (1974), found a close positive relationship between biomass and the highest mean monthly, ambient air temperature. Lee and Stewart (1981) found that water temperature was an important discriminant parameter in a study of populations of wild rice.

Mean dissolved oxygen as it relates to the redox potential

is an indicator of the availability of many of the productivity-limiting nutrients discussed above (Gosselink and Turner 1978). Phosphorus becomes more available as solubilization is promoted by the reduction of ferric iron and release of PO_4^{-3} from ferricphosphate (Sloey *et al.* 1978). Dissolved oxygen levels also effect the microbial release of potentially toxic sulphides (Joshi *et al.* 1975) and the rate of microbial release of available nitrogen, mineralization (Avnimelech 1971).

The loading of these nutrients and variability of the other potentially limiting, limnological parameters discussed above, in Lake C and parts of Lake A (Table 2.4.1), had no effect on the phytobenthic environment, where light availability, was the major limiting growth factor for aquatic macrophytes. They are however, likely to be of importance in determining aquatic macrophyte distribution, where the BKME has been diluted sufficiently, for light conditions to improve to the point where recolonization can occur. For this reason, the data from the 3 sectors of Lake A was useful to observe these effects, where a significant BKME dilution gradient was noted across the lake.

The plots of the mean total biomass and species diversity (Fig. 2.4.3), indicated a similar significant graduation for both variables ($F< 0.05$) across lake A, with the lowest levels being at the source of BKME entry, and the highest at the furthest end from the source.

The increase in biomass across Lake A, and the relatively high levels in sector 2.3, compared to the control lake, Lake B,

indicated that conditions for aquatic macrophyte growth, improved across lake A, as the distance increased from the entry point of BKME into the lake. The improved conditions were observed in the plots for secchi depth, colour, turbidity and total nitrogen, across Lake A (Fig. 2.4.4).

The amount of variability in the total biomass, also graduated upwards across Lake A, with the lowest variability occurring in the sector closest to the BKME leakage and the largest amount of variation occurring in the sector, furthest away from the BKME leakage into the lake (Fig. 2.4.3). Thus when BKME is diluted to the point where light conditions allow the growth of aquatic macrophytes, there is a rapid growth of a number of different species, that inherently vary in biomass.

Light conditions in lake waters, were measured indirectly by measuring secchi depth, colour and turbidity. Secchi depth increased across Lake A, as the BKME became diluted with Lake A water and the inflow of Lake B at the southern end, sector 2.3 (Fig. 2.3.1), which was reflected in decreased water turbidity and colour values (Fig. 2.4.4). Previous studies of wetlands, where light conditions have deteriorated due to poor management of catchment areas, have shown the association between turbidity and the depth distribution, density and productivity of submerged aquatic and marine macrophytes (Orth and Moore 1983; Cambridge and McComb 1984; Carter and Rybicki 1990; Larkum and West 1990).

The sediment nutrient analysis, indicated that BKME had been historically responsible for loading sediments of receiving lakes

with organic fractions, particularly nitrogen when compared against the control lake B (Table 2.4.2).

The loading of total sediment nitrogen in all parts of Lake A, indicated that sediment nutrient availability was not as important a limiting factor for aquatic macrophyte growth as was light availability. As with the three lake data set discussed above, water clarity was the most significant factor limiting aquatic macrophyte productivity in Lake A. Dissolved and sediment nutrient conditions, temperature, conductivity and the chemical parameters that determined availability of nutrients, such as the anoxic state of the benthic environment and alkalinity, became increasingly important in limiting productivity in sections of the lake, where suitable light conditions existed.

The positive effects discussed above, of nutrient loading of N, P and K, on aquatic macrophyte productivity, were displayed more clearly in sector 2.3 of Lake A (Fig. 2.4.5). Mean total biomass and species diversity were highest in this sector of Lake A and mean total biomass of this sector was higher than the control lake.

The discriminant analysis of the data was useful in determining functions that could discriminate between the three lakes, which differed historically only in their exposure to BKME.

The functions shown in Table 2.4.4a,b for the water quality data, indicate the clear separation that was given by the two functions, from the three lake group data set. As accuracy of

group classification was already 100%, division of the data set into five lake groups, did not aid in the classification (Figures 2.4.5a,b), nor did the division decrease the accuracy.

The stepwise canonical discriminant function coefficients (Table 2.4.5), indicated the water quality parameters measured for this data set, gave a more accurate separation of the three lakes, than the sediment quality parameters. Secchi depth, turbidity and the seven other water quality parameters, were selected by the programme to build the discriminant functions. This result suggested further research on the effects of BKME on lake chemistry should be weighted towards measuring these parameters. The apparent absence of sediment chemistry parameters from the discriminant function for this data set (Table 2.4.5), may seem incongruous in the light of the dominant role of sediment nutrients, in aquatic macrophyte nutrient assimilation (Klopatek 1975; 1978), particularly for emergent species and the inclusion of sediment nutrient parameters in the discriminant functions of the "sediment data set" (Table 2.4.9). This may be explained by the mechanism of discriminant analysis, which discards parameters that are highly correlated with other parameters (Green 1971). This phenomena was observed and discussed in Lee and Stewart (1981). A comparison of the relative levels of nutrients between the three lakes for the sediment and water columns (Tables 2.4.1, 2.4.2), indicated there was a high correlation between the two matrices' and the above reasoning of redundant parameters within the discriminant analysis programme,

was a likely explanation. The potential of nutrient levels within the water column to indicate a limit to productivity of aquatic macrophytes has been by shown by Boyd and Hess (1970), who demonstrated positive correlations between concentrations of P, Ca, Mg, K and Na, in water of collecting sites and percentages of these elements in shoots of *Typha latifolia*.

The concentration of the two ions (Na and Mg), in the water column were the most discriminating chemical parameters between the three lakes, in the water quality data set. These ions influence the alkalinity of a water body (Wetzel 1975) and Moyle (1944), observed that alkalinity seemed to influence the distribution of commercial stands of wild rice.

Secchi depth and the causative parameter, turbidity, were the most discriminating physical variables in the discriminate analysis of the data from the three lakes. The significance of light conditions in determining productivity of aquatic and marine macrophytes, has been shown in a number of studies (Orth and Moore 1983; Cambridge and McComb 1984; Carter and Rybicki 1990; Larkum and West 1990).

The plots of the vegetative parameters, biomass and species diversity against the first discriminant function (fig's. 2.4.6a,b), gave little more information about the effect of BKME, on aquatic macrophyte growth, than the mean +- SE plots, in figures (2.4.1) and (2.4.3), except that in the discriminant analysis, the "causative parameters" have been filtered to use the most discriminating parameters and the "cause-effect" more

accurately defined. The results indicated the link between BKME and changes to aquatic macrophyte growth, could be estimated in future research, from fewer chemical and physical water quality tests, particularly those measuring the alkalinity, conductivity and water clarity.

2.5.2 Sediment Data Set

Similar relationships existed for the measured water/sediment quality parameters in the three lakes, for the sediment data set (Table 2.4.7) as for the water quality data set (Tables 2.4.2, 2.4.3 and 2.4.4). Secchi depth and dissolved oxygen levels, were significantly reduced in both lakes A and C, the former, associated with the high colour levels due to dissolved organic acids and turbidity, the latter associated with the high BOD (RAP 1991).

The mean water temperature, sediment calcium and sediment total nitrogen, are all elevated in both lakes A and C, compared to the control lake. As with the secchi depth and dissolved oxygen, these results seem clearly associated with the contamination of BKME in these lakes and their effects on aquatic macrophyte productivity has already been discussed.

When the Lake A data were analyzed as a complete set, there was not a significant difference in mean biomass between Lake A and Lake B ($P<0.05$).

As in the water quality data set, dividing Lake A into 3

equal sections allowed the large variation in all parameters to be identified with a section of the lake. Also, similar to the water quality data subset, by far the largest amount of variation in biomass occurred from the sector of Lake A, that was furthest from the leakage of BKME into the lake, sector 2.3 (Fig. 2.4.7b). Each sector of Lake A, was significantly different from the other two, while the sector closest to the leakage of BKME, sector 2.1, was not significantly different from the mean biomass of Lake C and the sector furthest from the leakage, sector 2.3, was not significantly different from the control lake, Lake B at $P < 0.05$.

The BKME is clearly diluted across lake A and the negative effects to aquatic plant growth appear to follow this dilution.

BKME exposure was associated with complete removal of all vegetation at high concentrations, as in Lake C and the section of Lake A, that was closest to the leakage into the lake.

When BKME was diluted, as indicated by the graduation in secchi depth across Lake A (Fig. 2.4.8b), growth of aquatic macrophytes occurred, limited by light availability, available sediment nutrient levels (Fig. 2.4.9) and other parameters that influence nutrient availability, such as alkalinity and dissolved oxygen levels. The toxic levels of some ions such as sodium and chloride may also be contributing to distribution of the aquatic macrophyte populations (Table 2.4.1).

Further evidence of the light-limiting effect of BKME, was present in the results of biomass samples of the emergent aquatic macrophyte, *Typha latifolia*. The biomass of colonies in the

sector farthest from the BKME leakage into Lake A, was higher than for any vegetation samples taken in the control lake. The significant productivity of aquatic macrophytes in sector 2.3, suggested that, as with the water quality data set, the BKME is diluted in this sector of the lake, due to the inflow from Lake B and the dilution of the stream entering from the opposite end of the lake (Fig.2.3.1). The aquatic macrophytes growing in this sector are not as limited for light, as in sectors 1 and 2. Loading of the receiving lake sediment, with nitrogenous, phosphorus and potassium fractions in the BKME, (Table 2.4.6), lead to successful colonization and growth of opportunistic, emergent aquatic macrophytes in the parts of the lake where available light was above a critical value.

Typha latifolia was particularly noted, as a fast growing, opportunistic aquatic, found only in Lake A, which grew prolifically in floating islands and in dense, benthic anchored colonies, close to the shore-line.

Lake B had a total of 16 aquatic macrophytes identified in the survey of vegetation (Table 2.4.7), seven of these species were unique to Lake B. In contrast to the unique species of Lake A, all of these, with the exception of *Calamagrostis purpurace*, were either submersed, or floating leaf aquatics. The relatively clear water conditions of Lake B, appear to allow the growth of a greater variety of submersed and floating leaf macrophytes than the light depleted conditions existing in much of Lake A.

The inclusion of a greater number of sample points, but

fewer water quality parameters in the discriminant analysis of the sediment data set, made little difference to the accuracy of the classification of the data points into the 3 lake groups, from the discriminant analysis (Table 2.4.8).

The distribution of lake groups based on discriminant analysis of the parameters measured in the sediment data set (Table 2.4.6), shown in figure (Fig.2.4.11a), indicated the similarity in the chemical/physical characteristics, between Lakes A and C, while the control lake, Lake B, is clearly discriminated by the discriminant function 1 along the x axis.

Dividing Lake A into three sections to give five lake groups (Table 2.4.8b), slightly decreased the accuracy of the classification from 98.45% to 96.12%, but had the advantage of more accurately displaying and explaining the source of variation in the measured parameters.

The graduation of groups 2.1, 2.2 and 2.3, from Lake A, along the x axis (Fig. 2.4.11b), represented the graduation of the effects of BKME, as it was diluted from the source of leakage into Lake A.

Function 1, discriminated along the x axis, but gave little more information on the relationship between the presence of BKME and the vegetation parameters measured in this study, than the mean +- SE.

The increased role of secchi depth in discriminating between the three lakes in this data set, is explained by the absence of water quality parameters.

The large amount of variation that existed in the vegetation parameters in Lake A, was reflected in the spread of the data points for this lake in these figures, compared to lake B.

The relevance of the discriminant functions in relating the impacts of BKME on the limnological parameters used in the analysis (Table 2.4.9), to aquatic macrophyte biomass and species diversity in Lake A, is indicated by the regression coefficients for the plots of biomass and species diversity, against the first discriminant function. Function 1 explains 96.4 % of the discriminant variation for the sediment data set. When the 2 outlying points, corresponding to the highest biomasses for *Typha latifolia* in Lake A (Fig. 2.4.12a), are removed from the regression analysis, the regression coefficients obtained are respectively 0.67 and 0.54 at $P < 0.05$ for mean biomass and species diversity. As was noted earlier, function 1 is a strong indicator of the impact of BKME on secchi depth and the correlation between discriminant function 1 and biomass, supports the previous result of the multiple regression analysis, indicating secchi depth to be a significant predictor of the impact of BKME on biomass. Although not as highly correlated, the relationship between function 1 and species diversity, indicates secchi depth may also be useful in predicting the impact of BKME on species diversity. These correlations would be expected to be higher if the analysis was carried out on indidual species, thus reducing the variability of biomass measurements inherent in a data set including biomass from multiple species.

2.6 CONCLUSIONS

The effects of BKME, which were graphically displayed in the complete absence of any aquatic vegetation within Lake C, were objectively assessed, by comparing this lake with the two other lakes in the study area that differ in their exposure.

1. Similar trends of a decreased secchi depth and the related parameters of water colour and turbidity with increasing levels of BKME were found in both data sets. The BKME, which is darkly stained with dissolved lignin-derived components, reduced the availability of light to the point where it became the most important limiting parameter of plant growth. BKME reduced plant biomass and diversity in Lake A, but when diluted to a level where light availability increased above a critical threshold, recolonization of aquatic macrophytes occurred.

2. The high conductivity of BKME and the associated ion concentrations, particularly calcium, sodium, chloride and magnesium and potassium in the water column, have the potential to limit growth in rehabilitation programmes of lakes exposed to this effluent. The levels do not prevent vegetative propagation when sufficient light is available, but further studies are required to determine the effects of the high conductivity on natural recolonization of lakes exposed to BKME.

3. BKME was responsible for loading the receiving waters and sediments with organic nutrients, particularly nitrogen fractions. The organic loading encouraged the recolonization of opportunistic, emergent aquatic macrophytes in regions where the BKME was diluted to the level that allowed plant growth.

4. Discriminant analysis of the independent parameters, using a lake value as the group definer, gave a clear discrimination between the three lakes and between the 3 lake sectors within Lake A. The Lake A sector closest to the leakage of BKME, was shown to be most similar to Lake C, while the sector furthest from the leakage, was shown to be most similar to the control lake, Lake B.

5. Water quality parameters were selectively chosen by the analysis software, as giving a more accurate classification of the lake groups, than the sediment parameters. Future research could be narrowed to measuring only these parameters. Secchi depth was the only parameter that was common to all discriminate analysis sets and appears to be the best measure of BKME presence.

6. Multiple regression analysis of the data sets was difficult to interpret due to the large amount of variation in the biomass measurements. This was particularly evident within Lake A. Division of the Lake A data into three sectors enabled a

classification of the total variation of all parameters into sectors that could be more accurately related to the levels of BKME. The levels of variation of biomass, showed a trend across Lake A that was related to an improvement in light availability. As conditions improved to the point where enough light was available for plant growth, opportunistic aquatic macrophytes recolonized in scattered patches. The biomass of these patches of vegetation were the highest of both lakes containing aquatic macrophytes, indicating the limiting nature of nutrient availability in unpolluted lakes.

7. The conditions of high water clarity, in the control lake, Lake B, allowed a more even composition of submersed, floating leaved and emergent species of aquatic macrophytes, than Lake A. Emergent aquatic macrophytes were favoured in Lake A, due to their ability to produce photosynthetically active foliage above the water column.

8. BKME negatively affected species diversity. A graduation in diversity occurred not only between the three lakes in the ascending order of C,A,B, but also, within Lake A, in the ascending order of sectors away from the entry of BKME into the lake.

3. THE EFFECT OF WATER DEPTH ON THE GROWTH OF AQUATIC MACROPHYTES IN A LAKE USED FOR RECEIVING BLEACHED KRAFT MILL EFFLUENT (BKME).

3.1 ABSTRACT

A complete random experimental design was used to assess the ability of two submersed and six emergent aquatic macrophyte species, to grow in depth controlled conditions within a lake where all indigenous aquatic species had died, following the exposure to Bleached Kraft Mill Effluent (BKME).

All species were obtained from a lake that had been naturally recolonized, following the construction 12 years previously, of a barrier to separate it from the BKME stream. All plants were transplanted into buckets containing BKME-exposed sediment. These buckets were then submersed in the exposed lake, 6 cm below the mean secchi depth of 12 cm and 6 cm above.

All species grew at the 6 cm level, with two species, *Carex aquatilis* and *Eleocharis smallii*, attaining greater than 50% of the biomass of parent plant communities after 4 months.

Four species remained alive at the 18cm level after 4 months, with only two species, *Carex aquatilis* and *Eleocharis smallii*, attaining greater than 10% of the biomass of parent plant communities at this depth.

3.2 INTRODUCTION

Rehabilitation of aquatic lakes exposed to BKME is a difficult undertaking, due to the high colour of the effluent, which leads to the extinction of available light before it reaches submersed foliage of aquatic macrophytes. It was concluded from the first section of this study, that the availability of light was the major factor limiting growth of aquatic macrophytes within lakes exposed to BKME.

In order to evaluate the potential for rehabilitation of Lake C, which was totally devoid of aquatic vegetation, greenhouse and in-situ and trials of plant growth in Lake C water and sediment were conducted.

The nutrient analysis of sediment and water, as presented in chapter 2, indicated that nutrients are not limiting growth of aquatic macrophytes when compared to other northern wetlands (Klopatek 1978). The recolonization of 13 species of aquatic macrophytes within regions of Lake A, where the BKME was diluted sufficiently, was also evidence of the potential of recolonization of lakes exposed to BKME.

The measured secchi depth of the water, as it relates to the attenuation of photosynthetically available light (Walker 1982), has been used to explain radical changes to aquatic macrophyte distribution and composition (Canfield *et al.* 1985; Chambers and Kalff 1985, Kautsky 1992, Zimmerman *et al.* 1994). The was confirmed by the distribution of biomass in the three sectors of

Lake A in section 2 of this report (see Fig. 2.4.6), which was related to the impact of BKME on the light regime of the water column. The primary causes of light deterioration, leading to impacts on growth of marine and aquatic vegetation have been found to be eutrophication (Ozimek 1978; Spence 1980; Cambridge and McComb 1984), water level fluctuation (Cook and Powers 1958), erosion and dredging (Carter and Rybicki 1990) and the exposure to BKME (Kautsky *et al.* 1988).

The maximum depth of macrophyte colonization has been shown to respond to decreased light penetration (Spence 1982), while the depth of colonization of marine phytobenthic algal populations have been linked with the exposure to BKME (Chambers and Kalff 1985).

This study was conducted to determine i) if aquatic macrophytes could grow in water exposed to BKME at depths where sufficient light was available, and ii) to identify favourable species for future rehabilitation programmes.

3.3 METHODS

As decribed above (Sect. 2.3.1) a survey was carried out in the summer of 1993, of the aquatic macrophyte populations of all three lakes, A, B and C (Fig. 3.4.1), in the study area (Fig. 2.1). Fifty quadrats (20 m x 20 m) were randomly selected from the vegetated regions of the three lakes and estimates of percent cover from visual inspection of 2 replicate, 3 m x 3 m

subquadrats within the main quadrat were collected. These were then averaged for the 50 quadrats per lake.

Samples of all aquatic macrophytes and fringing terrestrials, were collected from each of the 50 quadrats. The two dominant submersed and six emergent aquatic macrophyte species that had recolonized Lake A, subsequent to its being bypassed from the effluent stream, were selected from Lake A, for in-situ trials of plant growth in Lake C water and sediment. In the late summer of 1993, 7 species were removed by spade from a standard area of 0.082 m^2, which represented half the area of an aquatic macrophyte sampler, described in Marshall and Lee (1994). Table 3.4.2 lists these species, which were taken to a green house in coolers and transplanted into plastic buckets containing Lake A sediment. These buckets were initially placed into large tanks and filled with tap water to a depth of 6 cm above the sediment level. The tap water was left standing for 24 hours before the plants were placed in the tanks to allow water to warm to room temperature and for the dissipation of any chloride residual. Tap water was used to maximize the chances of the plants surviving the transplanting process.

All species, with the exception of *Utricularia vulgaris*, were successfully grown for three months. After three months, the tap water was replaced by Lake C water, at the same depth.

Observations of vigour were made during a four month period and these results (Table 3.4.2), were used to help select the species shown below, for *in-situ* trials of aquatic macrophyte

growth within Lake C.

The species chosen for the growth trials in Lake C, were :*Eleocharis smallii; Calamagrostis canadensis; Carex aquatilis; Typha latifolia; Scirpus validus; Sparganium emersum; Potamogeton pusillus* and *Utricularia vulgaris*.

In the spring of 1994, the 8 aquatic macrophyte species were collected using the same methods as for the greenhouse study and transported to Lake C in coolers. The plants were washed in Lake C water and transplanted into 10 litre plastic buckets, containing Lake C sediment. The buckets, which were attached to the floating wooden rafts, were then lowered to one of two depths, 6 cm below the surface, or 18 cm below the surface.

Four replicates each of 0.082m^2 area, of each species collected from the parent populations in Lake A, were placed at both depths on the raft in Lake C, in a complete random design.

The 3 m x 3 m rafts were covered by netting to protect against interference from birds and foraging animals and anchored in Lake C.

Measurements of height were made after 4 months of growth and compared with the measurements of parent plants, from where the plant samples were initially collected from Lake A. Also at this time, all plants were harvested from both the buckets and from parent communities of Lake A. Parent biomass samples were collected from the same surface area (0.082 m^2) as was used to collect the initial samples.

The results were analyzed using ANOVA in SSPS 6.1.

3.4 RESULTS

3.4.1 Vegetation Survey

The results of the vegetation survey of the three lakes are shown in table 3.4.1. The dominant fringing terrestrial macrophytes in all lakes were *Alnus incana* (speckled or tag alder) and *Cornus stolonifera* (red osier dogwood). The dominant emergent macrophytes in Lake A and B, were *Carex aquatilis* (sedge), *Eleocharis smallii* (spike rush), and *Scirpus validus* (softstem bulrush). *Typha latifolia* (common cattail) and the grass *Calamagrostis canadensis* (bluejoint), were growing in thick isolated populations in Lake A. Two floating leaf macrophytes, *Potamogeton natans* (floating-leaved pond weed) and *Sparganium emersum*, were found growing in Lake A, while Lake B, had 6 floating leaf species.

The dominant submersed macrophyte in both Lakes A and B, was *Utricularia vulgaris* with *Potamogeton pusilis* growing in limited sections of the northern half of Lake A.

Lake C was devoid of aquatic macrophyte growth, although terrestrial grasses were growing on exposed islands and to within a few centimetres of the water line.

Table 3.4.1. The results of the vegetation survey of Lakes A,B and C, showing the composition of fringing, emergent and submersed aquatic macrophyte species with estimates of % cover.

SPECIES	VEGETATION TYPE	LAKE	% COVER
Alnus incana	fringing terrestrial	A,B,C	2,2,2
Calamagrostis canadensis	emergent macrophyte	A,B	2,<2
Calamagrostis purpurasce	emergent macrophyte	B	< 2
Carex aquatilis	emergent macrophyte	A,B	5, 5
Carex lacustris	emergent macrophyte	A,B	2, 2
Cornus stolonifora	fringing terrestrial	A,B,C	<2,<2,<2
Drepanocladus sp.	submersed macrophyte	B	< 2
Eleocharis acicularis	submersed macrophyte	B	< 2
Eleocharis smallii	emergent macrophyte	A,B	2,2
Equasetum fluviatile	fringing terrestrial	A,B	<2,<2
Glyceria borealis	floating leaf macro.	B	<2
Nuphar advena	floating leaf macro.	B	<2
Polygonum amphibium	floating leaf macro.	B	<2
Potamogeton alpinus	floating leaf macro.	B	<2
Potamogeton pusillus	submersed macrophyte	A	<2
Potamogeton natans	floating leaf macro.	A	<2
Sagittaria cuneata	submersed macrophyte	A	<2
Salix discolor	fringing terrestrial	A,B,C	2,2,2
Scirpus acutus	emergent macrophyte	A	<2
Scirpus validus	emergent macrophyte	A,B	<2,<2
Sparganium emersum	floating leaf macro.	A,B	<2,2
Sparganium fluctuans	floating leaf macro.	B	<2
Typha latifolia	emergent macrophyte	A	2
Utricularia vulgaris	submersed macrophyte	A,B	20,5

3.4.2 GREENHOUSE GROWTH EXPERIMENT

Growth results are shown in Table 3.4.2, for the aquatic macrophytes removed from Lake A and grown in Lake C water and sediment for 4 months.

Table 3.4.2. Growth characteristics of Lake A plants grown in the greenhouse, with height attained from the starting point of 2 cm. above the sediment in the four months of growth in Lake C water and sediment.

SPECIES	VEGETATIVE GROWTH	COMMENTS
Calamagrostis canadensis	1 meter	normal growth to maturity
Carex aquatilis	1 meter	normal growth to maturity
Eleocharis smallii	0.5 meter	10 - 15 stems to maturity
Potamogeton pusillus	4 cm	stunted, thin necrotic shoots
Utricularia vulgaris	2 - 3 cm	slow, thin vegetative growth
Typha latifolia	4 - 10 cm	slow, thin vegetative growth
Potamogeton natans	4 cm	stunted, thin necrotic shoots

The emergent aquatics macrophytes grew vigorously, with three species, *Calamagrostis canadensis*, *Carex aquatilis* and *Eleocharis smallii* developing through to flowering. Neither the two submersed macrophytes, *Utricularia vulgaris* and *Potamogeton pusillus*, nor the floating leaf macrophyte, *Potamogeton natans*, increased in size by more than 5 cm, from the initial transplanted specimens. Growth that occurred in these plants was chlorotic and stunted.

3.4.3 DEPTH EXPERIMENT

The mean secchi depth recorded for Lake C was 12 cm.

The effect of depth on mean biomass was significant with sig. of $F = 0.001$ at $P<0.01$. There was a clear benefit to

productivity of aquatic macrophytes grown at a depth above the mean secchi depth, than below (Fig. 3.4.1).

All 8 aquatic macrophyte species grew at the 6 cm depth, while only 4 species, *Carex aquatilis, Eleocharis smallii, Potamogeton birchtoldi* and *Utricularia vulgaris*, grew at the 18cm depth (Fig. 3.4.2).

The effect of plant species on biomass was also significant, with a sig. of F = 0.002 (P<0.01). The emergent species, *Typha latifolia*, attained the highest mean biomass at the 6 cm depth (Fig. 3.4.2) and was significantly greater in biomass than the other seven species (P<0.01). The two submersed species *Potamogeton pusillus* and *Utricularia vulgaris* were classed with the emergent *Sparganeum emersum* as having significantly less biomass than the other five species (P<0.01) (Fig.3.4.3).

The most successful species in terms of addition of biomass compared to parent populations in Lake A at both depths, during the 4 month growing period, were *Carex aquatilis*, attaining 64.5% of mean parent population biomass at 6 cm and 15.5% at 18cm and *Eleocharis smallii*, attaining 51% at 6 cm and 15.5% at 18cm (Fig.3.4.3).

There was a statistically significant difference between the biomass gained compared to parent populations, of emergent species and the 2 submersed species at the 6 cm depth (P<0.01). The least successful emergent grown at 6 cm, was *Typha latifolia*, which attained 26% of the biomass of parent populations. The two submersed species, *Potamogeton pusillus* and *Utricularia vulgaris*,

recorded mean percentage biomass of parent populations of 10.75 % and 7.5 % at the 6 cm depth respectively (Fig.3.4.3).

The mean height attained by the 8 aquatic macrophyte species, expressed as a percentage of mean parent population height (Fig. 3.4.4), shows less spread between species than the graphs of the % biomass compared to parent populations, but similar overall species distribution.

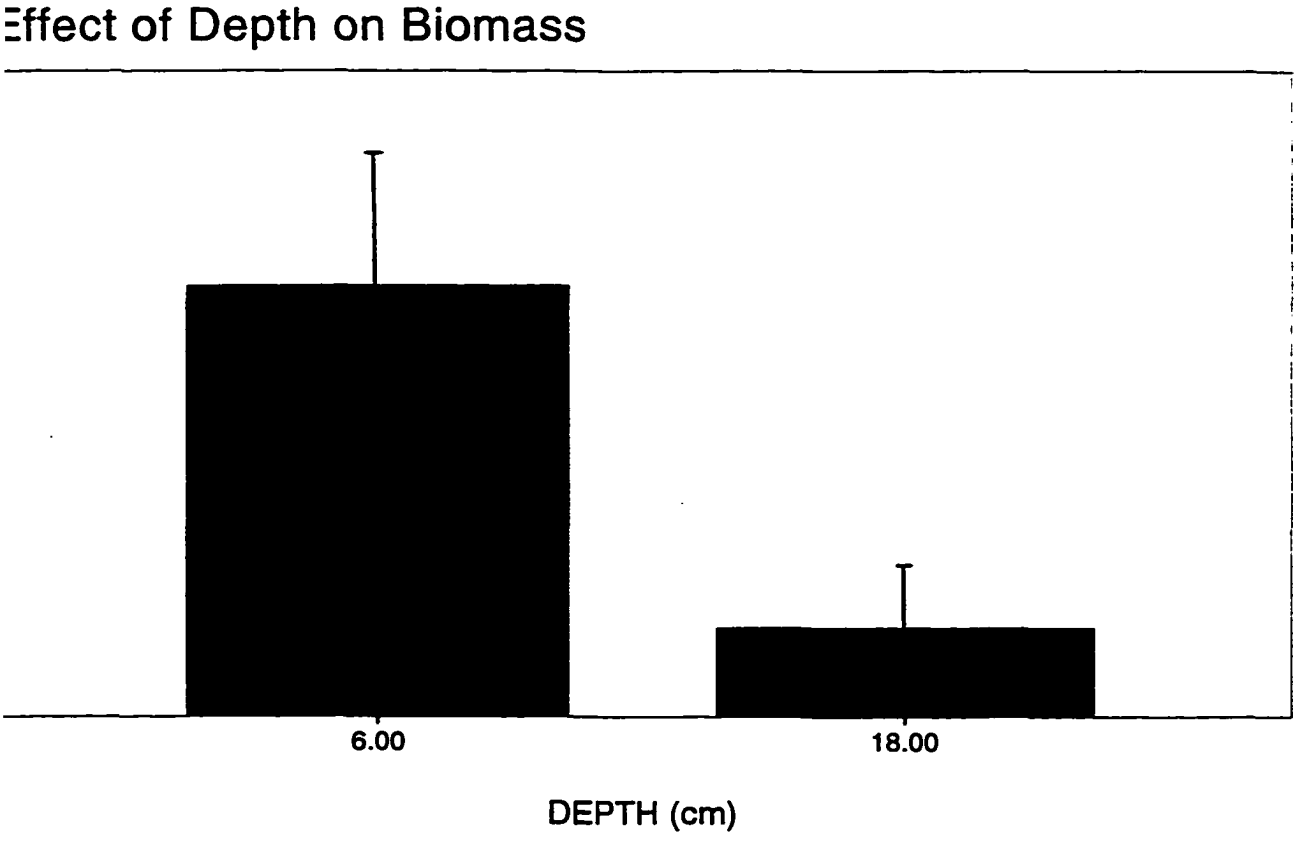
The two emergents, *Carex aquatilis* and *Eleocharis smallii* attained the greatest height as a percentage of parent populations, with 74% and 79% respectively. These two emergents were significantly more vigorous using percentage height of parent populations as a measure at $P<0.01$, although this measure indicated there was less significant difference between the aquatic macrophyte species, than the measure of percent biomass of parent populations.

All emergents attained mean heights closer to parent populations than submersed species.

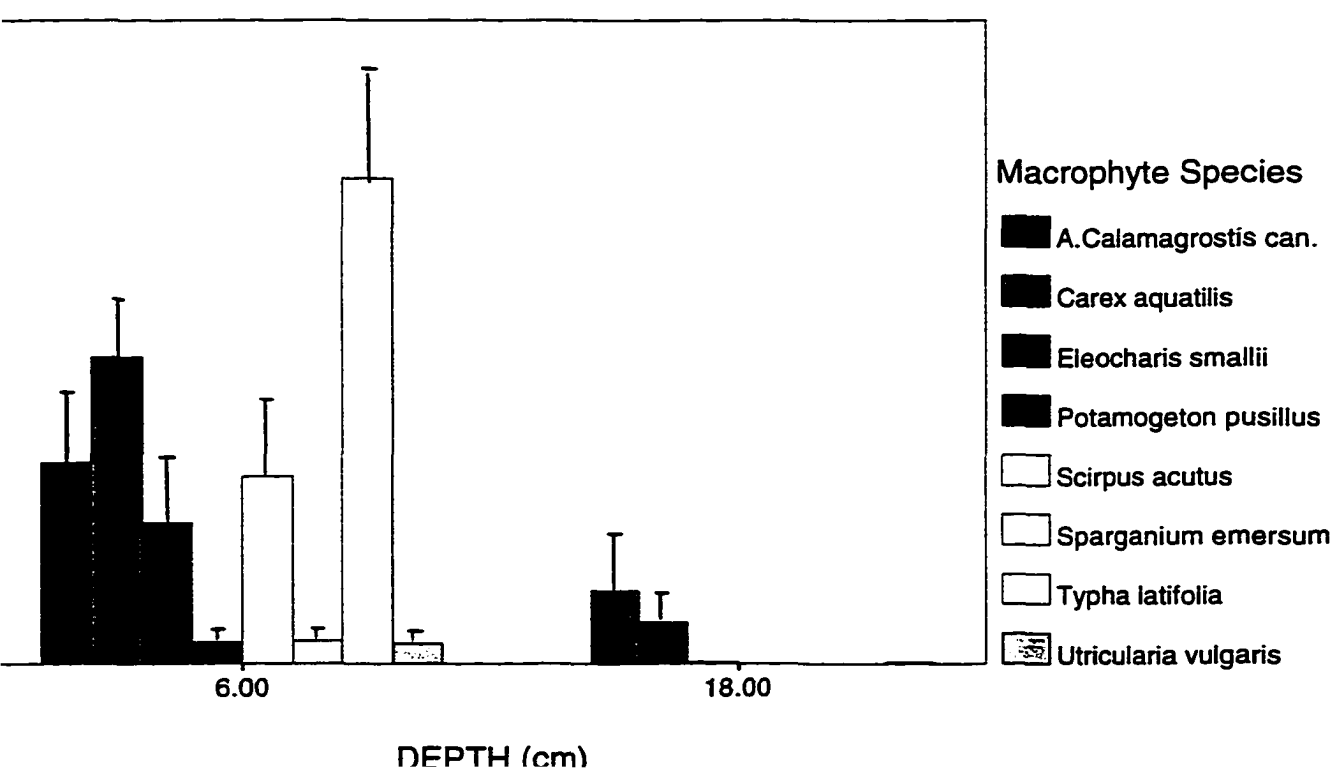
Table 3.4.3. Vegtative characteristics of parent populations from Lake A and transplanted samples from Lake C depth experiment.

Species	Parent Ht.cm	Parent Biomass.g/m2
Calamagrostis	110	610.98
Carex	100	539.02
Eleocharis	65	314.63
Potamogeton	40	287.80
Scirpus	95	482.93
Sparganium	100	69.51
Typha	205	2120.73
Utricularia	55	320.73

3.4.1. Mean biomass with standard error, for all eight macrophytes grown at 6 and 18cm.



3.4.2. Mean biomass of all eight aquatic macrophyte grown at 6 and 18 cm with standard errors.



3.5 DISCUSSION

3.5.1 Vegetation Survey

Results of the vegetation survey of the three lakes highlighted the diversity of aquatic vegetation that had been lost from Lake C, through exposure to BKME. The absence of aquatic macrophytes in Lake C seems highly likely to be a result of the high concentration of BKME, leading to inadequate levels of available light for plant propagation.

The growth limiting-effect that the high colour content of BKME has on aquatic macrophytes, has already been discussed in Chapter 2. The correlation between the secchi depth and the causative parameters of water colour and turbidity, with the productivity and species diversity of the aquatic macrophyte populations have been shown in chapter 2. BKME has been shown in a previous study of the phytobenthic biota to influence the density, distribution and diversity of marine vegetation in the receiving waters surrounding a pulp paper mill on the Baltic coast (Lehtinen et al. 1988, Kautsky 1992)

The possibility of fractions of BKME being toxic to the aquatic vegetation, (Lehtinen *et al.* 1988; Kautsky *et al.* 1988; Kautsky 1992) was not ruled out, nor was the effect of the low dissolved oxygen levels in the water column (Table 3.4.2). The resulting anoxic benthic conditions have been shown to lead to possible anaerobic synthesis of potentially toxic sulphides

(Joshi *et al.* 1975) as well as reducing the rate of microbial release of available nitrogen (Avnimelech 1971). Other factors such as the high alkalinity (Table 2.4.2) have been found to effect aquatic macrophyte distribution (Moyle 1944; Lee and Stewart 1981) probably due to the effect on transformation reactions and availability of limiting nutrients, particularly phosphorus (Sloey *et al.* 1978). Extreme and sudden fluctuations in conductivity, has also been shown to both reduce species diversity and total biomass of aquatic macrophytes within freshwater wetlands (Stone *et al.* 1978). The successful propagation, however, of various species in Lake C water and sediment, in both the greenhouse growth trial (Table 3.4.2) and the *in-situ* study (Table 3.4.3), as well as the growth of aquatic macrophytes in some sections of Lake A, as reported in Chapter 2, indicated that these factors were not limiting factors of growth, but that high colour content was more likely.

There were marked differences in species composition and density between Lakes A and B, most of which have been discussed in Chapter 2. Lake A, which was devoid of aquatic macrophyte populations when it was used as a settling pond for the BKME stream (RAP 1991), has been recolonized by aquatic macrophytes since it was bypassed in the early 1980's.

The conditions existing in Lake A have appeared to favour the recolonization by emergent species, over the submersed and floating leaf species. In contrast to Lake B, the control lake, which has 7 emergent species, 6 floating leaf species and 3

submersed species, Lake A has 9 emergent species, 2 floating leaf species and 2 submersed species. Of the unique species to Lake A, the emergent macrophyte, *Typha latifolia*, was noted in Chapter 2 to be a fast-growing opportunistic plant that was able to thrive in lake conditions where BKME had been diluted. The loading of the sediment with organic and inorganic nutrients originating from the BKME (Table 2.4.2) and increased availability of nutrient fractions, have been shown to increase productivity in aquatic macrophytes (Tamm 1954; Gore 1963; Goodman and Perkins 1968). The biomass of aquatic vegetation sampled from regions of Lake A, where the BKME had been diluted and where *Typha latifolia* was growing, were higher than any quadrats of the control lake. The results of the greenhouse trials (Table 3.4.2) and later the *in-situ* trials within Lake C, indicated however, other emergents were more tolerant of BKME than *Typha latifolia*.

3.5.2 Greenhouse Growth Trial

The results of the initial greenhouse trials for species growing in Lake C water and sediment supported the aquatic vegetation survey, which indicated that emergent species colonize and grow more successfully, under the conditions of lakes exposed to BKME. These preliminary results suggested that only vegetation that had foliage above the water level will grow satisfactorily in lake water containing BKME.

The 3 emergents, *Calamogrostis canadensis*, *Carex aquatilis*

and *Eleocharis smallii*, were the most successful of the aquatic macrophyte species grown under greenhouse conditions using Lake C sediment and water. All 3 species grew to maturity and flowered, whereas all other species, including the 2 floating leaf species collected from Lake A, recorded only minimal or zero growth and displayed symptoms of stress such as chlorotic, thin shoots.

3.5.3 Depth Experiment

More success was recorded for the Lake C *in-situ* study, as all 8 of the selected aquatic species from Lake A, were successfully propagated at the 6 cm depth, including the 2 submersed species, *Potamogeton pusillus* and *Utricularia vulgaris* (Fig. 3.4.2).

Depth had a significant effect on the overall productivity of the aquatic macrophytes at $F < 0.05$ (Fig. 3.4.1). The light conditions that existed at the 6 cm depth, which was 6 cm above the average secchi depth recorded for Lake C (Table 2.4.7), significantly favoured the growth of the aquatic macrophytes over the deeper level of 18 cm.

At the lower depth of 18 cm, only 4 of the 8 transplanted species survived over the four month trial. Interestingly, the 4 species included not only the 2 emergents which were most productive compared to parent populations in Lake A, *Carex aquatilis* and *Eleocharis smallii*(Fig. 3.4.3), but also the 2 submersed species named above. While both of these submersed

species were significantly less productive compared to the other 6 emergent species at the 6 cm depth, it may be they are better able to survive the poor light conditions that are incurred on exposure to BKME. This result was not totally unexpected, as *Utricularia vulgaris* was the most abundant aquatic macrophyte species in Lake A (Table 3.4.1) and *Potamogeton pusillus*, was the only aquatic macrophyte found in sector 2.1 of Lake A (Fig. 2.3.1), the sector that was closest to the source of BKME into that lake.

Transplanted emergent aquatic species were significantly more successful in coping with the conditions existing in Lake C, at the 6 cm depth, than the submersed species. At the lower depth of 18 cm, both submersed species were significantly less productive, using the parent populations in Lake A as a base comparison, than the 2 emergent species that survived at that depth (Fig. 3.4.3).

The two submersed species, named above, recorded the lowest mean biomasses of all 8 aquatic species (Fig. 3.4.2). Comparing the biomasses of aquatic species with the parent populations (Fig. 3.4.3) is a more accurate indication of relative vigour. The 2 submersed species were the least vigorous of the 8 aquatic species tested compared to parent populations. This result indicated the benefit to the survival and growth of aquatic plants in lakes exposed to BKME, if they are adapted to having foliage above the water level, where the problem of light availability is not the issue it is to submersed species.

It is also likely that emergent aquatic macrophytes reach regions of the water column that experience light levels above a critical level required for the photosynthetic process to commence, sooner than floating leaf and submersed species.

The most successful species, in terms of biomass relative to parent populations in Lake A, were the 2 emergent species, *Carex aquatilis* and *Eleocharis smallii*. Both species gained biomasses over 50% of parent populations and survived at both depths. These 2 species would seem to be the best suited to future rehabilitation programmes for Lake C.

The results of mean height of species, relative to parent populations was not as useful in discriminating between species, as the biomass relative to parent populations, although the same order of relative vigour was obtained (Fig. 3.4.4). This was particularly evident with the submersed species, which appeared to be more vigorous at both depths, than when the mean percentage biomass of parent data is used. The measurement of height also indicated that all the species that grew at the lower depth of 18 cm, gained comparative heights closer to those measured at the shallower depth of 6 cm. The results suggest that the aquatic macrophyte species that produce vegetative shoots that manage to grow to maturity at the lower depth, gained heights close to those growing at 6 cm. The disparity of the height gained with the biomass gained, is the result of much fewer shoots of the species actually survive to maturity at the lower depth.

Both the biomass and height measurements indicate that

aquatic macrophytes of this region propagate more successfully when growing at a depth shallower than the mean secchi depth. This result would have important implications for planned rehabilitation programmes for lakes exposed to BKME, especially where lake depths and/or secchi depths change due to effluent quality variation or seasonal runoff variation.

3.6 CONCLUSIONS

The aquatic macrophytes that were found to be dominant in recolonizing Lake A, following it having been removed from the BKME stream, were transplanted into Lake C at two depths. The results indicated that secchi depth is an important indicator not only of the ability of an aquatic macrophyte to grow, but also of the potential vigour, or ability to gain biomass and height relative to the same species growing in conditions where light availability is not a limiting factor. The major conclusions from this study are:

1. The most important factor limiting aquatic macrophyte growth in lakes exposed to BKME appears to be the availability of light. Plants can be successfully grown in exposed lakes, if the depth can be maintained at a level where plants can attain sufficient amounts of light.

2. Secchi depth is an important water quality parameter that has been shown to be an important indicator of the vigour of aquatic macrophytes. The measurement of this parameter is important in determining the depth ranges that aquatic macrophytes could tolerate in rehabilitation of lakes exposed to BKME.

3. The emergent aquatic macrophytes were more successful in growing in a lake exposed to BKME, than the submersed or floating

leaf species. This is most likely due to photosynthetically active parts of the plants being above the water surface and a shorter period of time taken for foliage to reach photo-active regions of the water column.

4. The emergent species *Carex aquatilis* and *Eleocharis smallii*, were the most successful aquatic species, in terms of biomass gained within a growing season.

5. The measurement of biomass is a more accurate indicator of the vigour of aquatic macrophytes growing in lakes exposed to BKME than height gained, although both parameters may be useful in selection of aquatic macrophyte species best suited for rehabilitation programmes of lakes exposed to BKME.

4. A SURVEY OF POLYCHLORINATED DIBENZO-p-DIOXINS AND POLYCHLORINATED DIBENZOFURANS, IN AQUATIC MACROPHYTES.

4.1 ABSTRACT

A survey was conducted of the concentration of polychlorinated-p-dioxins/furans (PCDD/PCDF) in sediment and aquatic vegetation growing in three separate lakes (A,B and C). The three lakes are connected by a common creek, but vary in exposure to Bleached Kraft Mill Effluent (BKME), from an effluent chain of a pulp and paper mill in Canada's northern shore of Lake Superior. Lake A was bypassed from the effluent stream in the early 1980's, while Lake B has never been a part of the effluent stream and Lake C is currently in the effluent stream.

The concentration of PCDD/PCDF congeners were found to be higher in the submersed aquatic species, than the emergent species in all lakes surveyed, although the emergent species had higher total loadings due to greater biomasses.

The PCDD/PCDF homologue profiles were similar for the lake sediments and the aquatic vegetation.

There was a clear PCDD/PCDF concentration gradient in the sediment and vegetation growing in the three lakes, with Lake B being the lowest and Lake C being the highest. This gradient reflected the historical and current exposure from the BKME and the release of these chemicals from the sediment reservoir.

4.2 INTRODUCTION

In long-term management of wetlands that have been used for the handling, storage or disposal of effluent from pulp and paper mills, it is important to understand the loading and fate of the chlorinated organic chemicals that have been reported in the effluent and receiving sediment and waters.

This study was carried out to determine the potential for aquatic macrophytes, exposed to BKME, to adsorb significant levels of PCDD's and PCDF's.

4.2.1 Sources of TCDD\TCDF's

The study area, as outlined in section 1.2, forms an "Area Of Concern" that has been so classified, due to impacts of the release of BKME from the Kimberly Clark pulp paper mill at Terrace Bay.

The stage 1 report of the Environmental Conditions and Problem Definition (RAP 1991), indicated that effluent being discharged into Blackbird Creek from the Kimberly-Clark mill contained significant sources of Adsorbable Organic Halides), including TCDD and TCDF, cadmium, chromium, nickel and zinc.

Given the persistence of inorganic metals and some of the polychlorinated organics (Muir et. al. 1985b, Miller and Ze 1987),detected in the BKME and receiving sediment, it is possible that many are contributing to the biotic loads that have been detected (RAP 1991).

One of the most studied source of TCDD's and TCDF's has been the effluent and products from paper pulp mills (Amendola *et al.* 1989; Kuehl *et al.* 1987; Wiberg *et al.* 1989; Kitumen and Salkinoja-Salonen 1989; and Rappe 1993). The dioxin problem in the pulp bleaching was first reported by Rappe *et al.*(1987), when unusually high levels of PCDD's and PCDF's were detected in sediment and crab hepatopancreas.

The results of a comprehensive screening study of the discharge of chlorinated compounds from 5 paper mills in the USA, (EPA #1 1988), indicated that 2,3,7,8-TCDD and 2,3,7,8-TCDF were the principal PCDD and PCDF congeners, contained in the effluent (Whittemore 1990), and alerted the US and Canadian government authorities, to the serious level of contamination of the water systems, that act as receiving pools for paper mill effluent.

A later study into the discharge of PCDD's PCDF's from paper mills in the USA, confirmed the release of 2,3,7,8-TCDD and 2,3,7,8-TCDF in three export vectors (bleached pulp, sludge and effluent) from all 104 paper mills that practised chlorine bleaching of chemically produced pulps (Whittemore *et al.* 1990). The Canadian National Dioxin Sampling Program, a 4 year study to determine the extent of dioxin and furan release form pulp, paper mills, revealed the contamination of finfish, shellfish and other wildlife with PCDD's and PCDF's at marine and fresh water sites in the vicinity of the 46 Canadian pulp and paper mills using the chlorine bleaching process (Whittle *et al.* 1993). Since then, PCDD's and PCDF's have been detected in bleached paper products

(Beck *et al.* 1989; Wiberg *et al.* 1989; and LeBel *et al.* 1992), in bleach plant pulp and effluents (Clement *et al.* 1989; Whittemore *et al.* 1990; Kitunen and Salkinoja-Salonen 1992;), in receiving water sediments and sludge associated with the mills (Sherman *et al.* 1990; Balk *et al.* 1993; Paasivirta *et al.* 1993) and in biota living in mill receiving water (Munkittrick *et al.* 1992; Andersson *et al.* 1988; Cooper *et al.* 1992 and Bauer *et al.* 1992).

4.2.2 Contamination Levels

The results of a study into the contamination of receiving waters surrounding pulp and paper mills in Canada (Muller and Halliburton 1990; Whittle *et al.* 1993), indicated that the congeners, 2,3,7,8-TCDD and 2,3,7,8-TCDF were significant contaminants in both top and bottom feeding fresh water fish and all bivalves tested.

The levels of 2,3,7,8-TCDD in lake trout samples were found to exceed 100 pg/g (t), while total T4CDD levels were measured in excess of 1200 pg/g (t).

In response to the results of the Canadian study, the Canadian Government, using health evaluations based on the tolerable daily intake of dioxins and furans in terms of total 2,3,7,8-TCDD equivalents of 10 pg/kg body wt./day, closed the crustacean fisheries in the vicinity of three coastal mills in British Columbia. These areas were expanded following further sampling programs the following year (Muller and Haliburton

1990). Further health advisories were issued in most areas affected by paper mill effluent involved in the Canadian study.

Ontario pulp and paper mills were also examined for their PCDD and PCDF output in a separate study (Clement *et al.* 1989). This study concluded that the PCDD/PCDF congener distribution pattern varied between mills. In Bleached-Kraft mills, the TCDF congeners were present in the highest concentration (up to 3.2 b), while total TCDD was also detected up to 0.3 t.

4.2.3 Formation of Contaminants

There is general agreement amongst dioxin research teams, that PCDD and PCDF congeners are anthropogenic in origin (Safe 1990; Hutzinger and Fiedler 1993; Rappe 1993). There is also some suggestion in the literature of natural, ubiquitous precursors (unchlorinated analogs), as well as contaminant precursors, in the pulp production, other than the chlorine bleaching stage, such as dibenzo-p-dioxin (DBD) and dibenzofuran (DBF) in the defoamer oil (Berry *et al.* 1989).

A possible mechanism was suggested by Hrutfiord and Negri (1992) for the chemical formation of PCDD's and PCDF's. The original precursors are suggested to be unsubstituted phenols, such as p-coumaryl alcohol and p-hydroxybenzoic acid which oxidatively couple to form a biphenyl. The phenol undergoing coupling, is substituted in the 4-position with a moiety that can be displaced by Cl during bleaching. Enolization of one carbonyl

to form a phenolic -OH group then occurs, which adds across the adjacent carbonyl group, eliminating water, to form the aromatic DBF. The DBD analog may also be formed by the initial oxidative coupling of the same precursors to form a biphenyl ether. Further oxidation causes ring closure, via a cation radical which couples and eliminates a proton. Again, the chlorine bleaching process leads to the displacement of moieties on the side chains to form the final PCDD congeners.

4.2.4 Absorption by Vegetation

Current studies suggest that, while some absorption of highly lipophilic chlorinated hydrocarbons into the root tissue occurs (Briggs *et. al.* 1982; Bacci and Gaggi 1985,1986; Reischl *et. al.* 1989; Lovett Doust *et al.* 1994), translocation from the roots up to the shoots, is unlikely to be a source of foliar absorption (Briggs *et. al.* 1982; Reischl *et. al.* 1989).

The most widely accepted route of foliar absorption of PCDD's and PCDF's is through the leaves (Bacci and Gaggi 1987; Bacci *et al.* 1992), via the lipid cuticle layer, or the stomata (Kerler and Schonherr 1988a, 1988b,:Reischl et. al. 1989).

Deposition routes of these chemicals onto leaf surfaces is possibly via airborne particulates, although this is not well understood. More likely the most important route is via adsorption of vapours onto the leaf surface, as this process occurs at a much faster rate (Reischl et. al. 1989).

Although PCDD's and PCDF's have a very low water solubility

as well as low vapour pressures (Eitzer and Hites 1986; Shiu et. al. 1988) and are rapidly partitioned to the dissolved and particulate organic matter fractions of the water column and sediment, they have been measured in the gaseous state within an aquatic environment (Corbet et. al. 1988). This study estimated that 0.5 % of total added 1,3,6,8-T_4CDD to a pond, was lost through vaporization from the water surface.

Volatilization of these chemicals following slow diffusion from sediments and decayed plant material on the pond bottom into the water column may be a significant route of PCDD's PCDF's entry into the vapour state (Corbet et. al. 1988).

4.3. METHODS

Data was collected for the two year period 1993-1994. In June, 1993 samples of the dominant submersed and emergent aquatic plant species from Lake A were collected and transported to a greenhouse for an initial survey of the levels of PCDD's/PCDF's in Lake C sediment and vegetation.

The plant roots were then washed, before being transplanted into 14 litre plastic buckets containing Lake C sediment and water. The buckets were then placed in large tanks containing Lake C water. The plants were allowed to grow in the greenhouse for a period of three months before the two species, one submersed, *Utricularia vulgaris* and one emergent, *Calamagrostis canadensis*, were sampled for PCDD\PCDF analysis at the Toronto

Ministry for the Environment and Energy (OMOEE) laboratory, using the OMOEE protocol for PCDD\PCDF analysis in vegetation. Lake C sediment was also analyzed at the same laboratory using the OMOEE protocol for industrial waste and sediment. Total toxicity concentrations are calculated as the equivalent concentration of the most toxic congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and given here as international toxicity equivalent (I-Teq.) (Rappe 1991).

The 2 submersed aquatic macrophyte species found in Lake A, *Potamogeton pusillus* and *Utricularia vulgaris* and the 4 emergent species, *Calamagrostis canadensis, Carex aquatilis, Typha latifolia* and *Eleocharis smallii* were selected for a growth trial in Lake C. These plant species from Lake A were collected in early June 1994 and placed into plastic bags. The plants were then transported to Lake C, the root systems washed in Lake C water and transplanted into 10 litre plastic buckets containing Lake C sediment. The buckets were then suspended at a depth of 6 cm below the lake surface, from two large floating rafts that were secured to the lake bottom.

In mid September 1994, 4 replicates of these plants together with plants from fifty random sites within Lakes A and B, were collected and the foliar portions bulked for PCDD\PCDF sample analysis at the OMOEE laboratory in Toronto, following the same protocol as for the greenhouse trial.

Sediment samples from all three lakes, were also collected at the same time. These were then bulked and all samples were

placed on ice, before transport to the Ministry of the Environment Laboratories, Toronto for analysis of the PCDD\PCDF content.

Due to the cost of PCDD/PCDF analysis, and the absence of precedent analysis results for aquatic macrophytes, confirming their ability to absorb these chemicals, only one species, *Carex aquatilis* was sampled from all three lakes. This species was chosen as it was the dominant emergent species in lakes A and C, was easier to sample than the submersed species and was more successful in propagation in Lake C conditions.

4.4 RESULTS

4.4.1 Survey of Greenhouse Sediment and Vegetation

The early results of the greenhouse survey of 2 aquatic macrophytes, one submersed species, *Utricularia vulgaris*, and one emergent species, *Calamagrostis canadensis* in 1993 (Table 4.4.1), indicated that only *Utricularia vulgaris*, absorbed measurable levels of some of the congeners in the foliage, whereas *Calamagrostis canadensis* had absorbed measurable levels into the roots, but not the foliage.

Selective absorption of tetra-furans and octa-dioxins took place that was consistent in both species sampled.

The PCDD/PCDF homologue distributions in the 2 aquatic species sampled (Figs. 4.4.1, 4.4.2), indicated a similarity in absorption profiles, between the aquatic vegetation grown in Lake C water and sediment and with the Lake C sediment itself.

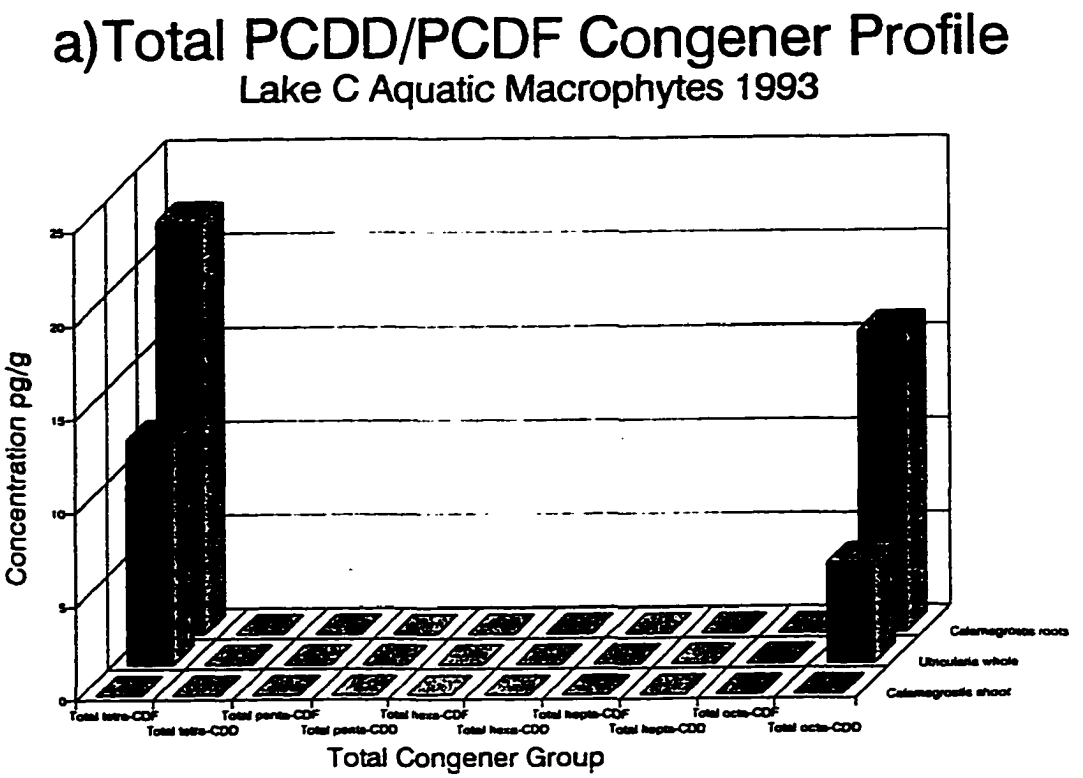
4.4.2 In-situ Survey of Water, Sediment and Vegetation

The 1994 survey of the concentration of PCDD/PCDF's in the lake sediments (Table 4.4.2), indicated a graduation in PCDD/PCDF concentrations from the lowest in Lake B, to the highest in Lake C, with Lake A being intermediate.

Table 4.4.1 Profile of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran congeners in two species of aquatic macrophytes and Lake C sediment.

Congener	Utricularia vulgaris Shoots (pg/g)	Calamagrostis canadensis Shoots (pg/g)	Calamagrostis canadensis Roots (pg/g)	Lake C Sediment (pg/g)
Total Tetra-CDD	0	0	0	12
Total Penta-CDD	0	0	0	0
Total Hexa-CDD	0	0	0	4.8
Total Hepta-CDD	0	0	0	28
Total Octa-CDD	5.3	0	16	68
Total Tetra-CDF	12	0	22	450
Total Penta-CDF	0	0	0	32
Total Hexa-CDF	0	0	0	2
Total Hepta-CDF	0	0	0	7.4
Total Octa-CDF	0	0	0	9.5
2378-tetra-CDF	8.4	0	14	230
2378-tetra-CDD	0	0	0	12
12378-penta-CDF	0	0	0	5.6
12378-penta-CDD	0	0	0	0
123478-hexa-CDD	0	0	0	0
123678-hexa-CDD	0	0	0	0
123789-hexa-CDD	0	0	0	0
1234678-hepta-CDD	0	0	0	12
23478-penta-CDF	0	0	0	11
123478-hexa-CDF	0	0	0	2
123678-hexa-CDF	0	0	0	0
234678-hexa-CDF	0	0	0	0
123789-hexa-CDF	0	0	0	0
1234678-hepta-CDF	0	0	0	2.5
1234789-hepta-CDF	0	0	0	0
International Toxic Equivalent (ITEQ)	0.84	0	1.4	41.125

Figure 4.4.1. Homologue distribution of PCDD/PCDF for the submersed aquatic macrophytes, *Utricularia vulgaris* and *Calamagrostis canadensis* grown in Lake C water and sediment (shoots and roots).



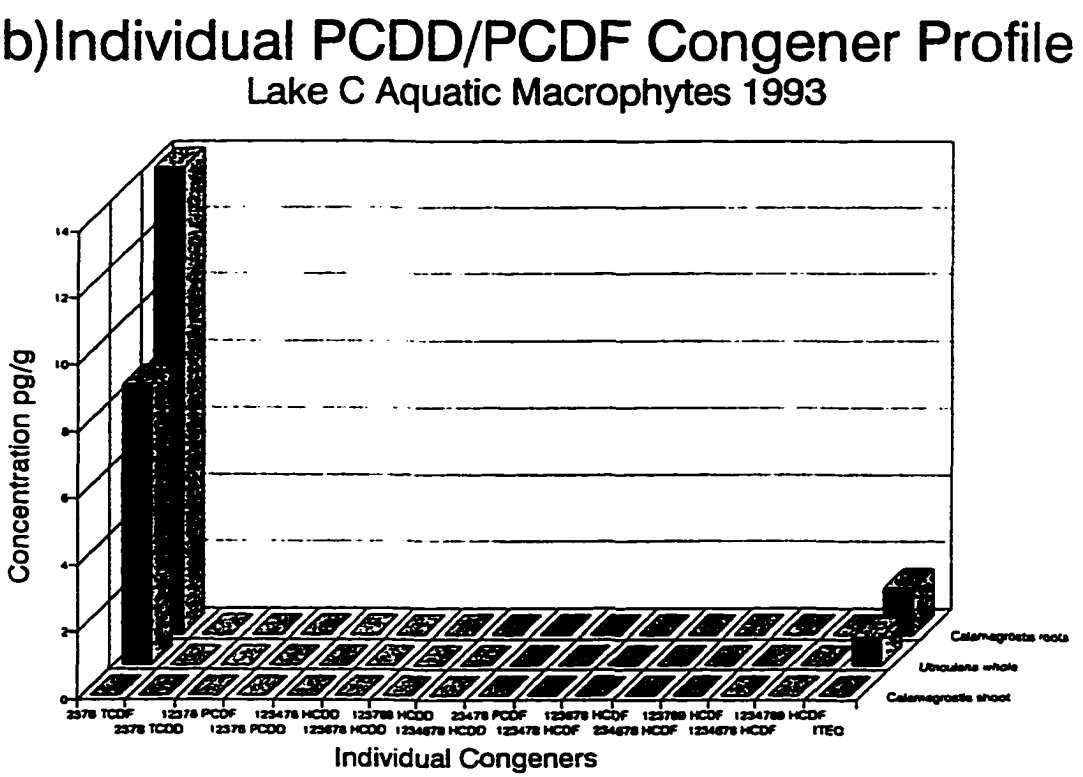
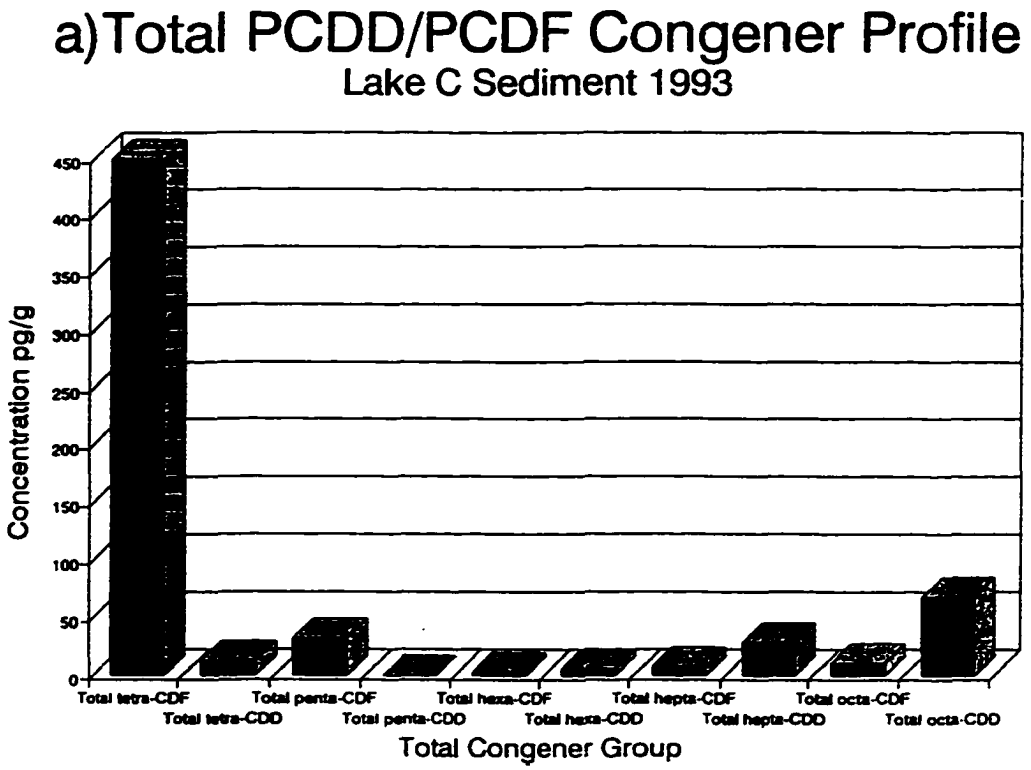


Figure 4.4.2a,b. PCDD/PCDF homologue profiles a)total congener groups a)individual congeners, for the 1993 sediment sampling of Lake C.



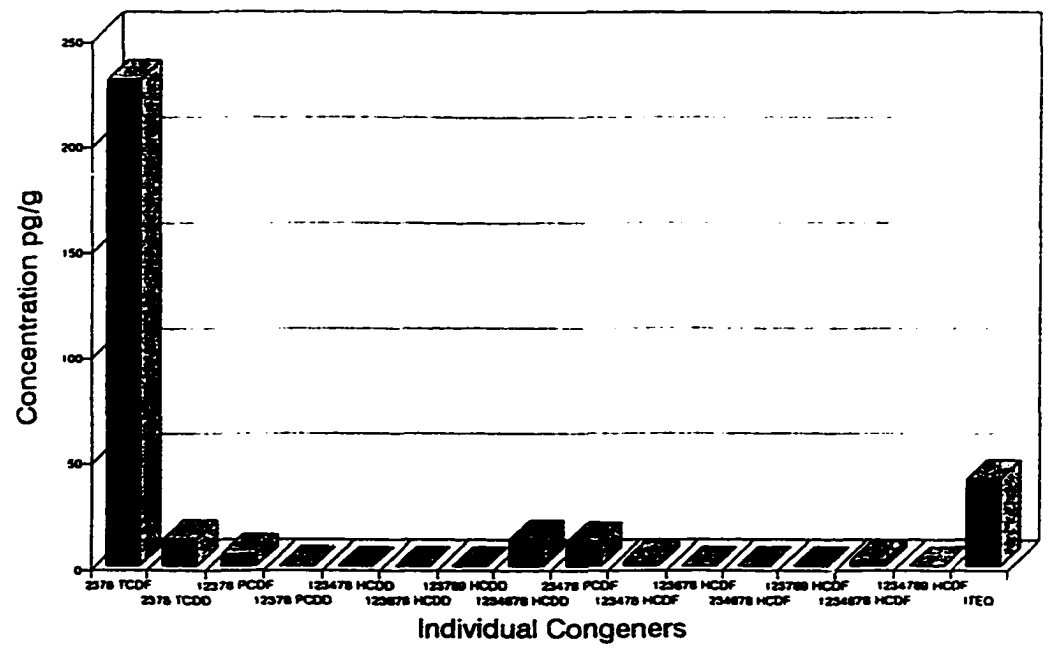


Table 4.4.2 Sediment Analysis for PCDD/PCDF's in Lakes A, B and C, showing the concentration of all congeners.International equivalent values are given in the bottom row.

Congener	Lake A sediment p pg/g dry	Lake B sediment pg/g dry	Lake C sediment p pg/g dry
Total tetra-CDF	240	1	1100
Total tetra-CDD	12	1	42
Total penta-CDF	6.8	1	77
Total penta-CDD	1	1	4.8
Total hexa-CDF	2	1.7	5
Total hexa-CDD	7.4	1.3	16
Total hepta-CDF	11	2	17
Total hepta-CDD	44	5.3	67
octa-CDF	15	3	22
octa-CDD	140	24	230
2378-tetra-CDF	130	1	600
2378-tetra-CDD	12	1	41
12378-penta-CDF	2	1	17
12378-penta-CDD	4	1	28
23478-penta-CDF	1	1	3
123478-hexa-CDF	2	1.7	5
123678-hexa-CDF	1	1	1
234678-hexa-CDF	1	1	1
123789-hexa-CDF	1	1	1
123478-hexa-CDD	1	1	1
123678-hexa-CDD	1	1	2
123789-hexa-CDD	2	1	4
1234678-hepta-CDF	4	2	6.4
1234789-hepta-CDF	1	1	1
1234678-hepta-CDD	23	5	32
IEQ	28.78	3	119.24

The graphs of the homologue distributions in the lake sediments (Fig. 4.4.3a,b), indicate the similarity of the distributions in lakes A and C, while the control lake, Lake B is significantly different, exhibiting a flat profile, with limited elevation of the octa-congeners. The major congener groups showing in the homologue distribution of lakes A and C, are the tetra-chlorinated group and the octa-chlorinated group.

The levels of total Octa-PCDD/PCDF's are higher in the sediment of lakes A and C than the control lake, Lake B. The levels and distribution of congeners in the sediment of Lake B resemble earlier results from a study of Lake Huron sediment (Czuczwa and Hites 1986) and are assumed, indicate the background distribution and levels of PCDD/PCDF's in the northern Great Lakes region.

The 1994 results of the *in-vivo* study in Lakes A and C, of PCDD/PCDF levels in the aquatic macrophyte populations (Table 4.4.3), confirmed that all plants tested, contained measurable levels of the PCDD\PCDF congeners.

Results of the 1994 survey indicated *Utricularia vulgaris* had the highest concentration of PCDD/PCDF congeners of the aquatic plants surveyed. This species had a total International Toxic Equivalent value (I-Teq) of 22.18 pg/g (dry wt.) in Lake A, compared to the mean of 12.63 pg/g (dry wt.) and an I-Teq value of 67.02 pg/g (dry wt.) in Lake C, compared to the mean of 29.67 pg/g (dry wt.) (Table 3). The next highest was another submersed plant, *Potamogeton pusillus*, which had a total I-Teq

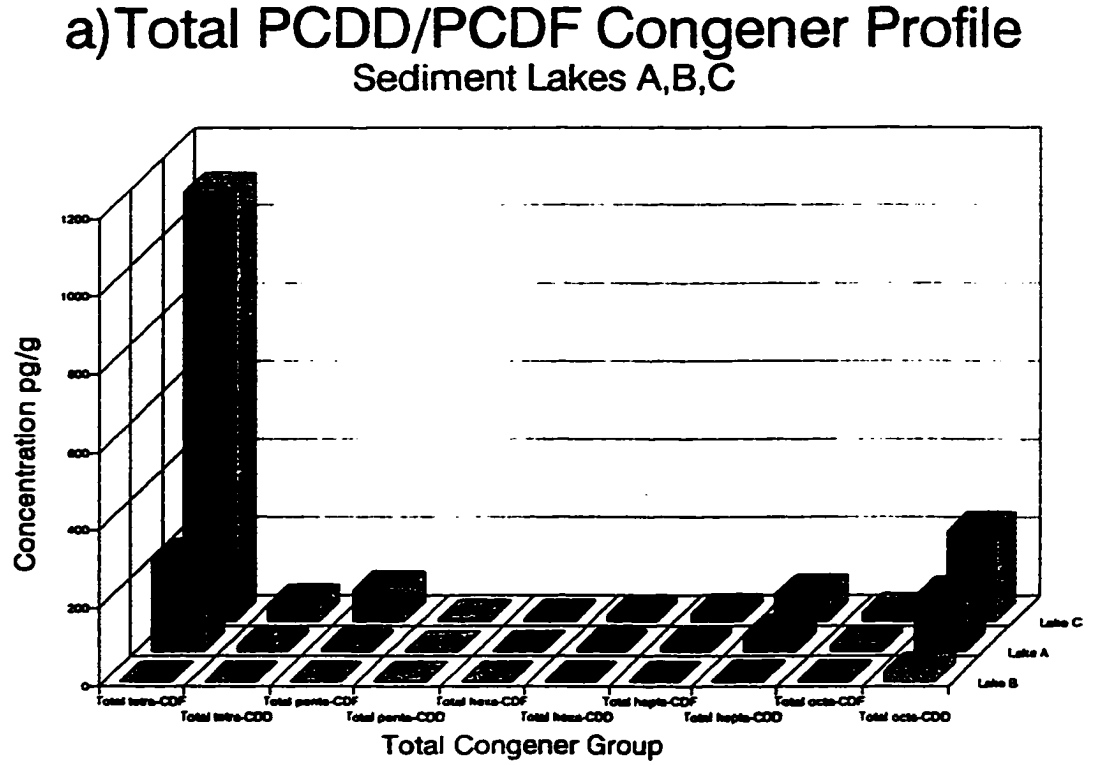
concentration of 46.16 pg/g (dry wt.), compared to the mean of 29.67 pg/g (dry wt.).

The emergent aquatic species all recorded lower concentrations of the PCDD/PCDF congeners, but higher total loadings when mean biomass was included (Table 4.4.3a). The lowest concentrations of PCDD/PCDF congeners were found in *Typha latifolia*, where the levels were just in the detectable range.(Fig. 3.4.4c,d).

The ratio of the concentration of the PCDD/PCDF congeners in the aquatic vegetation to the sediment in which the vegetation was growing, gave an estimation of the bioconcentration of these organochlorines. Highest bioconcentration occurred in the lower chlorinated congeners, particularly the tetra-, penta- and hexa-congener groups (Table 4.4.3b). The bioconcentration was higher in all plant species growing in Lake A, compared to Lake C.

There was a large variation in bioconcentration of PCDD/PCDF congeners and total ITEQ levels between species, with the highest being in the emergent species, *Eleocharis smallii* and the two submersed species, *Utricularia vulgaris* and *Potamogeton pusillus*. The highest bioconcentration was recorded in the emergent *Calamagrostis canadensis*, of 12.5 for the total congener group total penta-CDD.

Figures 4.4.3a,b. PCDD/PCDF homologue distribution a)total congener group b)individual congeners, for the sediment survey of lakes A, B and C.



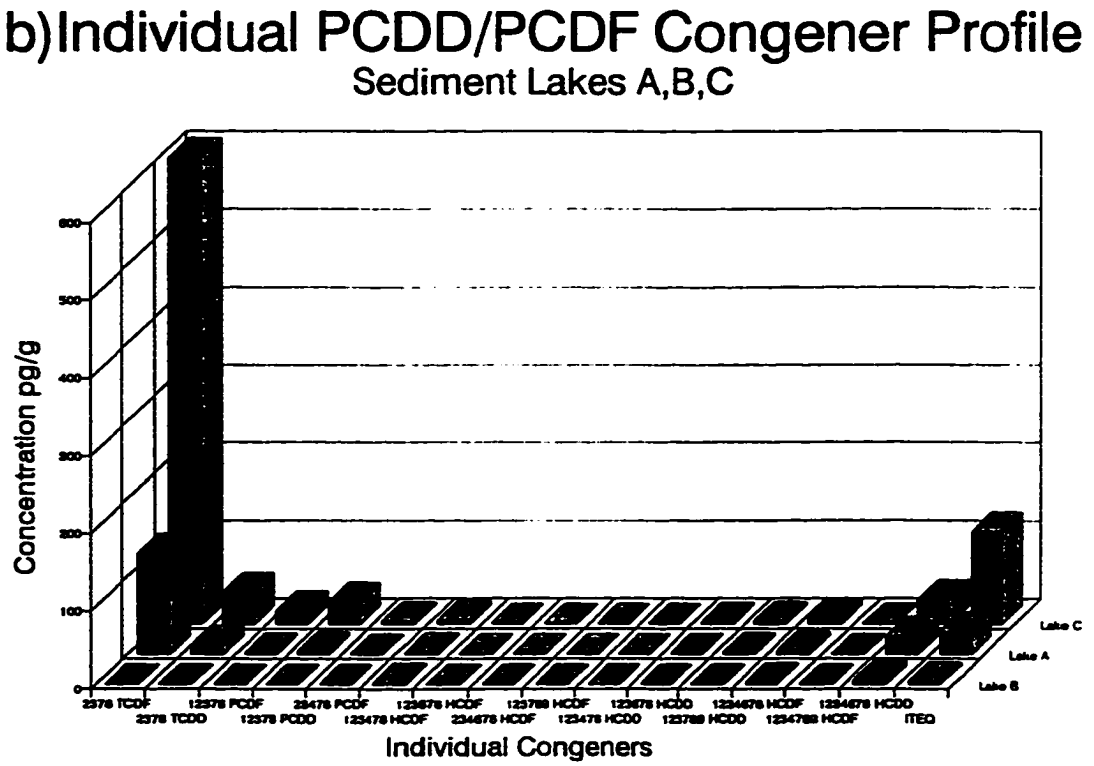


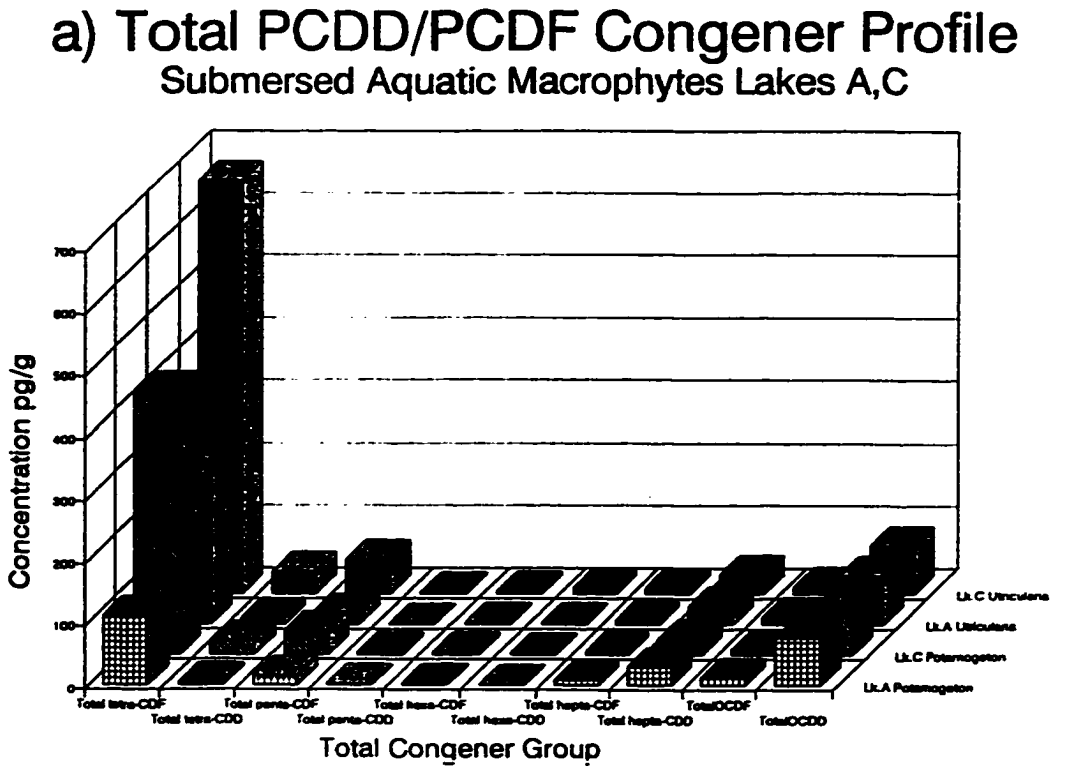
Table 4.4.3. PCDD/PCDF levels (pg/g dry wt.) in five of the dominant aquatic macrophyte species found in Lake A. The total ITEQ concentrations are multilpied by mean biomass to estimate total loads. Two of the species, *Utricularia vulgaris* and *Potamogeton pusillis* are submersed aquatics, while the remaining three are emergents.

Congener (pg/g dry wt.)	Utricularia vulgaris Lake A	Utricularia vulgaris Lake C	Potamogeton pusillus Lake A	Potamogeton pusillus Lake C	Eleocharis smallii Lake A	Eleocharis smalli Lake C	Calamagrostis canadensis Lake A	Calamagrostis canadensis Lake C	Typha latifolia Lake A	Typha latifolia Lake C
Total tetra-CDF	240	660	110	420	62	160	1.9	140	1	21
Total tetra-CDD	9.7	35	4.6	21	20	5	5	10	1	5
Total penta-CDF	17	54	15	38	9.8	6.1	1	4	1	1
Total penta-CDD	1	2.1	2	4	1	1	1	60	1	1
Total hexa-CDF	2.4	4.2	1.8	3.9	4	3	2	6	2	4
Total hexa-CDD	2	8.8	1	3.3	2	1	1	20	1	1
Total hepta-CDF	5.6	10	7.7	3.8	2	3.1	1	4	2	1
Total hepta-CDD	29	31	28	25	7.2	5.2	2.5	9.3	1	2.1
Total octa-CDF	11	11	12	8	5.6	7.6	1	10	3	2
Total octa-CDD	78	77	75	64	32	41	10	49	5	10
2378-tetra-CDF	100	320	47	220	44	78	3	70	1	13
2378-tetra-CDD	7.6	29	4.6	19	20	5	5	10	1	5
12378-penta-CDF	5	9.4	3.4	5	3.2	4	1	4	1	1
12378-penta-CDD	5.3	7.1	1.6	6.4	2.5	3.1	1	4	1	1
23478-penta-CDF	1	1	2	1	1	1	1	10	1	1
123478-hexa-CDF	2	4	2	2.8	2	1	1	3	1	1
123678-hexa-CDF	1	1	1	1	1	1	1	3	1	1
234678-hexa-CDF	2	2	3	2	4	3	2	3	2	4
123789-hexa-CDF	1	1	1	1	1	1	1	6	1	1
123478-hexa-CDD	1	1	1	1	1	1	1	9	1	1
123678-hexa-CDD	1	2	1	1.6	2	1	1	4	1	1
123789-hexa-CDD	2	2	1	1	2	1	1	6	1	1
1234678-hepta-CDF	3	4.6	3	3.8	2	2	1	2	1	1
1234789-hepta-CDF	1	1	1	1	1	1	1	3	2	1
1234678-hepta-CDD	14	14	14	12	7	6	3	6	1	2
ITEQ	22.18	67.02	12.45	46.16	27.71	16.04	7.2	27.71	2.99	8.39
Total ITEQ uptake (pg)	201.84	134.04	120.77	92.32	1003.1	192.48	137.52	211.7	499.33	369.16

Table 4.4.3b. Ratio of the total PCDD\PCDF congener concentration in the aquatic macrophyte species, to the sediment in which it was growing.

Congener (pg/g dry wt.)	Utricularia vulgaris Lake A	Utricularia vulgaris Lake C	Potamoget pusillus Lake A	Potamoget pusillus Lake C	Eleocharis smallii Lake A	Eleocharis smalli Lake C	Calamagro canadensi Lake A	Calamagro canadensi Lake C	Typha latifolia Lake A	Typha latifolia Lake C
Total tetra-CDF	1	0.6	0.5	0.4	0.3	0.1	0.001	0.1	0.004	0.02
Total tetra-CDD	0.8	0.8	0.4	0.5	1.7	0.1	0.4	0.2	0.1	0.1
Total penta-CDF	2.5	0.7	2.2	0.5	1.44	0.1	0.1	0.1	0.1	0.01
Total penta-CDD	1	0.4	2	0.8	1	0.2	1	12.5	1	0.2
Total hexa-CDF	1.2	0.8	0.9	0.8	2	0.6	1	1.2	1	0.8
Total hexa-CDD	0.3	0.6	0.1	0.2	0.3	0.1	0.1	1.25	0.1	0.06
Total hepta-CDF	0.5	0.6	0.7	0.2	0.2	0.2	0.1	0.2	0.2	0.06
Total hepta-CDD	0.7	0.5	0.6	0.4	0.2	0.1	0.1	0.1	0.02	0.03
Total octa-CDF	0.7	0.5	0.8	0.4	0.4	0.3	0.1	0.5	0.2	0.1
Total octa-CDD	0.6	0.3	0.5	0.3	0.2	0.2	0.1	0.2	0.03	0.04
ITEQ	0.8	0.6	0.4	0.4	0.96	0.1	0.3	0.2	0.1	0.07

Figure 4.4.4a,b. TCDD/TCDF homologue profiles a)total congener group b)individual congeners in the submersed aquatic macrophyte species grown in Lake C water and sediment.



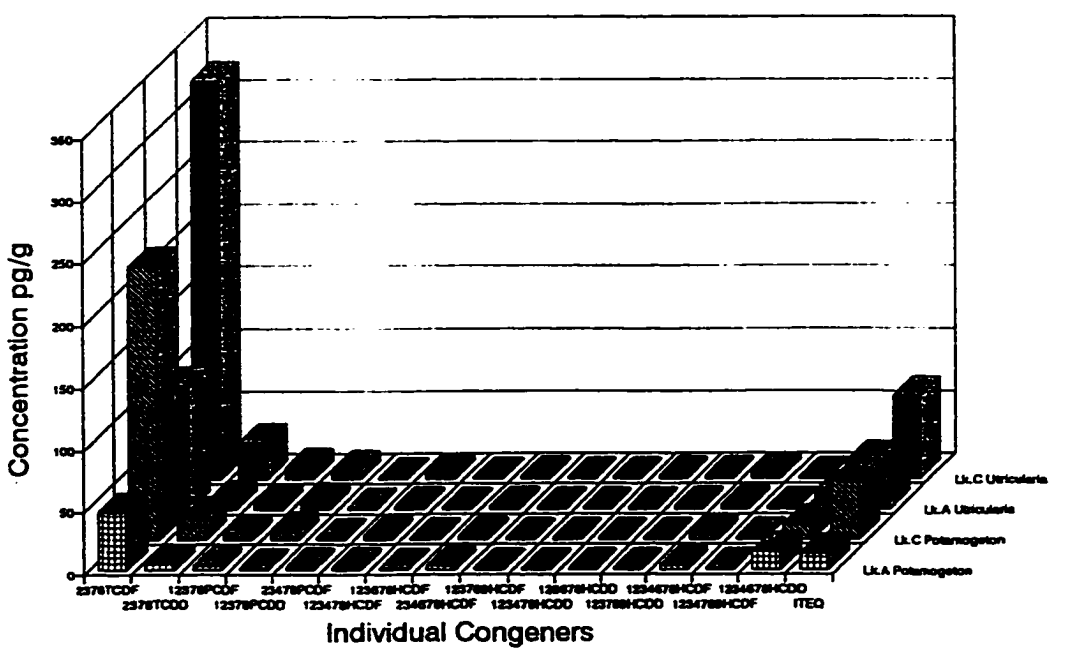
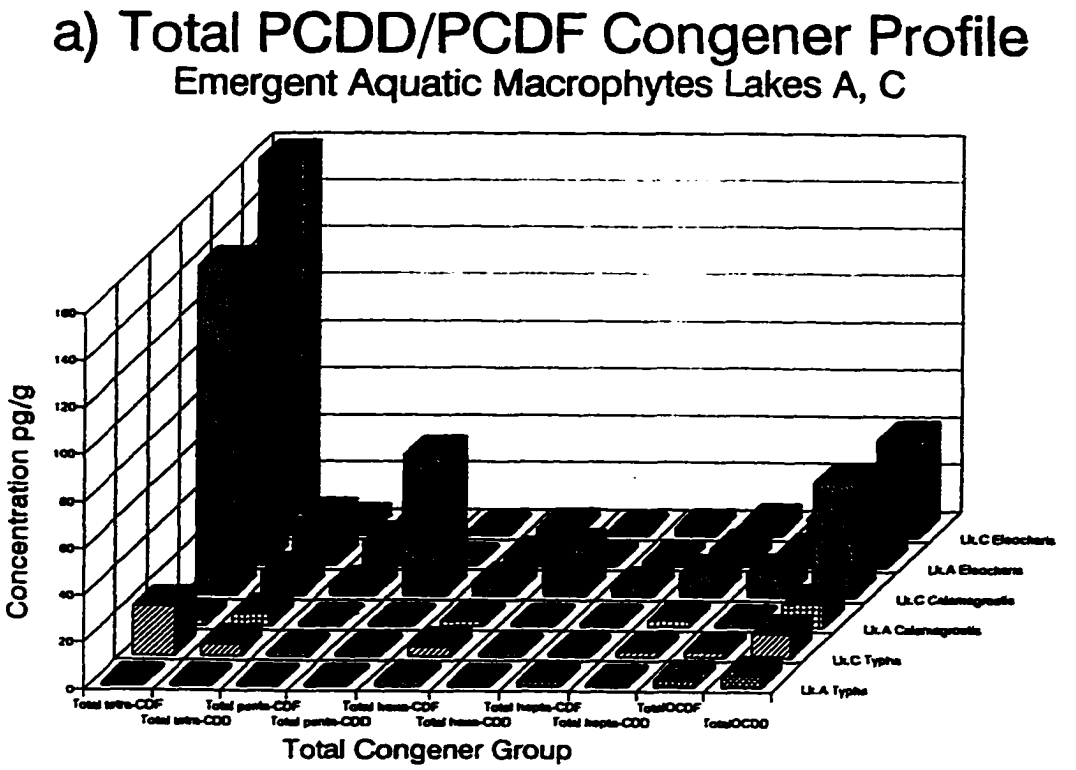


Figure 4.4.5a,b. TCDD/TCDF homologue profiles a)total congener group b)individual congeners in the emergent aquatic macrophyte species grown in Lake C water and sediment.



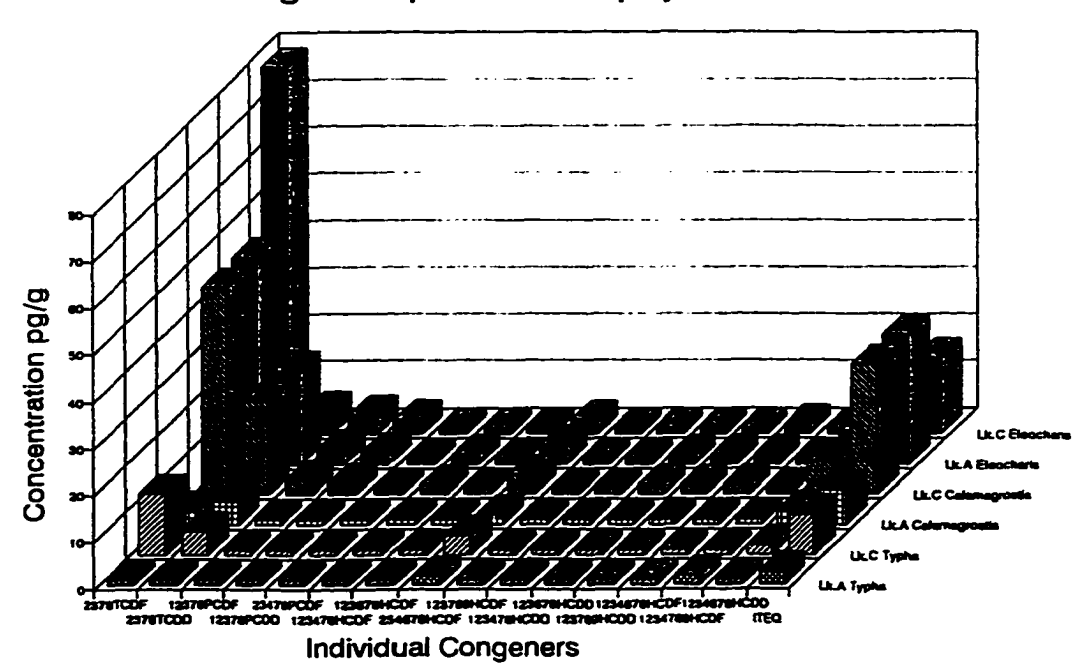
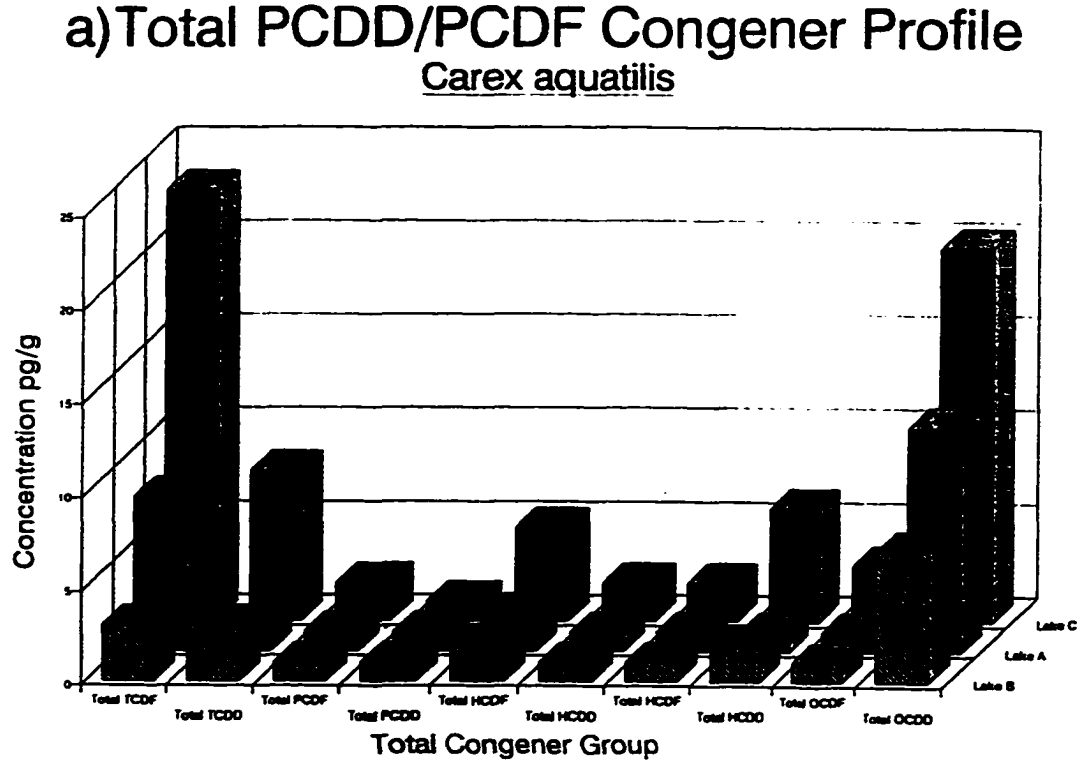
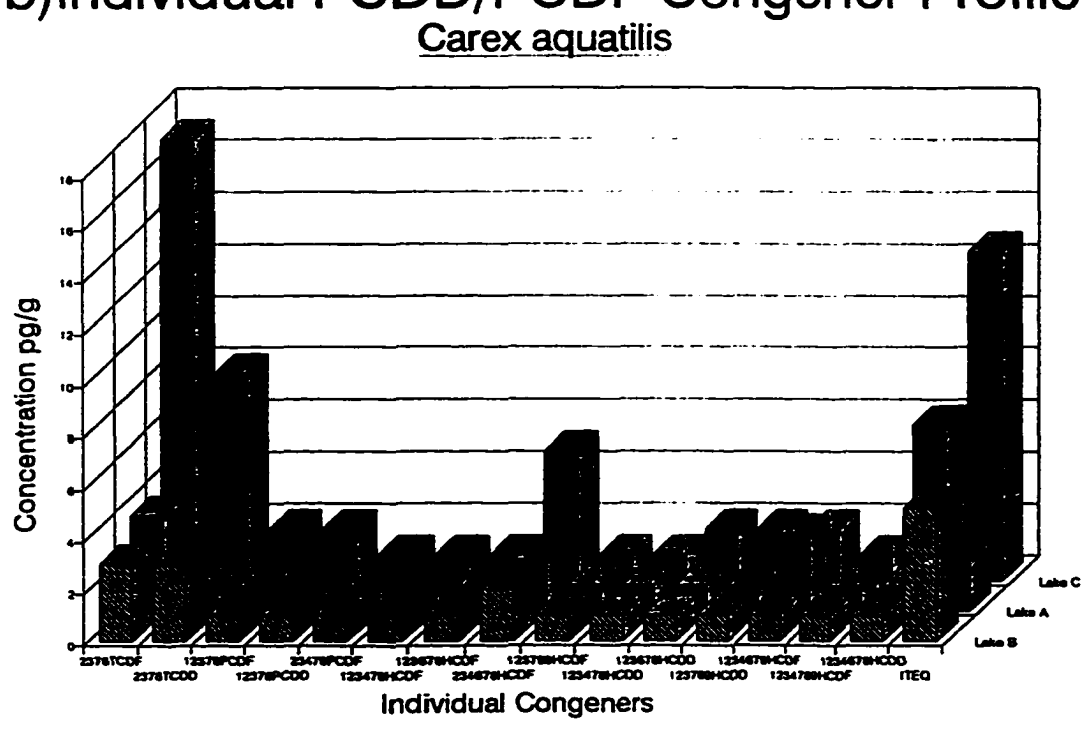


Figure 4.4.6a,b. PCDD/PCDF homologue distribution a) total congener group b) individual congeners, in the aquatic macrophyte *Carex aquatilis* from Lakes A, B and Raft Experiment in Lake C.





The concentrations of PCDD/PCDF congeners found in the various species of aquatic macrophytes, were consistent between species (Fig. 4.4.4) and closely reflected the profiles found in the sediments (Fig. 4.4.3a,b).

The survey of the PCDD/PCDF contamination of aquatic macrophytes (Table 4.4.3a), indicates a loading of a majority of the congeners in the macrophytes growing in Lake C water and sediment. This corresponds with the continuous exposure of Lake C to BKME, since the pulp paper mill in Terrace Bay was converted to the kraft bleaching process in 1972 (RAP 1991).

The tetra-furans were also elevated in the macrophytes growing in Lake A, indicating the source is likely to be BKME and reflects the historic exposure to BKME, prior to 1982 when the effluent stream freely entered the lake and the limited current exposure to BKME, following the breaching of the Lake A barrier, on Blackbird Creek.

The distribution of PCDD/PCDF homologues in *Carex aquatilis*, the dominant emergent species in both lakes A and B and the only species to be sampled in all three lakes, is consistent with the other aquatic macrophyte species shown in Figures (4.4.4, 4.4.5). The dominant congener groups are tetra-chlorinated dioxins and furans and the octa-chlorinated groups.

4.5 DISCUSSION

4.5.1 Survey of Greenhouse Trial Vegetation

The early results of the survey of aquatic macrophytes growing in Lake C water and sediment in the greenhouse, indicated that absorption of PCDD/PCDF congeners into foliage of the 2 species sampled was limited (Table 4.4.1). Absorption profiles of the congeners were consistent for both aquatic species, with only the tetra-furans and octa-dioxins being measurable. These results were consistent with a contaminant source of paper mill effluent, where the tetra-congeners have been found to dominate congener profiles (Rappe *et al.* 1987; Clement *et al.* 1989; Whittmore 1990).

Although no previous studies of aquatic plant uptake of these chemicals have been reported, the congener profiles, as well as the I-TEq levels, were consistent with those obtained from *in-vitro* studies of the transfer of PCDD's/PCDF's, from contaminated soils to terrestrial food and fodder crop plants (Hulster and Marschner 1993).

The similarity in PCDD/PCDF homologue profiles between the 2 aquatic macrophyte species and the Lake C sediment in which they were grown for 4 months, suggested a common source of contamination. The profiles suggest that the BKME is the major source, although the presence of octa-congeners is consistent with atmospheric deposition (Czuczwa and Hites 1986).

4.5.2 Field Survey of Water, Sediment and Vegetation

There was a clear graduation in PCDD\PCDF sediment concentration between the three lakes with Lake B being the least contaminated, Lake A being intermediate and Lake C being the most highly contaminated. The results strongly reflect the results for the survey of water colour and the inverse for the secchi depth which displayed the same relationship and indicates the original source of contamination as being the BKME.

These results fit well with the historical and current exposure of the three lakes to BKME. The control lake, B has never been exposed to the PCDD/PCDF fractions of BKME and are consistent with earlier measurements of the background levels, due to atmospheric deposition in the Great Lakes region (Czuczwa and Hites 1986).

Lake A has intermediate levels of PCDD/PCDF, following continuous exposure to BKME between 1948 and the early 1980's and periodic exposure since the construction of the barrier following flood events. Lake C had the highest levels of PCDD/PCDF's reflecting the continuous exposure since the mill opened in 1948.

The graphs of the PCDD/PCDF homologue distributions (Fig. 4.4.3a,b), indicate the similarity of the distributions in lakes A and C. It is aarent these two lakes have a common source of PCDD's/PCDF's.

The major congener group showing in the homologue distribution of lakes A and C, is the tetra-chlorinated group, which is consistent with the congener profile pulp paper mill

effluent (Clement *et al.*1989). The octa-chlorinated congener group were also elevated, which is consistent with atmospheric deposition (Czucwa *et al.* 1985; Czuczwa and Hites 1986)

The levels of total Octa-PCDD/PCDF's are higher in lakes A and C than the control lake, Lake B, indicating the BKME is the major source of all congener groups, including the higher chlorinated groups. As Lake B has never been included in the effluent stream from the paper mill, it is assumed the analysis results for this lake, indicate the background distribution and levels of PCDD/PCDF's in the Great Lakes region. These results are consistent with the results of an earlier study by (Czuczwa and Hites 1986), in which the sediments of Lake Huron were surveyed.

The major congener groups showing in the PCDD/PCDF homologue distribution in the sediments of lakes A and C, are the tetra-chlorinated groups, which is consistent with the earlier greenhouse trials and with studies in receiving waters of BKME (Clement *et al.* 1989) and the octa-chlorinated group which has been found to be the dominant group in atmospheric deposition (Czuczwa and Hites 1986; Hutzinger and Fiedler 1993).

The PCDD/PCDF congener profiles and congener concentrations found in the aquatic macrophyte species grown on rafts in Lake C as well as those growing in Lake A, confirmed that all plants tested, contained measurable levels of the PCDD\PCDF congeners. Concentrations were similar to those obtained in the *in-vitro* study of terrestrial food and fodder crop plants (Hulster and

Marschner 1993).

The two submersed species *Utricularia vulgaris* and *Potamogeton pusillus* had the highest concentrations for PCDD\PCDF congeners of all species surveyed (Table 4.4.3). Both these species also had consistently high bioconcentration factors compared to the emergent species for all of the PCDD/PCDF congener groups, with the exception of the emergent *Eleocharis smallii*. This could indicate that submersed aquatic macrophytes have a higher capacity to absorb PCDD's/PCDF's than emergent species, although this is not supported by the results for the total loadings of the species. The total PCDD/PCDF loadings indicate the emergent species absorb greater total amounts of these organics than the submersed species and the lower concentrations in the emergent species is partly a result of a dilution effect, caused by the higher biomasses attained by these species (Table 4.4.3a).

Variation was shown between species, for both concentration of the organics in the foliage and total loading (Table 4.4.3). Further testing is required to delineate experimental error, from real variation which may exist due to a variation in lipid content in the surface layer of the stem and leaves between species, or more likely to a variation in total surface area of the species which is exposed to the PCDD\PCDF sources in the water column.

This would also be reasonably expected, if a major source of PCDD's/PCDF's contamination, originated through volatilization

from sediment layers, below the water column. This source has been discussed in studies by Servos et. al. (1992a,1992b) and aears a likely source, as a covering of bubbles on the surface of Lake C, originating from the sediment, was noted during sampling. It is unlikely, due to the low vapour pressure of PCDD/PCDF's, especially the higher chlorinated groups (Shiu *et al.* 1988), that these gas bubbles were entirely a result of volatilization of PCDD/PCDF. However, the benthic gas production, especially under the near anoxic conditions indicated by the low dissolved oxygen concentration and warm temperatures, recorded for lakes A and C (Table 3.4.7), would likely resuspend particulate organic matter that has bound PCDD/PCDF fractions. This would mean that plants with a greater vegetative surface area in contact with bubbles of volatilized PCDD/PCDF gas making their way to the water surface, would be likely to absorb higher levels. The same reasoning may explain the results in table (4.4.1), which show that the levels of PCDD's/PCDF's found in the roots of the emergent plant, *Calamagrostis canadensis*, were higher than the foliar portion. Root absorption of PCDD's\PCDF's by aquatic plants, may be a more important source of overall plant loading than foliar absorption, due to the higher contact with the sediment undergoing the volatilization and biotransformation processes as discussed in Servos et. al. (1992b). This may well be true only for emergent plants, as the levels found in the foliar portion of the submersed plants, aear closer to the levels found in the actual sediment (Table 4.4.1).

The bioconcentration of the PCDD/PCDF congeners originating from the sediment, in the aquatic macrophyte species was consistently higher in those growing in Lake A, with the one exception of the emergent *Calamagrostis canadensis*. This species was found to have a level of total penta-CDD, 12.5 times higher than in the Lake C sediment in which it was propagated (Table 4.4.3b). This may indicate that the actual concentrations of these chemicals in sedimentary deposits below the sample depth of 20 cm, were higher than the levels detected in the sediment samples for Lake A.

The similarity between the vegetation and sediment TCDD/TCDF profiles, indicates either a common source of contamination by atmospheric deposition, already noted in the Great Lakes region (Czuczwa and Hites 1986), or, that the major source of plant contamination is from the release of PCDD/PCDF from the lake sediment reservoir. The release of PCDD/PCDF from sediment reservoirs by the processes of volatilization and biotransformation, is discussed by Servos *et al.* (1992b) and Corbet *et al.* (1988). Even with the absence of statistical analysis of variation in this survey of PCDD/PCDF levels, it seems improbable that the values of total loss of sediment PCDD/PCDF by volatilization, estimated by Servos of 0.05%, could account for the levels found in the aquatic vegetation in this study. In the two lakes contaminated by BKME, the values of PCDD/PCDF found in aquatic vegetation expressed in ITEQ, were around 10 % of the values obtained for the sediment.

The 1991 RAP report on the environmental conditions of this study area, indicated that substantial in-filling of lakes A and C had occurred as a result of the highly organic nature of the effluent from the mill (RAP 1991). A very deep organic sediment existed in these two lakes and a covering of bubbles on the surface of Lake C, has been observed.It is possible that the highly anoxic conditions, combined with the elevated temperatures noted in these two lakes (Table 2.4.7), increased the rate at which PCDD/PCDF was released from the sediment reservoir.

The similarity between the profiles of PCDD/PCDF congeners for the sediment samples of the lakes (Fig. 4.4.3) and the aquatic macrophyte vegetation (Fig. 4.4.4), indicates that the major source of contamination of PCDD/PCDF's is common for both matrices. The fact that the lower chlorinated congeners are elevated and the 2 exposed lakes, A and C have higher levels of the higher chlorinated congeners than the control lake, Lake B, indicated that the dominant source of contamination, is likely to be mill effluent. The background levels found in *Carex aquatilis* from Lake B, the control lake, indicated that atmospheric deposition of PCDD/PCDF is also contributing to vegetation loading, though almost a magnitude less.

As with the results of analysis of the aquatic macrophyte species shown in Figure 4.4.4, the distribution of the PCDD/PCDf homologues is consistent with a major source of contamination from the BKME from the pulp paper mill at Terrace Bay and a secondary source of atmospheric deposition from distant thermal

emissions as indicated by elevated levels of octa-chlorinated congeners in Lake B, the control lake.

4.6 CONCLUSIONS

The survey is an indication of the levels of PCDD's/PCDF's in the dominant aquatic macrophyte populations exposed to BKME and possible explanations have been given.

The results from this survey have important implications for planned rehabilitation programmes for lakes exposed to BKME. There aear to be three management options that are available if future studies confirm the variability in uptake and bioconcentration of PCDD's/PCDF's between species : a) aquatic macrophyte species may be selected to contain the organochlorines as much as possible within the sediment, water column and atmosphere, that is to select those species that show the least absorption and bioconcentration of organochlorines.

b) aquatic macrophyte species may be selected to absorb the highest levels of organochlorines, followed by mechanical harvesting and thermal destruction.

c) aquatic macrophytes may be chosen solely on their relative ability to survive and grow under the conditions observed in lakes exposed to BKME.

The major conclusions from this study are reported below:

1. Foliage tissue from the dominant aquatic plants of the lakes within the study area were found to have significant levels of PCDD's/PCDF's. The levels were consistent with previous *in-vitro* studies of uptake of these chemicals by terrestrial fodder and food crop plants.

2. Significant bioconcentration of the lower-chlorinated PCDD/PCDF congeners over the sediment concentrations were recorded in many of the plant samples from both Lake A and Lake C.

3. The PCDD/PCDF homologue profiles are similar for the sediment and vegetation in Lakes A and C, indicating the likely primary source was the paper mill effluent, while the current source is volatilization from the sediment reservoir.

4. There is a clear graduation in PCDD/PCDF concentration between the three lakes, both in the sediment and in the vegetation. This fits very neatly with the historical and current exposure of the three lakes from the Bleached Kraft Mill effluent.

5. The submersed aquatic plants surveyed, had higher concentrations of PCDD/PCDF congeners, than the dominant emergent species. This is suggested to be partly due to the dilution of

the organics by the higher biomasses attained by emergent species. Total loading of the PCDD/PCDF congeners is likely correlated to total surface area of the species that is in contact with the water column, although variation in lipid content of the foliage may be important.

6. Results of the 1993 survey indicated that root absorption of PCDD/PCDF may be a more significant source of total loading than foliar absorption in emergent aquatic vegetation, even though little translocation occurs to the foliage from the roots. Root absorption is not as likely to be the major source of plant uptake in submersed aquatic macrophytes, due to the greater contact of foliage with resuspended TCDD/TCDF fractions.

7. The survey indicated that variation occurs between the concentration of total PCDD/PCDF in aquatic plant species, although there is little difference in total loading of species. Further sampling is required to determine if this is due to experimental error or real differences in the biological concentration factors between species.

5. GENERAL SUMMARY AND CONCLUSIONS

BKME has been shown to impact aquatic macrophytes in a number of ways. The most graphic is the total loss of all species growing in lakes used for BKME disposal. The major reason seems likely to be deterioration in water quality, due to the high colour content. The high colour prevents light from penetrating the water column, preventing photosynthetic activity in germinating seed banks and vegetative shoots of established plant populations.

The toxicity of components of BKME that have been associated with reduced marine vegetation, such as chlorate, are unlikely to be major factors in preventing aquatic macrophyte growth, nor is the near anoxic conditions of the effluent, or the elevated conductivity and temperature observed in the lakes exposed to BKME, likely to be significant factors, as six of the dominant emergent and two submersed species from Lake A, were successfully grown in a lake used for BKME disposal.

In BKME diluted conditions, where light availability improved above a critical threshold, the loading of organic nutrients, particularly total nitrogen, allowed oortunistic, emergent macrophyte species, to obtain total biomasses greater than the same species growing in control conditions.

Aquatic macrophyte species were successfully propagated in Lake C water and sediment, when the depth of water was artificially maintained above the average secchi depth of the

lake. Clearly then, if light conditions can be improved in lakes currently exposed to BKME, by methods such as lowering regions of the lake depth to less than the average secchi depth, recolonization of certain of the lake's aquatic macrophyte population could be expected. Selection of the emergent species that performed most favourably in the "depth experiment", in chapter 4 of this report, notably, *Carex aquatilis* and *Eleocharis smallii*, would be advised in any rehabilitation programme. The survey of PCDD/PCDF concentrations in selected aquatic macrophyte species grown in Lake C, indicated that emergent species accumulate lower concentrations of these contaminants, but similar or even higher total loads due to higher biomasses. These results, together with the observation of variation of absorption of these organic chemicals between emergent species, cannot be differentiated from experimental error, until further sampling allows an analysis of the variation.

Under natural recolonization processes of lakes removed from permanent BKME exposure, it aears that not only is aquatic macrophyte species diversity reduced, but that species composition changes in favour of emergent species. This would seem to be due to selection pressure exerted by the poor light conditions inherent in lakes exposed to BKME.

Surveys of the PCDD/PCDF levels of the aquatic macrophytes exposed to BKME, indicated that aquatic vegetation does absorb significant amounts of these organic chemicals. Further, the similarity of the homologue profiles of the sediment and the

vegetation, as well as the elevated levels of the lower chlorinated congeners, indicated the major source of aquatic vegetation was from the pulp paper mill effluent.

It aears that lakes historically exposed to BKME have a reservoir of dioxins and furans in the sediment layers which, under anoxic conditions, is being released into the water column and atmosphere.

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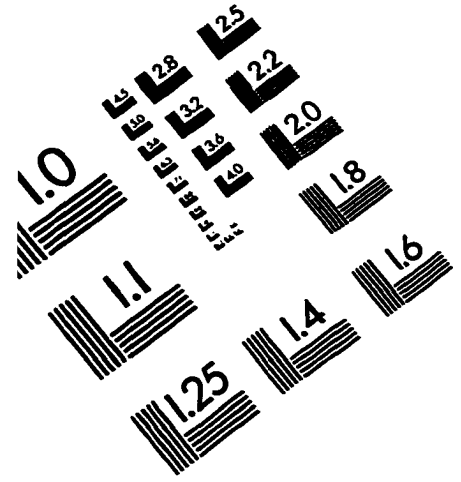
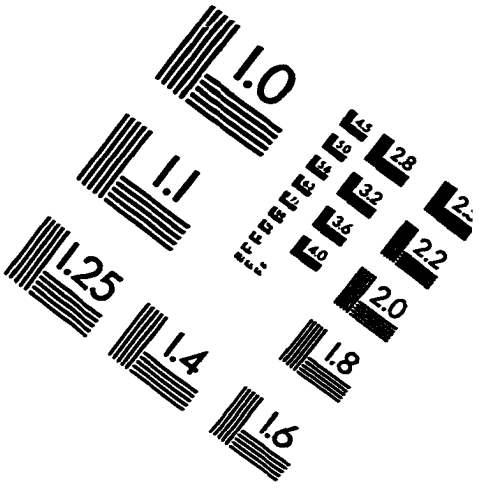
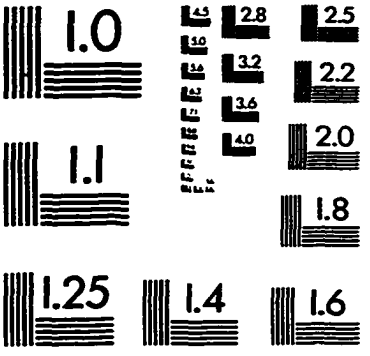
Aendix 1. Discharge of priority pollutants and wastewater characteristics for the Kimberly-Clarke mill for 1990, 1994 and 1995 (RAP 1991; OMOEE 1996).

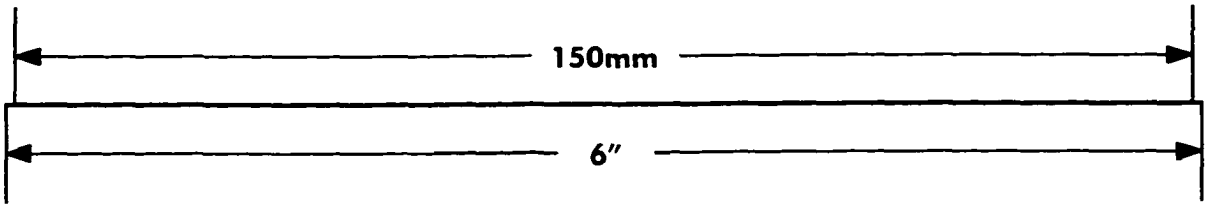
Parameter	1990	1994	1995
Average Effluent Loading			
Flow (m^3/d)	94,900	102,275	107,753
BOD_5 (t/d)	1,400	2.8	2.7
Suspended Solids (t/d)	4,100	4.1	4.9
Adsorbable Organic Halides (AOX) (kg/d)	1942	1286	1399
Toxicity	non-lethal	non-lethal	non-lethal
Chlorinated Organics	(pg/g)		
Total Tetrachloro-Dioxin (TCDD)	180	N/A	1
Total Tetrachloro-Furan (TCDF)	360	N/A	77
Total Pentachloro-Furan (TCDF)	6000	N/A	22
Total Heptachloro-Dioxin	6000	N/A	2
Metals (mg/L)			
Aluminium	0.465	N/A	0.643
Cadmium	0.0005	N/A	0.001
Chromium	0.1633	N/A	0.324
Copper	0.00167	N/A	0.00195
Lead	0.005	N/A	0.0651
Nickel	0.01333	N/A	0.002
Zinc	0.08654	N/A	0.0938
Phenols (ng/L)			
Phenol	N/A	N/A	0.2

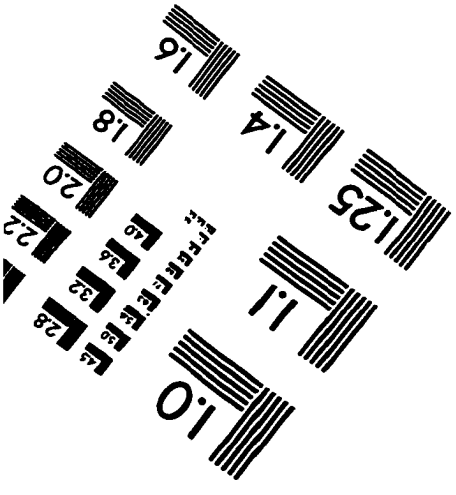
IMAGE EVALUATION
TEST TARGET (QA-3)

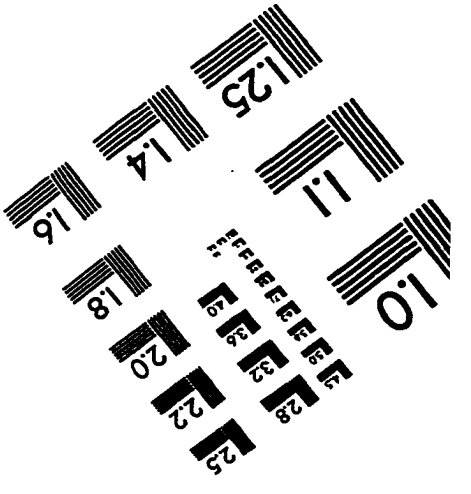












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