DIAGENESIS AND TRANSFORMATION OF AQUATIC DISSOLVED ORGANIC MATTER IN NOVA SCOTIA FRESHWATERS

By

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"All this science, I don't understand

it's just my job five days a week"

John, E. and B. Taupin (1972) Rocket Man. MCA Records.

ABSTRACT

The acid-base and structural chemistry of freshwater dissolved organic matter (DOM) is known to vary with time and location. The purpose of this dissertation is to show how the chemical quality of DOM in temperate streams, wetlands and lakes of the Kejimkujik National Park area in central Nova Scotia varies over an annual cycle and how the changes in quality are related to DOM formation and diagenesis.

New techniques were developed and used to better define the chemical quality DOM. A titration method described by Brassard et al. (1990) allowed the description of the acid-base characteristics of DOM. A reverse osmosis method is also described for the concentration of DOM without its fractionation to allow structural determination by ^{13}C NMR of whole material.

The results indicate that the chemical quality of DOM found in freshwaters is not similar to that found in soils. This difference suggests that interactions between soils, biology and hydrology modify the DOM in streams. A laboratory experiment suggests that the most likely pathway of DOM formation is the breakdown of plant structural material into aliphatic material with subsequent aromatic formation via semi-quinone and quinone.

Comparison of incubation experiments with field results also shows that DOM acidity in natural water decreases with time caused by biological and chemical oxidative processes.

Theoretical considerations indicate that the acidity of DOM does not follow the simple relationship suggested by Oliver et al. (1983) because of differences in source material and diagenetic processes, as well as the influence of inorganic cations and anions which until now have been assumed to be uncomplexed with organic matter.

ACKNOWLEDGEMENTS

To complete a work of this scope in such a short time would have been impossible without the help of a large number of friends and colleagues. Permission to take up academic leave with half salary was forced out of the Environment Canada bureaucracy by my Regional Director Steve Fenety. In this, he was ably seconded by Tom Pollock and Doug Cullen of Water Quality Branch, Inland Waters Directorate in Moncton, and Floyd Elder of the National Water Research Institute in Burlington, Ontario. Abbey Ouellet, Ann Morton and Louise Boulter kept my life simple at work. Further funding for the research was obtained over two years from the O'Brien Foundation of New Brunswick, and Mount Allison University of Sackville, N.B.

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The field work was conducted at Kejimkujik National Park. Thanks are

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CHAPTER 1

DISSOLVED ORGANIC MATTER IN FRESHWATER

1.0 Introduction

Dissolved organic matter (DOM) is often an important constituent of freshwaters, as it often contributes large numbers of anionic functional sites. DOM can contribute up to 50% of anions in streams and lakes (Clair and Freedman 1986, Freedman and Clair 1987). Though important, DOM cannot easily be chemically defined making predictions of acid-base interactions, complexation reactions with cations, and structural chemistry difficult to explain.

DOM promotes weathering processes in nature (Cronan 1985). Its presence causes problems in public water supplies as carcinogenic trihalomethanes are formed when brownwaters are chlorinated (Oliver and Visser 1980), as well as in clogging of filtration systems. Freshwater DOM is also an important part of the world's carbon cycle, as it is eventually lost to the ocean floor and forms an important sink for this element (Meybeck 1982) with important implications in global warming. It may also be a growth enhancer to soil (and maybe aquatic) bacteria (Visser 1985). The quality of DOM is also important in establishing ecosystem types in freshwater environments (McArthur and Marzolf 1986, Tranvik and Sieburth 1989).

Because of these ramifications to geology, environment and public health, it is important to understand the variation in chemistry and structure of the aquatic organic matter formed in nature, as well as the physical, chemical and biological factors which modify it.

In this dissertation, I attempt to better understand two aspects of freshwater DOM: its acid-base and its structural chemistry. The data collected is used to determine: i) what natural variability in DOM can be expected in freshwaters ii) what effect this variability has on solution acid-base chemistry, and iii) how the measured variability can be related to sources of DOM and diagenetic processes.

1.1 What is DOM?

Two kinds of organic matter are usually referred to in aquatic studies: particulate, and dissolved organic matter (POM and DOM). POM is assumed to be composed mostly of incompletely broken down plant matter, and as such maintains the original plant material's chemical structure. It will not be further discussed in this work.

DOM is defined as the organic fraction passing through a 0.45 μm glass-fibre filter (Thurman 1985). It is assumed to originate from the recombination of small organic molecules formed by the biological and chemical breakdown of plant material in soils and water, as well as from phytoplankton excretions. Dissolved organic carbon (DOC) will be used to describe the result of the analytical technique which estimates total carbon content. To further complicate the picture, DOM is also called "humic

material", as it is assumed to originate in the soil humus layer. Though this term will not be used in this thesis, it is commonly used in the literature.

1.2 Formation, structure and transport of DOM

Transport of DOM from soils to water courses has not been extensively studied, but is thought to be due mostly to runoff and water movement in soils. Clair and Freedman (1986), and Lazerte and Dillon (1984) show that most carbon export from temperate basins occurs during high flow periods, which are also times when biological activity is lower than usual. This fact suggests that DOM is generated in soils during the late spring to early fall when runoff is minimal, and then is later transported into water courses during rain and snowmelt events.

The structure and composition of the DOM transported to freshwaters is poorly understood. It has been described as being composed of macromolecules formed by polymerization reactions of smaller molecules produced by the biological degradation or autolysis of plant cells (Vandenbroucke et al. 1985). The macromolecules range in molecular weight from 0.1 to several hundred kilodaltons (Cole et al. 1984, Wershaw and Aiken 1985).

Four main theories of DOM formation have gained a certain amount of support over the years, and these have been summarized by Stevenson (1982):

i) incompletely broken down lignin,

ii) phenolic aldehyde formation from lignin,

iii) phenolic aldehyde formation from non-lignin material,

iv) sugar-amine condensation.

The lignin theory is based on the hypothesis that the nitrogen content of humic substances is due to the condensation of amino acids produced by microbial metabolism of plant lignin. As lignin is an aromatic polymer, partial breakdown into smaller molecules exposes sites which complex with extracellular amino acids, and thus form reactive complexes. As lignin breakdown continues, more humic substances should be produced.

Hypothesis ii is superficially similar to the lignin theory, but assumes a complete breakdown of lignin to form unstable polyphenols which convert to quinones, which then combine with amino acids, reforming small humic molecules. The third theory is similar to the second, except that the material forming the phenols is aliphatic. In both cases, it is understood that the newly formed humic molecules will complex with each other at free radical sites to form ever larger molecules.

The sugar-amine condensation theory involves the addition of an amine to the aldehyde group of a sugar molecule to form a Schiff base, which eventually forms aromatic compounds with reactive sites. This is the so-called 'browning' reaction, common in food preparations, eg. beer production, and apple bruising.

Of these potential pathways, the second is thought to be the most important, as it is a reaction known to occur under a range of conditions,

unlike the sugar-amine reaction (Stevenson 1982). Lignin is the most common organic compound in most soils, and therefore its breakdown to form polyphenols is thought to be more likely. Direct lignin breakdown into humic substances has lost favour in recent years, as humic substances are formed in environments low in lignin, such as moss bogs. The direct lignin mechanism is thought to be possible under specific conditions however.

The chemical structure of DOM is still a matter of controversy. Initial models for soil humic material (Schnitzer and Khan 1972) hypothesized that it was composed principally of aromatic rings. Stuermer and Payne (1978) who examined DOM derived from marine waters, produced a model suggesting a structure with a greater carbohydrate fraction than that postulated by Schnitzer and Khan (1972). As more information becomes available, it appears that there is no unique structure for DOM, but that it may span a number of potential arrangements which include in varying proportions, aromatic, aliphatic, carbonyl, carboxyl and phenolic groups. The challenge to scientists in the field of natural organic acids has thus changed from elucidating what the structure of humic compounds is, to trying to understand what structural differences can be ascribed to different environments of formation and to subsequent diagenesis.

Part of the reason determination of structure has been difficult is that "to measure a process or a state of matter is to disturb it". This is especially true in the study of aquatic DOM. In soil and water literature, two terms are commonly used: fulvic and humic acids (the latter is different from humic "material"). Humic acids are the fraction of the DOM precipitated at pH 2, while fulvic acids are the fraction not precipitated, but removed from solution

using an anion adsorption column (Lenheer 1981). DOM can further be fractionated by exchanging the liquid going through the anion columns with a cation exchange resin to remove the positively charged material (Bourbonniere 1989). Though this method has been widely employed, it's application in water is of limited utility, as the various separated fractions may take on properties related to the separation procedure. Because of this objection, whole unfractionated aquatic material was used here. This approach insured that the information gathered explained the behaviour of the total DOM and not simply some operationally defined fraction.

Physical separation techniques are more likely to avoid the pitfalls of DOM fractionation, as they theoretically produce concentrates quite similar to the original material in solution. Until recently however, physical separation methods have been too time consuming for use on larger volumes of water. In addition, Buffle et al. (1978) showed that some physical separation methods were influenced by electrostatic effects at membrane surfaces.

The development of reverse osmosis membrane technologies has allowed a new approach to sample concentration. Its use is more acceptable than other filtration methods, as new types of membranes allow better retention of DOM with reduced membrane surface charge effects and at more manageable cost. RO differs from ultrafiltration (UF), described by Spiteller (1987) and Buffle et al. (1978), in that the nominal filtration membrane pore sizes are smaller; thus the osmotic pressure difference between concentrate and filtrate necessitates higher filtration pressures. The theory and use of RO have been well documented (e.g. Sourirajan, 1981), allowing users of the technology to optimize both operational procedures and membrane selection,

depending upon specific need.

A number of studies have documented the use of RO in removing organic contaminants from freshwater sources. Deinzer et al. (1975), Reinhard et al. (1986), Baier et al. (1987) and Lynch and Smith (1987), using various types of membranes, reported removal efficiencies of model organic compounds ranging from 67 to 99% depending upon the specific compound and membrane type. Odegaard and Koottatep (1982) and Reinhard et al. (1986) have also shown total organic carbon removal efficiencies ranging from 80 to 100% in the former study and 89 to 99% in the latter, again depending on the membrane selected and operating conditions. Lynch and Smith (1987) found that conventional cellulose acetate membranes poorly recovered a number of model compounds, suggesting inadequate retention, as well as adsorption of the contaminants on the membrane itself. They found however, that polysulfonate membranes composed of more highly crosslinked material, were less likely to allow passage of molecules above the nominal pore size, and that DOM was also less likely to adsorb on the membrane surface. Moreover, RO allows the processing of relatively large volumes of sample, so that more sample can be recovered with a minimum of effort. Disadvantages of the method may include the potential for interaction between the concentrate and the membrane, and the retention of inorganic ions which may have to be removed by ion exchange, depending on the use that is made of the concentrate. On the whole, I decided that the potential advantages outweighed the disadvantages, especially in comparison to the adsorption chromatography method, so that I chose to assess RO for the concentration of aquatic DOM from sample streams.

There are few studies which characterize seasonal and environmental changes of DOM in freshwater systems. Part of the reason for this is not only the difficulty inherent in its collection and concentration, but also the lack of commonly accepted standard methods for structural analysis. Recent advances in nmr spectroscopy technology (Preston and Blackwell 1985, Schnitzer and Preston 1986) now permit more accurate structural definition. Thus, a main goal of this work was to describe changes in DOM structural chemistry from different hydrosystems over a seasonal cycle to see if patterns of change emerged which could be related to its formation and diagenesis.

1.3 Acid-base Chemistry of DOM and its measurement

Because of the presence of exposed carboxylic and phenolic acid sites on DOM, it is negatively charged, though the presence of amino acids at much lesser concentrations also provide positively charged sites. Characterizing the acid-base chemistry of DOM in natural solutions is difficult due to the DOM's weak polyprotic nature, and due to the chemically complicated solutions in which they are found. Because of these difficulties, the techniques used in the past to quantify its acid-base chemistry were subject to poorly understood interferences. Moreover, conceptual problems in describing the acid-base chemistry of macromolecules have been inadequately addressed until now, making analysis difficult.

During the last twenty years, theoretical advances have allowed a better understanding of the functional group composition of natural organic acids, allowing the development of more sophisticated analytical techniques for their

description. Studies by Gamble (1970, 1972), Perdue et al. (1980,1984), Marinsky and Ephraim (1986), Ephraim et al. (1986), Buffle (1988) and Altmann and Buffle (1988) have developed new frameworks modifying the way we look at natural organic acids, and have allowed the development of laboratory techniques for their accurate description.

The first work successfully describing the acid-base characterization of DOM in natural waters (as opposed to purified samples) was probably by Kramer and Davies (1988) based on an approach developed by Tobler and Engels (1983). Using sample titration data from the U.S. Environmental Protection Agency Eastern Lakes Survey, they calculated total concentration-pK_a affinity spectra for a number of lake samples. By measuring the dissolved inorganic carbon (DIC) contribution and pH, they then calculated the bicarbonate and carbonate contributions to the solutions, and by difference measured the organic anion site distribution.

Their approach was based on the assumption that the organic acid sites could be treated as a mixture of monoprotic acids, an assumption which Perdue (1985) disagreed with as being suitable for describing the proton binding capacity of humus because of electrostatic effects. However, work by Dzombak et al. (1986) seems to show that for metals at least (and most likely for protons), either the continuous distribution approach favoured by Perdue et al. (1984), or the discrete affinity spectrum method used by Kramer and Davies (1986) is suitable, depending on the objectives of the study, and the method of analysis.

There were a number of problems with the Kramer and Davies method which first had to be dealt with. First, the titration data had to be of

sufficient quality to allow consistent interpretation to be done. They rejected approximately half the samples they studied due to erratic data. Secondly, they fitted the titration curve data to produce affinity spectra by iteration, which is slow and inefficient. Kramer and Brassard (1989) and Brassard et al. (1990) refined the initial approach in a number of ways which allowed consistent results useful description of the physical chemistry of DOM.

Because of the difficuities inherent in titration techniques alluded to above, a number of approaches have been developed to indirectly estimate organic acidity of water samples. In order to calculate the carboxylic acid content of DOM, Oliver et al (1983) isolated and purified the fulvic acid fraction from total water DOM from two sites, and then carefully titrated the extract to measure the reactive acid sites. They then back calculated the contribution of these acid sites to the reconstituted DOM. They calculated the carboxylic content of "typical" aquatic DOC to be approx. 10 μeq of carboxylic acid sites per milligram of carbon. They also developed a way of calculating a mass action dissociation constant which allowed an estimation of the dissociated acid fraction. There are a number of shortcomings to this method. The most important one was explored by P. Takats (McMaster Univ. pers. com. 1990) who showed that by separating the DOM into fulvic and humic acid fractions, a number of acid functional sites which are not normally available for proton exchange, become so after fractionation. This has the effect of causing an overestimation of the total carboxylic acidity of DOM, and thus its contribution to the solution acidity. Because of the use of purified material for their titrations, Howell and Pollock (1986) calculated that the carboxylic acid values estimated by the Oliver et al. (1983) method could be

50 percent greater than actual site concentrations.

Another technique used to calculate organic anion concentration is by difference, the so-called anion deficit approach (Driscoll et al. 1989). This involves calculating a charge balance from all measurable cations, including Al which is corrected for the fraction complexed and acid anions. The difference between the positively and negatively charged ions, which in brownwaters always shows an anion deficit, is assumed to be due to the carboxylic acid sites on DOM. Driscoll et al. (1989) calculated the organic acid contribution for a large number of samples to be roughly equal to that calculated using the Oliver et al. (1983) method. Though outwardly simple, and corroborative of another method, use of this approach is always questionable due to the complexation of DOM with metals which is not measured by most analytical methods used to obtain the ion balance parameters. For example, Clair and Komadina (1984) who used the Chelex-100 speciation method (Campbell et al. 1983), showed that all Al in 19 samples from six Nova Scotia streams was complexed with DOC, as opposed to the results of Driscoll et al. who measured a labile Al fraction, even in highly organic waters. Moreover, Clair and Freedman (1986) showed significant correlations between DOC and Ca, Mg, Fe, Mn, and Al in water collected weekly over 3 to 5 years from four sites in Nova Scotia. Though not proof of complexation, these data suggested that the base cations were at least partially associated with the organic matter, reducing the number of low Pk_a sites available for proton exchange, and thus acid buffering. Perhaps more importantly, the technique is subject to problems of incomplete definition of metal species and potential multiple analytical errors, especially in waters low in dissolved salts.

1.4 Thesis Objective.

From indirect and direct studies, it could be deduced that the structure and chemistry of natural organic acids varied from site to site, and within sites, at different times of the year. In this thesis, I a) describe an improved method for concentrating DOM from freshwaters to allow its structural determination; b) describe the changes which were measured in the acid-base and structural chemistry of DOM from four aquatic environments; c) report on results from two laboratory experiments designed to explain how these differences occurred, i.e., which formation theory described above is most likely to be operating; d) based on field and laboratory results, I explain potential diagenetic pathways of DOM once it has reached freshwater bodies; and e) I show how organic and mineral acids interact in natural solutions. This information was then taken into account to construct an electroneutrality model used to predict how increases or decreases in acid precipitation change the overall acidity of surface waters high in DOM.

CHAPTER 2

FIELD AND EXPERIMENTAL METHODS

2.0 Introduction

The Kejimkujik National Park area of south central Nova Scotia has been the focus of Environment Canada's acid precipitation research in Atlantic Canada since the late 1970's. The special emphasis of the work done there was and is still, to understand the chemistry and biology of anthropogenic acidification in aquatic environments already impacted by naturally organic material. As much information had already been accumulated on environmental conditions in this area (see Kerekes 1989), I thus decided to pursue research on the formation and diagenesis of DOM in the Kejimkujik National Park region where excellent research facilities were available. Appendix A describes the geography, physiography, and geology of the area.

2.1 Site Selection

Soils in this region are very shallow which allows much of precipitation in the area to leave the basins as runoff. Moreover, drainage conditions are often poor, resulting in high incidences of wet and boggy soils. Because of these conditions, surface waters are generally high in DOM with values

ranging from 5 to 40 mg L^{-1} (Kerekes et al. 1984, Clair and Freedman 1986, Freedman and Clair 1987, Howell 1989). Figure 2.1 shows the four sites chosen to provide conditions where DOM quality and quantity should be sufficiently different from each other to provide contrasting results. This variability allowed the assessment of different environmental conditions on DOM chemistry. The sites chosen were: Moose Pit Brook, a first order stream draining a mainly forested area, a treed mire (submerged bog) in the Moose Pit Brook basin, the Mersey River at Mill Falls, a fourth order stream, and Tupper Lake, a small shallow lake into which emptied Moose Pit Brook and other similar streams. Their selection was also based on the fact that they presented a continuum of environments of DOM formation and modification, as shown in Figure 2.1. For example, the treed mire or bog represented a typical DOM generating site, Moose Pit Brook represented the first order stream which emptied into a lake (in this case, Tupper Lake), and the Mersey River was selected to show a site where all basin DOM modification effects were integrated. Figure 2.2 shows the rationale for site selection.

Approximately 10% of the Moose Pit Brook basin area is covered by Sphagnum bogs, along with White Spruce (Picea glauca), Balsam Fir (Abies balsamea), Hemlock (Tsuiga canadensis) and White Pine (Pinus strobus) (DeGraeve and Peterson 1982). At the sampling site which is located at on a woodlands road, the basin area is approximately 16.7 km^2 and has a mean annual water discharge of 9,600 m^3ha^{-1} yr^{-1} (Freedman et al. 1985).

In the Moose Pit Brook basin, a treed mire, a type of shallow bog with open water, was chosen to represent a wetland where DOM formation occurred. Partly created by the building of a logging road through a

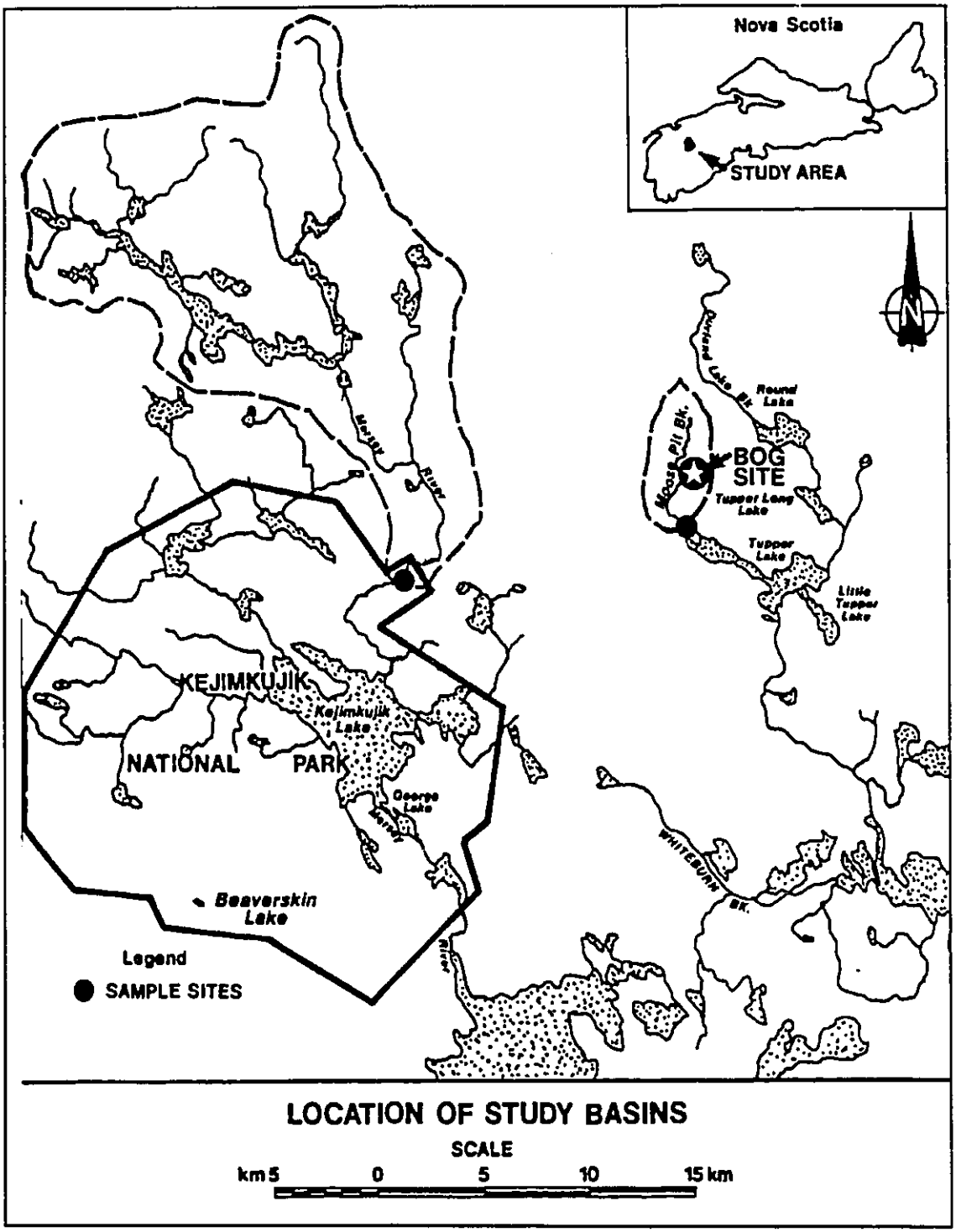

Figure 2.1 Location of field study sites. The Mersey River station is represented by the point at the Park Boundary, the Moose Pit Brook site is the point below the Bog site, and above Tupper Lake.

depression, it extended over a two or three hectare area. No water inlets or outlets were seen around the site, so it was probably fed by runoff and direct precipitation. The mire was surrounded by Sweet Gale shrubs (Myrica gale) and Sphagnum mosses and contained a large number of standing or fallen dead Spruce trees. No previous water chemistry data existed for the location, but as it was easily reached in even the worst of weather, and as it seemed to be a typical environment in the basin, it was chosen for sampling.

The Mersey River was the third site selected. It was a fourth order stream, and was seen to represent a hydrological condition somewhat between a first order stream and a lake. The site is located approximately 10 km from Moose Pit Brook and its basin is similar in both geology, geography and vegetation. Its basin covers 295 km^2 and on average, exports 8,300 m^3 ha^{-1} yr^{-1} of water (Freedman et al. 1985).

The fourth site chosen was Tupper Lake. Lakes are thought to contain DOM different from that found in streams because autochthonous DOM production by plankton should produce compounds different from those originating in soils (Hessen et al. 1990). Tupper Lake receives its water from Moose Pit Brook and a number of similar streams which drain approximately 150 km^2 of wooded and bog area. Again, there was no previous information on the site chemistry or bathometry, though the lake itself is relatively shallow. In southwest Nova Scotia, a number of similar glacial scour lakes exist (Kerekes and Schwinghamer 1973), and as the site was located near Moose Pit Brook, it was deemed to be representative of local conditions, and thus a reasonable site to select.

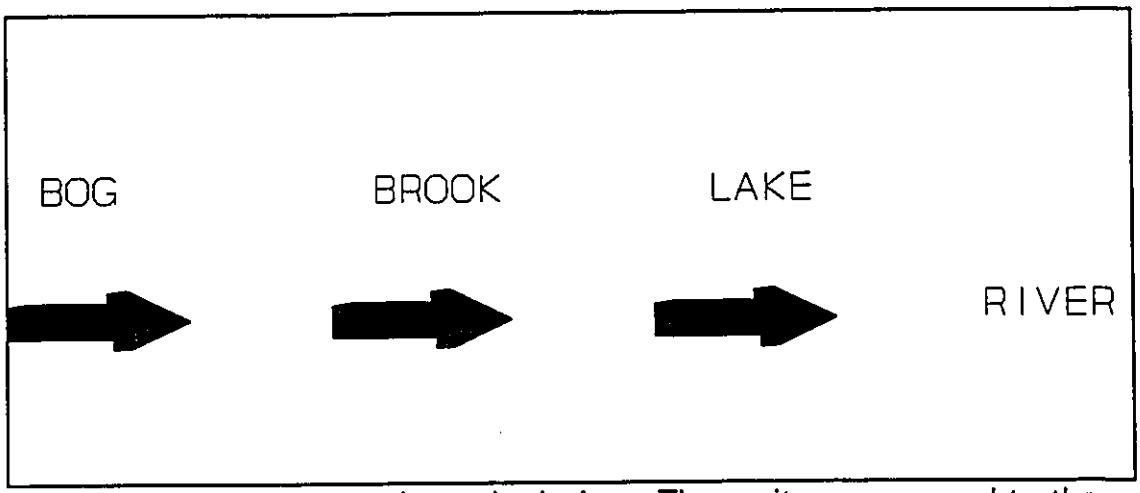

Figure 2.2 Site sequence in study design. These sites correspond to those identified in Figure 2.1.

The sampling times for this study were chosen based on the region's hydrological cycle. The main physical factors involved in the modification of DOM quantity and quality in freshwaters are temperature which controls its biological formation and modification, and hydrology which controls the flow of DOM out of soils into surface waters. In southwest Nova Scotia, DOM concentrations are highest in the summer at low water flow periods, and lowest in the winter-early spring when river discharge is at its maximum (Clair and Freedman 1986). Figure 2.3 shows the mean monthly discharge curves over a period of years for the Mersey River, the Westfield River at the mouth of Tupper Lake, and Moose Pit Brook at its mouth (Water Resources Branch, Environment Canada, Data Summary) which I used to determine sampling times. The triangles in the figure indicate the times selected for sampling. These were: a) in the early winter, when soil and water biological activity as well as runoff are low, b) in the late winter - early spring, when water runoff is high and biological activity still low, c) in the late spring, and d) mid summer

when DOM is being modified by chemical and biological processes under low runoff conditions, and e) in the mid to late fall, when runoff increases again, and modification of DOM by biological and chemical processes is reduced.

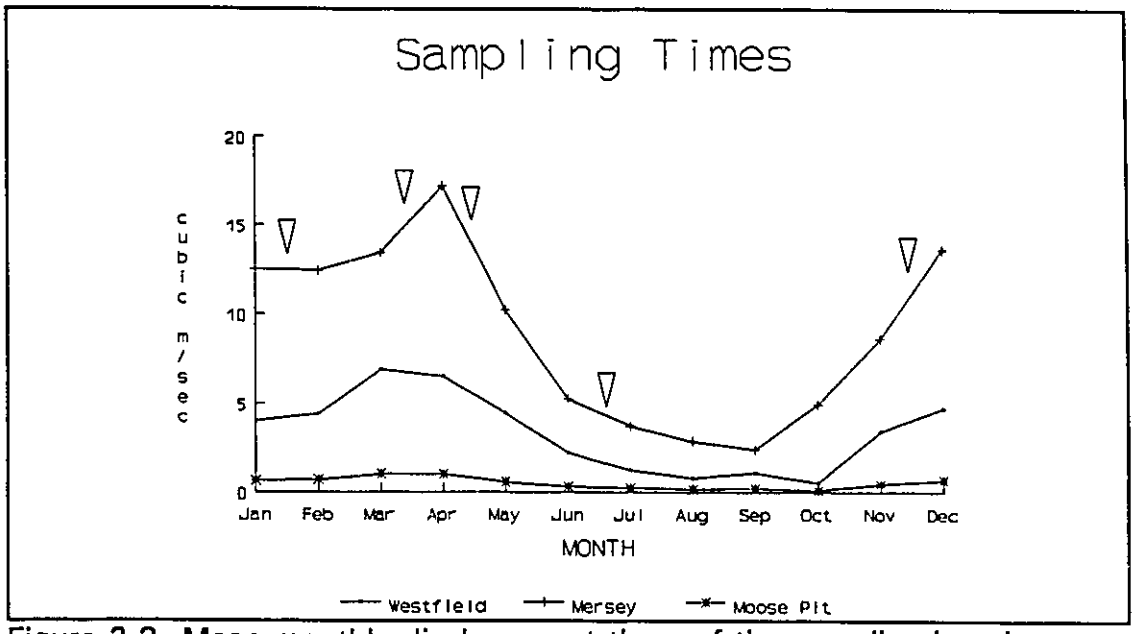

Figure 2.3 Mean monthly discharges at three of the sampling locations. Arrows show selected sampling times.

2.2 Field Sampling Procedure

At each sampling time, a 1 L sample of stream water was collected per station in an acid washed, triple rinsed polyethylene bottle. The bottles were sent to McMaster University, where the water was analyzed for calcium, magnesium, potassium by atomic adsorption, sulfate and chloride by ion chromatography (IC), pH potentiometrically, and DOC and dissolved inorganic carbon (DIC) with a Dohrmann carbon analyzer. Every sampling triplicate samples were collected at one site. The sample was also acid and base titrated to measure its acid-base characteristics (see Chapter 3).

Usually, analyses were done within one month of collection, though some samples could only be done more than two months after collection. One of the repercussions of the lag time was that some organic material was seen to have precipitated in a few of the sample bottles. In order to attempt to redissolve the organic matter, the samples were shaken hard before decanting into the titration vessel, and base titrated first, in order to dissolve the precipitate. It is not possible to know if redissolution occurred, but pH values of 11 or greater are known to dissolve humic matter from soils.

To get an idea of data reproducibility, triplicate samples were collected from sites chosen at random during the March, June and November samplings. The values were compared with each other to provide some idea of the natural and analytical variability expected from the samples.

Also at each sampling, 250 L of stream water were collected in clean polyethylene carboys, and transported immediately to a laboratory at the National Park, where DOM was concentrated within 12 hours using RO (see Chapter 3). This procedure was carried out for all sites visited, except for the two winter samplings, when the treed mire water was frozen.

2.3 Measurement of Biological Activity in Water

One of the most likely causes of DOM modification is biological activity in both soils and water. The detailed study of microbial populations in both of these media is beyond the scope of this study, but an attempt to quantify biological activity of water in the field and laboratory was made in order to try to relate this activity to changes measured in the chemistry of DOM. The

main objective of this portion of the study was then to see if specific enzymes could be correlated to measured changes in DOM chemistry.

The measurement of biological activity in soils is a field of research requiring equipment and techniques not easily available. Soil enzymes have been studied over the last few years (Skujins 1978), but data interpretation is still in a primitive stage, is oriented towards agricultural applications, and is itself a very fertile source of future discoveries best left to others. The measurement of biological activity in water on the other hand, is somewhat more straightforward due to the lower level of complexity of the medium. The most widely used method in limnology involves the use of oxygen measurements but the technique is more applicable to pollution and high biological productivity situations (Warren 1971), and is not very sensitive in oligotrophic environments. Moreover, the technique is not specific, and cannot describe the types of biological reactions occurring in the solution.

In order to properly measure the effects of aquatic bacteria and fungi on DOM, a new technique adapted from medical technology was used which involved the measurement of enzyme activity. As enzymes can often be related to specific metabolic processes, I thought that DOM modification pathways could be identified by knowing which enzymes were the most prevalent. The technique used involves using the semi - quantitative API ZYM® system (Analytab Prod., Plainview, N.Y.) used to scan a series of 19 different enzymes. The test system consists of strips with a series of microcupules containing dehydrated chromogenic enzyme substrates (Table 2.1). The principles involved in producing the reactions are described in the Analytab procedures (1979). The presence of specific enzymes in the tested

water causes a colour reaction whose intensity is related to a standard chart for comparison. A number of these enzymes are medically related and should not appear in freshwater, but several others are produced during all types of biologically mediated reactions, and should be found in water, even if in small quantities.

Table 2.1 Enzymes measured using the API ZYM method.

Enzyme number	Description
1	Control
2	Alkaline phosphatase
3	Esterase (C4)
4	Esterase lipase (C8)
5	Lipase (C14)
6	Leucine amino-peptidase
7	Valine amino-peptidase
8	Cystine amino-peptidase
9	Trypsin
10	Chymotropsin
11	Acid phosphatase
12	Phospho-hydrolase
13	α-galactosidase
14	β-galactosidase
15	β-glucoronidase
16	α-glucosidase
17	β-glucosidase
18	N-acetyl-β-gluco-aminidase
19	α-mannosidase
20	α-fucosidase

In practice, the procedure involves placing 2-3 mL of water into each microcupule of the test strip, and incubating for four hours at 35°C. At the end of this period, one drop of two proprietary reagents are added to each microcupule, and the strips are exposed to light for five minutes. The colour reaction registered is compared to a colour chart and a numeric value is

assigned. The numeric values are calibrated by the manufacturer to enzyme concentration levels. A colour value of 1 indicated 5 nanomoles; colour 2, 10 nanomoles; colour 3, 20; colour 4, 30; and colour 5, 40. The test was used on over 40 samples over the course of the research without discernable problems. In the field, it was difficult maintaining proper incubation temperatures, but a covered heating pad, monitored consistently with a thermometer seemed to provide a suitable environment for the incubation to proceed.

2.4 Parent Material Incubation

In June 1989, samples of fallen black spruce (Picea mariana) branches and Sphagnum mosses were collected near the treed mire site and placed into four 125 L plastic barrels (two barrels per substrate type). The barrels were filled with well water and the substrates allowed to soak for 3.5 months in the dark, to discourage algal growth. The water was stirred several times a week for the duration of the study. Black spruce and moss were chosen as they are both common in the study basins and because Sphagnum mosses are the main component of the wetlands found in the region. Moreover, the composition of both substrates is radically different. Woody plants structural material is mostly composed of lignin, high in aromatic structures. Mosses on the other hand, are mostly composed of carbohydrates. It was thought that pathways of DOM formation could be elucidated by studying the differences produced by such different starting materials. The DOM generated in the barrels was concentrated using RO at the end of the incubation period. The

concentrate was then processed in a similar to the stream samples.

2.5 Acid - Base Laboratory Incubations

I conducted three laboratory experiments with water from Moose Pit Brook in order to determine if changes in the DOM's acid-base chemistry could be detected. The first experiment (Exp. 1), began in November 1987, and consisted of six pump aerated, 10 L aquaria, three of which contained 300 g of 1 mm sieved sediment to a depth of ca 3 mm, and the remaining three without sediment. All aquaria were filled with 8 L of water which was sterilized of bacteria and fungus by filtering through a 0.2 μm glass fibre filter (Jones 1979). Twelve sterilized 1 L Erlenmeyer flasks were filled with 750 Ml of filtered water. Six contained a 2 mm deep layer of autoclaved sediment, while the remaining six were without sediment. The aquaria, but not the flasks were inoculated with unfiltered water to reintroduce heterotrophs and were kept covered to protect against contamination by dust and other debris. All containers were kept dark to suppress autotroph production of organic acids.

The set-up of the second experiment (Exp. 2) was similar to that of the first, except that only three aquaria and six sterile flasks were used, and all microcosms contained sediments. The water used for this experiment was collected in May 1988 from the same site as for the first experiment, but only the water and sediments used in the sterile flasks were 0.2 μm filtered. In both experiments, the aquaria were sampled at the beginning of the study and at roughly monthly intervals for 120 days, and sterile flask samples were

collected 6 to 8 weeks after the start of the experiment and at the end. Collected samples were stored in a dark location at 4°C prior to analysis.

The third experiment (Exp. 3) was conducted differently to correct shortcomings identified in the first two. Water was collected in the field on March 10, 1989, transported to the laboratory, and incubated in 24 clean, distilled water rinsed, 750 Ml Erlenmeyer flasks none of which contained sediment. Eight flasks contained 500 Ml of 0.2 μm sterilized water and the remaining 16 were filled with unfiltered water. Each of these two groups was divided in half, with four of the sterile flasks and eight of the non-sterile wrapped in aluminium foil to exclude light. Thus at the beginning of the experiment, there were four dark and four light sterile flasks, and eight dark and light non-sterile ones. Pairs of non-sterile samples were collected from both the light and dark bottles after 29, 50, 101, and 131 days from the beginning of the incubation, and after 50 and 131 days from the sterile flasks. Each flask was manually shaken every other day to aerate it.

At all sampling times for the three experiments, 500 mL of water were collected and analyzed for the following parameters: potentiometric pH, acid neutralization capacity (ANC) by the Gran method, DIC and DOC using a Dohrmann carbon analyzer, as well as Na, Mg, K, Ca, SO_4, and Cl ions.

During the second experiment at each sampling time, one sample randomly chosen from each of the sterile and non-sterile set-ups was acid and base titrated to produce an affinity spectrum of the DOM (see Chapter 3). For the third experiment, all samples collected were analyzed for their pK_a - concentration spectra. Since duplicate samples were available for each treatment, these were first compared to each other, and if found to be similar,

they were averaged and the results reported as such. When discrepancies occurred between duplicates, these were compared to previous and subsequent samples, with the sample most likely to be incorrect, removed from the data interpretation. The probable cause for deviant samples seemed to be contamination from cleaning materials which were not properly rinsed out, as higher than average pH and ANC values were the only causes of sample rejection.

Initial water, 0.2 μm filtered water, and sediments from both aquaria and sterile flasks were analyzed for enzyme activity in Exp. 1 and Exp. 3. Enzymes were also detached from sediments in Exp. 1 by exposing 15 g of substrate in 15 ml of water to a Sorval sonifier at 50% capacity for 1 min. Biological uptake of oxygen from water and sediments from all containers was measured using a YSI Respirometer during Exp. 1 only. Table 2.2 summarizes the experimental setup for this portion of the work.

Table 2.2 Summary of procedures and analyses for the incubation studies.

<table>
<tr><td colspan="4">Experiment 1</td></tr>
<tr><td colspan="2">Dark, Non-sterile (aquaria)</td><td colspan="2">Dark, Sterile (flasks)</td></tr>
<tr><td>3 with sed.</td><td>3 no sediments</td><td>6 with sed.</td><td>6 no sed.</td></tr>
<tr><td colspan="2">Analyses:
Enzymes, pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 5 times (incl.initial)
aerated by bubbling</td><td colspan="2">Analyses:
Enzymes, pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 3 times (incl.initial)
aerated by shaking</td></tr>
<tr><td colspan="4">Experiment 2</td></tr>
<tr><td colspan="2">3 Dark Aquaria with sed.

Analyses: Affinity spectrum
pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 5 times (incl.initial)
aerated by bubbling</td><td colspan="2">6 Dark Flasks with sed.

Analyses: Affinity spectrum
pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 3 times (incl.initial)
aerated by shaking</td></tr>
<tr><td colspan="4">Experiment 3</td></tr>
<tr><td colspan="2">Non-sterile</td><td colspan="2">Sterile</td></tr>
<tr><td>8 light</td><td>8 dark</td><td>4 light</td><td>4 dark</td></tr>
<tr><td colspan="2">Analyses:
Affinity spectrum, Enzymes
pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 5 times (incl.initial)
aerated by shaking</td><td colspan="2">Analyses:
Affinity spectrum, Enzymes
pH, ANC, DIC,DOC,Na, Mg, K, Ca, SO_4^{-2}, Cl
Sampled 3 times (incl.initial)
aerated by shaking</td></tr>
</table>

2.6 Summary of Field and Experimental Procedures

The sampling strategy for this study was to provide both spatial and temporal data on the chemical quality of DOM from southwest Nova Scotia. Sites and sampling times were selected to provide a logical progression of DOM formation and modification in an area over a seasonal period. Methods were then elaborated to produce the major ion and acid-base chemistry of the study sites as accurately as possible. Quality control checks and procedures were also set in place to avoid spurious results due to contamination or poor analyses.

In order to better understand the results and causes of plant material breakdown which is responsible for the generation of DOM, I set up a study in a field laboratory to produce DOM from "typical" starting material, and used an enzyme detection technique to assess the intensity of biological activity in the waters being tested.

I studied the change in acid-base characteristics of DOM with time by incubating stream water under laboratory conditions. Samples were collected and analyzed for changes in major ion and DOM characteristics with time from three separate experiments.

CHAPTER 3

DOM CHARACTERIZATION METHODS

3.0 Introduction

A number of laboratory analytical techniques were used repeatedly over the course of this work to characterize DOM. The purpose of this Chapter is to describe the main methods which I used in this task. These are: a) the titration technique used to describe the acid-base character of the freshwater solutions studied b) the technique used for the concentration of DOM for its structural characterization and c) the use of ^{13}C Nuclear Magnetic Resonance (nmr) spectroscopic technique to elaborate the chemical structure of the DOM. Though described here, further explanations and analysis of the methods are also given in the chapters following.

3.1 Acid-Base Analysis of Freshwaters

3.1.1 Affinity Spectra

The development of the affinity spectrum method by Brassard et al. (1990) marked an easier way of measuring ligand concentrations of DOM. The method can deal with low ligand concentrations and is relatively non-destructive in that it does not require a preconcentration process which usually

modifies the acid-base properties of the material being studied. This study is the first which uses such a method in-depth, to directly measure the acidity of DOM in stream water.

The general theory and methods used for the affinity spectrum calculations described in this work have been described by Kramer et al. (1989) and Brassard et al. (1990). Collected samples are titrated in a closed system to allow for constant CO_2 concentration in the solution, with total dissolved salt concentration increased to 0.04 N by the addition of KCl for constant ionic strength. Thus the acid pK's generated are conditional constants. Samples were titrated by the addition of base (NaOH) or acid (HCl) with an ultraprecise microprocessor controlled microburette, at 0.05 pH steps. Both the titrants and pH probe were calibrated using the linear part of the titration curve. The sample is titrated while in a shielded, constant temperature bath at 25° C. The completed titration lasted approximately two hours. Additions were made at 5 minute intervals.

The titrant volume and solution pH values were recorded with a microprocessor. The data collected were processed to calculate acid neutralization capacity (ANC) using the Gran approach (Gran 1952), and the concentration - pK_a affinity spectrum titration data, using a linear analysis technique. This is a mathematical approach using a series of linear equations to optimize parameter values (in this case, pK_a concentrations) producing ANC values which are measured at each step of the titration.

Although the theory is documented, the actual application of the method to samples is complex. Initially, samples were acid titrated to a pH of 2.5, ANC was calculated, and then they were base titrated to pH 11. ANC

was recalculated with the base titration data, and it was found that the two values, which should have been the same, were often different because of DOM precipitation during the acid titration. The precipitated material did not immediately redissolve during the base titration, which changed the acid-base characteristics of the sample.

The titration approach was modified so that all samples were first base titrated to pH 11. This allowed for the redissolution of most DOM. Then, the sample was acid titrated to pH 3.0, and the affinity spectrum and ANC calculated with this curve. This modification better reflects the natural condition, as no DOM coagulation occurs. In general however, acid/base or base/acid titrations although carried out over long periods of time are not reversible.

Dissolved organic and inorganic carbon (DOC, DIC) were measured at the time of titration using a Dohrmann carbon analyzer. The DIC values were used with pH to calculate sample HCO_3^- and CO_3^{-2} and integrated in the affinity spectrum approach to allow the removal of the carbonato fraction of the spectrum. What is left is the affinity spectrum of all non-carbonate ligands in solution. As phosphorus and silica are at low concentrations in the waters analyzed, and DOC is high, it can be assumed that the resulting acid/base data can be interpreted for DOM only.

3.1.2 Indirect Method

In order to resolve the difficulties inherent in calculating anion deficits, I used a simple chemical equilibrium approach to estimate effective organic acid protolyte concentration in freshwater samples. One way around the

problem of measuring ionic cations and anions in solution, is to ignore direct calculation of ion balances and to apply the electroneutrality principle elaborated by Stumm and Morgan (1981) to samples which have passed rigorous quality control procedures. To start from ion balance considerations, we must expect:

$$[ANC] = [HCO_3^-] + 2[CO_3^{-2}] + [Or^-] + [OH^-] - [H^+] \quad (1)$$

where [ANC], the sample acid neutralization capacity is measured using the Gran Titration method, while $[Or^-]$ is the mean sample organic anion protolyte concentration. As a first approximation, no other ligand is considered, as Al and Fe oxides are assumed bound to the organic matter, and silicic acid which has a pK_a of approximately 9.5, does not significantly contribute to acid buffering of the types of freshwaters in question.

$$[HCO_3^-] + 2[CO_3^{-2}] = [DIC]^*(\alpha_1 + 2\alpha_2) \quad (2)$$

where α_1 and $2\alpha_2$ are the ionization fractions of bicarbonate and carbonate respectively, and [DIC] is the dissolved inorganic carbon concentration (Stumm and Morgan 1981). The ionization fractions of bicarbonate and carbonate are calculated using the following equations:

$$\alpha_1 = ([H^+]/K_1 + 1 + K_2/[H^+])^{-1} \quad (3a)$$

$$\alpha_2 = ([H^+]^2/K_1K_2 + [H^+]/K_2 + 1)^{-1} \quad (3b)$$

where K_1 and K_2 are the equilibrium constants for carbonic acid-bicarbonate, at temperature and ionic strength conditions. [ANC], [DIC] and $[H^+]$ can be measured. $[OH^-]$ is equal to $[H^+]/K_w$, where K_w, the dissociation constant for water is approximately equal to 10^{-14}. $[Or^-]$, the organic anion concentration which can react with hydrogen is then calculated by difference using Equation 1. $[Or^-]$ in turn, is a function of the total number of organic anion protolyte sites uncomplexed by metals or other ligands ($[C_{or}]$) at the reference pH and the mean sample organic acid dissociation constant.

$$[Or^-] = [C_{or}] * \alpha_{or} \quad (4)$$

and,

$$\alpha_{or} = \overline{K}_{or}/(\overline{K}_{or} + [H^+]) \quad (5)$$

assuming a mass action K can be treated as a monoprotic K.
K is calculated using the Oliver et al. (1983) algorithm:

$$pK_{or} = 0.96 + 0.90pH - 0.039(pH)^2 \quad (6)$$

The overall electroneutrality equation can then be stated as:

$$[ANC] = [HCO_3^-] + 2[CO_3^{-2}] + [OH^-] - [H^+] + [C_{or}](\overline{K}_{or}/\overline{K}_{or} + [H^+]) \quad (7).$$

where $[C_{or}](\overline{K}_{or}/\overline{K}_{or} + [H^+])$ calculates Or^-.

The mass action $\overline{K}_{or}$ value in this case is estimated at every titration step using the equation developed by Oliver et al. (1983) and C_{or}, the

concentration of organic protolyte exchange sites, is estimated from Eq. 4. From Eq. 7, it can be seen that changes in mineral acid or base inputs for a one site model, which change [ANC], can be related to changes in water pH.

3.2 DOM Concentration Methods

The Reverse Osmosis (RO) unit used to concentrate DOM from freshwater, was a modified maple sugar concentrator obtained from Les Equipements Lapierre, of St. Georges de Beauce, Québec. It was rated to pump 500 L of liquid*hr^{-1}, using a FILMTEC FT30 ® composite brackish water desalination membrane which had been previously tested by Lynch and Smith (1987). This membrane consists of three layers: a polyester support web, a microporous polysulfone interlayer, and a ultrathin barrier coating on the top surface. Figure 3.1 is a schematic of the complete RO system.

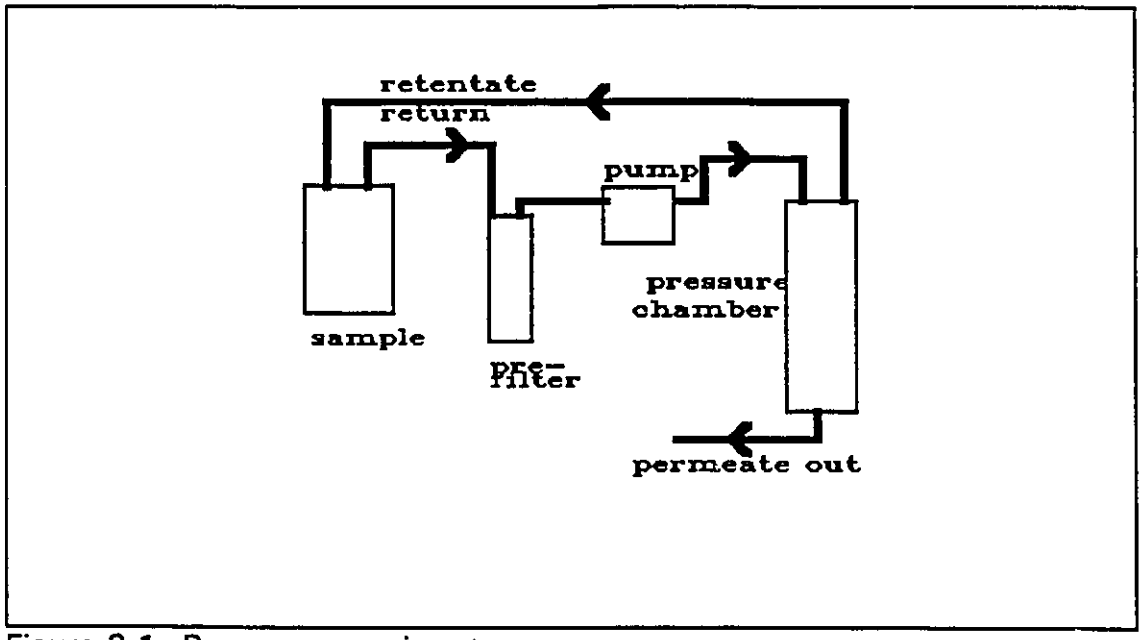

Figure 3.1 Reverse osmosis set-up.

Membrane preparation prior to processing samples involved pumping well water around the membrane for six hours to flush out solvents left by the manufacturing process, and to condition the membrane. It was further cleaned with 1% HCl, followed by a wash with a mild enzyme detergent to remove any organic material left behind during the earlier operation and prior to conducting a sampling program.

Over the course of this study, approximately twenty five 230 L water samples were collected at a number of sites in the Kejimkujik National Park area in southwestern Nova Scotia, Canada. These were transported in clean polyethylene 25 L carboys to a field laboratory where the RO apparatus was set up. Water was pumped from two 125 L plastic storage drums into the RO unit. The water was pre-filtered through a 5.0 μm filter to remove particulate matter and then pressurized by a 5 hp motor drain pump at between 20 and 33 bars pressure into the membrane vessel where a separate pump driven by a small electric motor kept the fluid circulating to reduce fouling. The material rejected by the membrane was returned to the supply tank from where it was recycled into the equipment. This process was continued until the supply tanks were empty and all concentrate was inside the pressure vessel. Four to five L of concentrate were usually produced, resulting in a 45 to 58 fold concentration factor. The concentrate was then drained into clean glass bottles.

Filtrate was collected at the beginning and at the end of the test, with most of the water either being discarded, or kept in a separate tank for later use in cleaning the membrane. The total recovery of DOM was calculated by diluting 25 mL of concentrate into a measured amount of distilled water for

analysis. Typical operation time to process 230 L of sample was between 1.25 to 1.5 hours.

Results of quality control tests (Clair et al. in press) indicated that the membrane retained over 96% of the DOM (measured as DOC) in solution (Table 3.1). The results also show that the membrane performed according to the manufacturer's specifications.

Table 3.1 Raw water, initial and final permeate sample concentrations in mg/L^{-1} and percent removal of DOC from June samples.

Site	Raw water	init. perm.	% rem.	final perm	% rem.
Moose Pit Brook	18.8	0.24	98.7	0.34	98.2
Moose Pit Bog	22.3	0.18	99.3	0.35	98.5
Tupper Lake	8.1	0.11	98.7	0.33	95.9
Mersey Lake	11.1	contaminated	n/a	0.30	97.3
Pebblelog.	9.9	0.19	98.0	0.43	95.6

The results also show that the filtrate DOM was described by affinity spectra suggesting carboxylic acid and mid-range sites. Examination of the membrane suggests that no irreversible liquid-membrane interactions occurred, as the membrane was very clean even after processing over 25 samples.

The liquid concentrate was taken to a laboratory at Mount Allison University, Sackville, N.B. and kept in a cold room at approximately 5°C until it was freeze-dried to form a powder (Malcolm 1976). The powder was stored in the dark, in a dessicating bell until used in analysis. This processing was completed for all samples within two weeks of the field sampling, using a LABCONCO® freeze drier at the Agriculture Canada Atlantic Pathology

Laboratory located on the University campus.

The powder samples were shipped to the Spectroscopy Laboratory at McMaster University and analyzed according to the procedures described in Section 3.3.

3.3 Sample Structural Analysis

3.3.1 Methods and Theory

Atomic nuclei which have odd mass or odd atomic numbers behave as if they were spinning, and thus have a quantized spin momentum. The number of spin states which nuclei have is determined by its nuclear spin number I, which is a constant for each type of nucleus and there are $2I+1$ spin states allowed for each (Pavia et al. 1979). For example, both ^{1}H and ^{13}C have $I = ½$, and thus have 2 spin states for their nuclei, $-½$ and $+½$. As the two spin states correspond to rotation in opposite directions, they generate nuclear magnetic moments of opposite signs equal to each other. But when a magnetic field is applied to these nuclei, all magnetic moments become aligned either with the field or against it. In this condition, the spin state which is aligned with the applied field is of lower energy than the other state, which is opposed, therefore the spin states split into unequally populated energy levels. In other words, the nuclear magnetic resonance phenomenon occurs when nuclei aligned with an applied field are induced to absorb energy and change their spin orientation with respect to the applied field (Pavia et al. 1979). The energy absorbed can be measured and must be equal to the energy difference between the two states involved.

The advantage of nuclear magnetic resonance (nmr) is that not every nucleus of the same element will resonate at the same frequency because of shielding by nearby electrons, and because of the different electronic environments they are found in. The greater the electron density near a nucleus, the greater the shielding, and the lower the frequency of precession. In order to standardize measurements, the resonance frequencies are compared to that of the protons of tetramethylsilane (TMS) whose methyl protons are the most shielded protons known. Values are calculated as:

$$\delta = \text{(shift from TMS in Hertz)/spectrometer freq. in Hertz)}$$

where δ is the chemical shift reported in parts per million (ppm), the spectrometer frequency is a function of the instrument, and the shift from TMS is measured.

The two nuclei of most importance to natural organic acid research are those of hydrogen (^{1}H) and of carbon-13 (^{13}C). Because of their relatively higher concentrations and ability to precess, proton nmr should be a more useful approach than ^{13}C. However, the narrow chemical shift range of the ^{1}H nmr spectrum (1 to 15 ppm), compared to that of ^{13}C (0 to 200 ppm) allows the analysis of the latter to provide more easily interpretable information, even though the sensitivity of the method is not as good as with the former approach. A discussion of the relative advantages and disadvantages of the two methods, as it relates to humic material is provided by Wershaw (1985). For the reason provided above, I decided to subject the samples entirely to ^{13}C nmr analysis.

The major difference found between nmr on humics compared to pure chemicals is the line broadening at the various assigned peak heights which occurs because of different relaxation times of the various carbon atoms. Recent work has tended to clarify and better define types of C producing various resonance effects. For example, Preston et al. (1984) produced information for the selection of optimal delay and acquisition times as well as pulse angles for both solid and liquid state humic samples.

Two approaches to ^{13}C nmr are possible: liquid and solid state, the latter also having two approaches: basic and cross polarization. Comparisons between the two techniques have been done by a number of authors. Schnitzer and Preston (1986) suggested that liquid state analyses provided better definition of the spectrum's carboxylic region than the former. Moreover, problems of quantification are better understood with the liquid state approach.

On the other hand, Schnitzer and Preston's (1986) conclusion was contradicted by Steelink et al. (1989) who reported that the solution state ^{13}C nmr spectra ascribed to aromatic carbon was probably incorrect due to problems in determining optimum pulse delays for defining that region of the spectrum. Moreover, reactive solvents such as NaOD must sometimes be used, which complicate interpretation of spectra (Wilson 1989). There is nevertheless a problem with the basic solid state approach which had to be resolved. This was due to the unequal distribution of electrons in organic molecules which results in different resonant frequencies (chemical shifts) of functional groups, as different orientations of the magnetic field are not averaged (anisotropy) (Wilson 1989). To compensate for this problem, as well

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as to deal with the coupling of ^{13}C and ^{1}H atoms which further complicates spectrum interpretation, magic angle and cross-polarization techniques have been developed (Hatcher and Orem 1986 and Wilson et al. 1983). This technique had been heavily used on solid materials such as coal and a number of interferences occurring with liquid state did not occur.

As there was some question about the relative advantages of the two approaches, both were tried on samples. Liquid state spectra were generated from water samples concentrated using RO. Even though DOC concentrations of the liquid concentrate were near 300 mg/L, these were still too dilute to produce spectra which showed any features. It was thus decided to freeze-dry the liquid concentrate into a powder and to use the CP/MAS ^{13}C technique on them, instead of rediluting in NaOD.

The nmr frequency of a given nucleus is measured relative to a standard, in the case of this work, tetramethylsilane and produces a spectrum describing resonance frequencies. Sufficent sample was available to fill the tube. The regions under the ^{13}C nmr spectrum have been assigned to various carbons based on studies of pure compounds (Gonzalez-Vila et al. 1983, Preston MS, Wershaw 1985). These compilations, though showing somewhat different ranges, are similar. I chose the assignments of Preston (MS) because it represents the most up to date compilation (Table 3.2). The breakdown of a typical spectrum is shown in the first figure of Appendix B.

The freeze-dried samples were analyzed by CP/MAS ^{13}C nmr at McMaster using a Bruker MSL100 spectrometer, operating at 25.18 Mhz and 2.35 Tesla. Contact time was 1 msec and recycle time, one second to obtain an adequate signal to noise ratio (Preston and Blackwell 1985). Typically,

overnight runs with between 71,000 and 140,000 scans were done. The percent aromaticity is calculated as the sum of the aromatic and phenolic fractions.

Table 3.2 Major ^{13}C nmr resonances of humic substances.*

Shift Range (abbrevia.) in ppm	Carbon assignment
0 to 50 (UNSUB)	Unsubstituted aliphatic carbons: CH, carbons in long chain saturated hydrocarbons/C, CH,CH_2 bound to aromatic rings/-CH_2-carbons in alicyclic and ethyl groups/aliphatic carbons of peptides/methyl carbons.
50 to 90 (SUB)	Substituted aliphatic carbons: OH, O and N substituted carbons in polysaccharides and amino acids, aliphatic ethers.
90 to 140 (AROM)	Aromatic carbons
140 to 160 (PHENOL)	Phenolic carbons
160 to 190 (CARBOX)	Carboxyl carbons in carboxyl, ester and amide groups.
190 to 210 (CARBONYL)	Carbons in aldehydes, ketones, and C=S groups (Carbonyls)

* Ranges compiled from Preston (1988), Wershaw (1985), and Gonzalez-Vila et al. (1983).

CHAPTER 4

FIELD RESULTS

4.0 Introduction

This Chapter describes the environmental, water chemistry and biological conditions extant in the study area over the study period. Climate and hydrology, as well as major ion chemistry are compared to historical data to see if the study year was different from other "normal" years. The biological results, as interpreted from the enzyme study will be used later to interpret changes in DOM quality which will be reported below.

4.1 Climate and Hydrology

The mean monthly noon temperature for the study area during 1989 is shown in Figure 4.1. Values are typical values for this part of Nova Scotia, with warm temperatures in the summer and relatively mild values in the winter, as temperature is rarely below -20°C (Watson 1977). These data show that possibilities exist for the biological decomposition of organic matter from early March to mid November in both soils and water, as these media are usually warmer than that of air in the spring and fall.

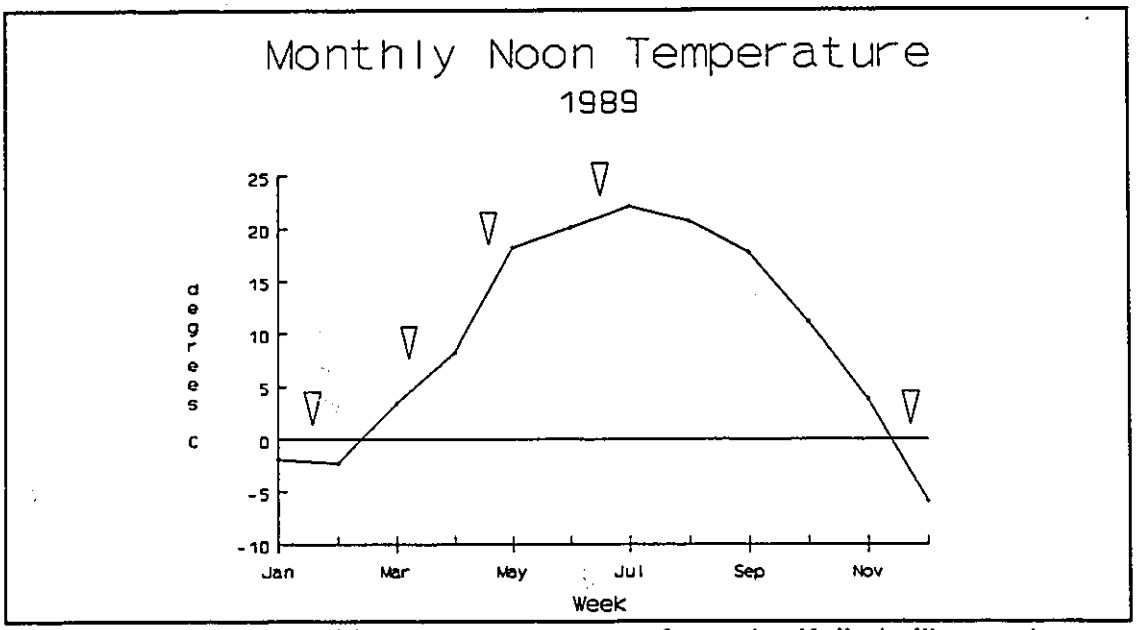

Figure 4.1 Mean monthly noon temperature from the Kejimkujik weather site. The triangles indicate sampling dates for this study.

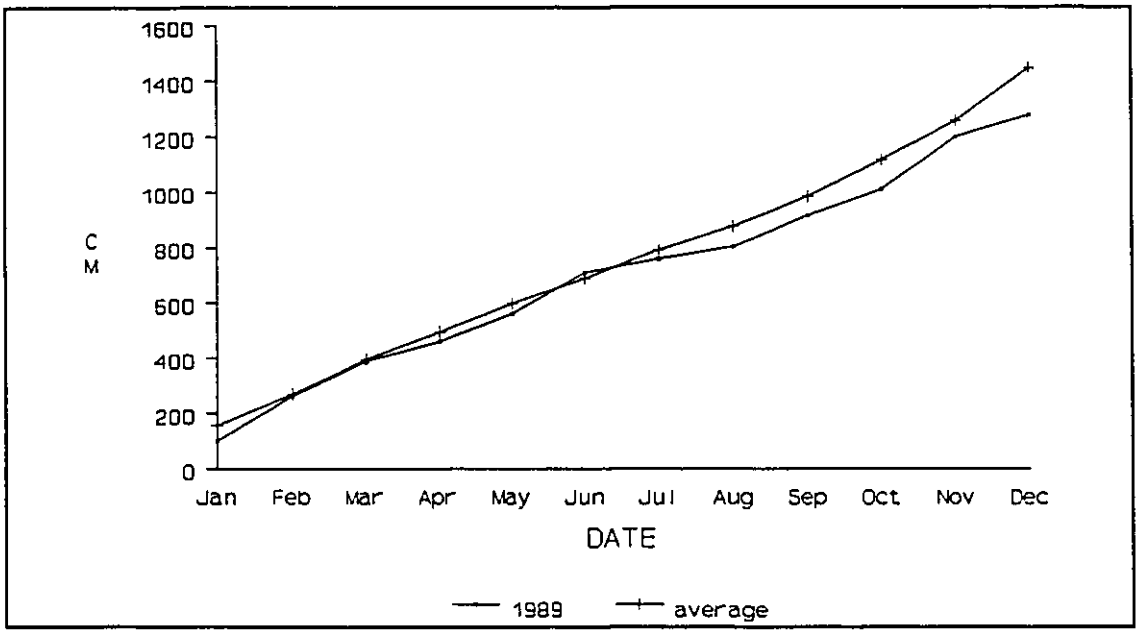

Figure 4.2 Cumulative precipitation at the Kejimkujik weather station in 1989, compared to the long-term mean.

Rainfall in 1989 was lower than usual (Figure 4.2). Approximately 1300 mm fell over the study period, compared to 1450 mm over the long term. This difference is within normal variability and is not considered to significantly affect biological activity.

Results show that 1989 water export from the basins was typical for the sites, with high water discharges occurring in the winter and spring, low values in the summer, increasing again in the late fall (Figure 4.3). The Figure also shows that Moose Pit Brook has greater water storage in the spring than the Mersey basin. This suggests that organic matter in the Moose Pit Brook basin should be in a somewhat different diagenetic environment in the late spring than soil organic matter in the Mersey basin.

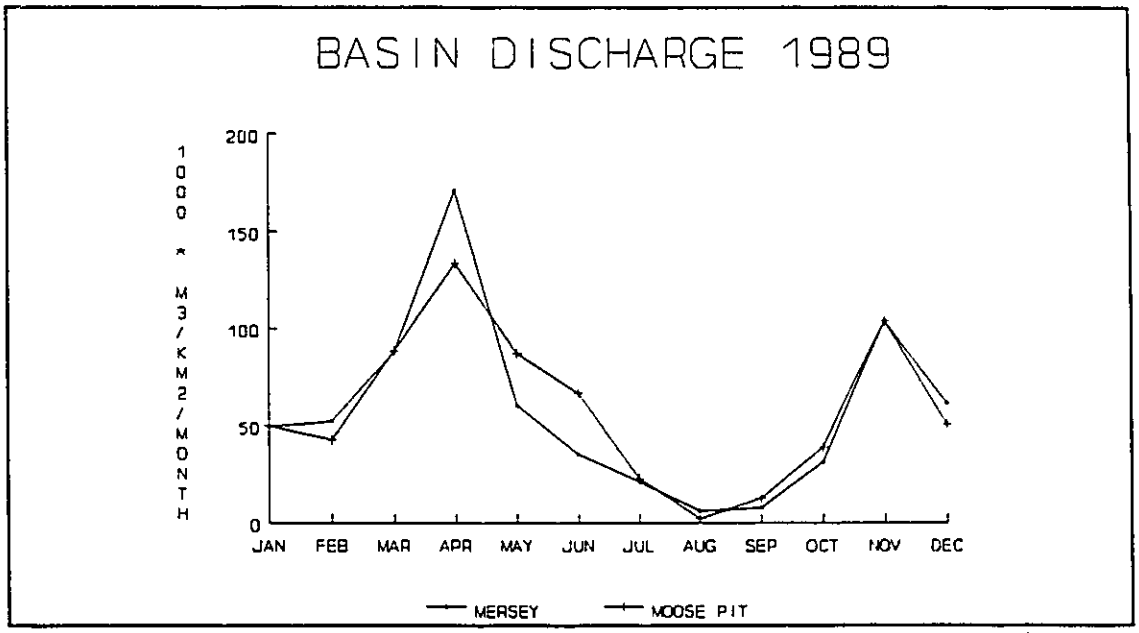

Figure 4.3 Basin discharge, normalized for surface area, at the two lotic sites.

4.2 Major Ion Chemistry

The pH at the Mersey and Moose Pit Brook sites, measured in the field is shown in Figure 4.4 (C.F. Luxton, pers. com.). Values are generally low compared to waters elsewhere in the Maritime Provinces (Clair et al. 1982), with Moose Pit Brook values always lower than those from the Mersey.

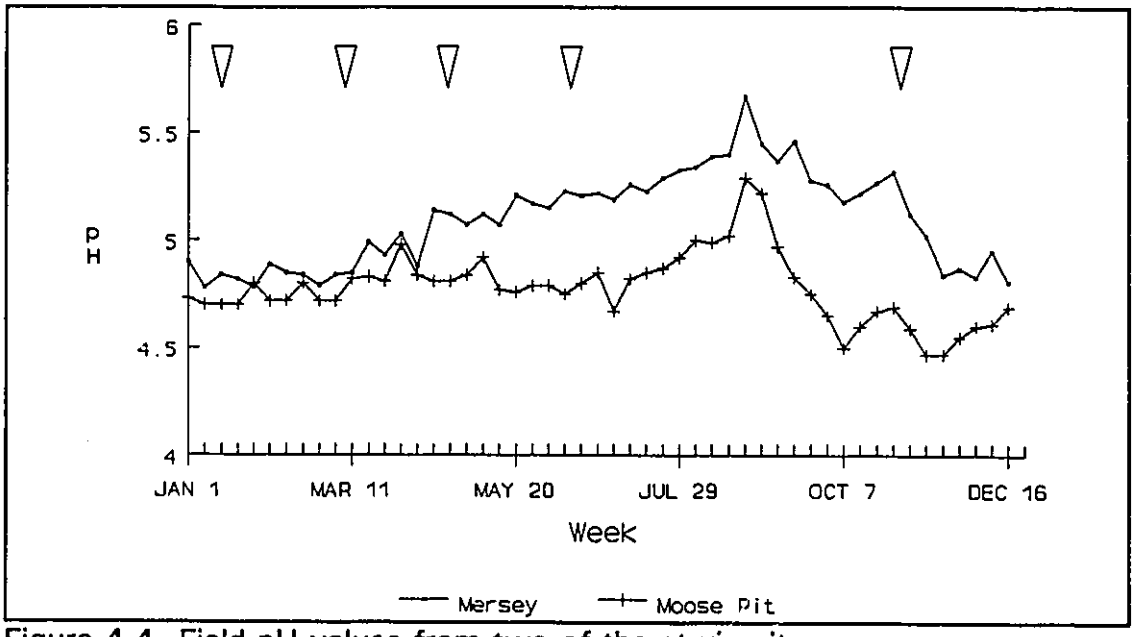

Figure 4.4 Field pH values from two of the study sites.

The trends in pH at the two sites generally follow each other, even though the difference between the two sites widens over the course of the year, narrowing again during times of high runoff in the winter, early spring and late fall. Therefore, the acidity or amount of natural organic acids must be greater in Moose Pit Brook than in the Mersey. The assumption of similar inorganic chemistry for the Mersey River and Moose Pit Brook is supported by

this study's major ion results (Table 4.1a, b) and from the work of Freedman et al. (1985), who used data collected weekly from March 1983 to May 1985. They found that the sum of base cations from the Mersey R. over a two year or longer period of time averaged 243.9 $\mu eq*L^{-1}$ compared to 237.2 $\mu eq*L^{-1}$ for the Moose Pit Brook. Average pH for the Mersey over this period was 4.9, and for Moose Pit Brook 4.6, while average SO_4^{-2} value was 54.3 $\mu eq*L^{-1}$ for the Mersey, compared to 52.8 for Moose Pit Brook.

The data complied in following reflect the major constituents. Ions such as NH_4, NO_3, PO_4, and silica contribute only trace amounts.

The values shown in Table 4.1 suggest that the situation has not changed since the early part of the decade at these two sites, and that mineral acidity, as represented by SO_4^{-2} and bedrock dissolution, represented by the sum of the base cations are similar for basins.

Table 4.1a Major ion chemistry from the Mersey River at the sampling times. Based on triplicate analyses, major ion values varied only by 10 percent of the mean and pH varied by 0.1 units.

Date	Na^+ $\mu eq * L^{-1}$	K^+ $\mu eq * L^{-1}$	Ca^{+2} $\mu eq * L^{-1}$	Mg^{+2} $\mu eq * L^{-1}$	pH
10/1/89	146.1	9.2	51.4	52.6	4.74
13/3/89	30.4	10.0	45.5	50.7	4.9
4/24/89	117.8	10.0	31.1	33.1	5.2
15/6/89	137.4	9.5	34.4	36.8	4.8
14/11/89	136.1	12.3	58.9	57.8	4.9

Date	Cl^- $\mu eq * L^{-1}$	SO_4^{-2} $\mu eq * L^{-1}$	ANC $\mu eq * L^{-1}$	DOC $mg * L^{-1}$	DIC $mg * L^{-1}$
10/1/89	145.8	58.1	7.3	13.9	0.7
13/3/89	85.7	61.6	11.6	8.4	0.5
24/4/89	155.1	55.2	10.8	6.8	0.8
15/6/89	166.1	53.7	28.7	11.1	1.5
14/11/89	142.1	64.5	15.8	11.7	0.7

Table 4.1b Major ion chemistry from Moose Pit Brook at the sampling times.

Date	Na^+ $\mu eq*L^{-1}$	K^+ $\mu eq*L^{-1}$	Ca^{+2} $\mu eq*L^{-1}$	Mg^{+2} $\mu eq*L^{-1}$	pH
1/01/89	132.6	30.3	41.3	31.7	4.1
13/3/89	106.5	21.9	32.8	24.2	4.9
4/24/89	100.4	20.1	30.1	23.7	4.7
15/6/89	93.0	28.1	28.5	20.5	4.6
14/11/89	114.8	12.6	49.8	25.8	4.3

Date	Cl^- $\mu eq*L^{-1}$	SO_4^{-2} $\mu eq*L^{-1}$	ANC $\mu eq*L^{-1}$	DOC $mg*L^{-1}$	DIC $mg*L^{-1}$
10/1/89	229.0	228.5	-47.7	9.3	1.0
13/3/89	179.1	147.7	14.3	7.0	1.0
24/4/89	178.1	147.7	4.5	10.4	1.3
15/6/89	128.2	55.4	3.8	22.3	1.1
14/11/89	176.1	163.1	-75.5	26.5	0.8

The Tupper Lake and Moose Pit Bog sites on the other hand show different chemistry than the two streams (Table 4.2a, b). Though there are no long term data sets to compare with, the data from Tupper Lake show significantly lower Na, and Cl values than from the two stream sites, while pH is somewhat higher. The reasons for the lower Na and Cl concentrations which are contributed by marine salts transported through the atmosphere are not apparent, and may have to do with conditions not relevant to this study.

The Bog was only sampled three times, so that patterns in the chemistry are more difficult to detect compared to the other three sites. However, most inorganic ions are lower reflecting the ombrotrophic nature of the site. K is fairly high in the water, probably due to leaching from plants, as has been reported occurring with precipitation draining from trees (throughfall) in the area (Percy 1989). The DOC varies a greater deal at this site, obviously a reflection of precipitation and evaporation and biogenic processes which cannot be addressed here.

DIC is quite high at the treed mire probably due to decomposition processes producing CO_2 which are occurring in the sediments, and the influence of bedrock carbonates. It should also be pointed out that the high DIC value could also be an artifact of the sample storage. Decomposition of organic matter and production to dissolved CO_2 is bound to occur in water samples which are high in DOM, so that the pH value in the field, where more CO_2 exchange between the water surface and the atmosphere occurs, may be quite lower due to escape of the gas.

Table 4.2a Major ion chemistry from Tupper Lake at the sampling times.

Date	Na^{+} $\mu eq*L^{-1}$	K^{+} $\mu eq*L^{-1}$	Ca^{+2} $\mu eq*L^{-1}$	Mg^{+2} $\mu eq*L^{-1}$	pH
1/01/89	114.4	10.5	40.6	48.3	4.8
13/3/89	98.3	10.7	35.5	43.5	5.0
4/24/89	89.6	11.7	34.6	37.5	5.2
15/6/89	103.0	13.6	59.0	39.8	5.2
14/11/89	100.4	10.4	35.9	42.4	5.3

Date	Cl^{-} $\mu eq*L^{-1}$	SO_4^{-2} $\mu eq*L^{-1}$	ANC $\mu eq*L^{-1}$	DOC $mg*L^{-1}$	DIC $mg*L^{-1}$
10/1/89	97.9	54.5	13.1	11.8	1.3
13/3/89	98.1	59.3	16.8	7.4	0.8
24/4/89	111.1	66.8	24.6	7.5	0.9
15/6/89	83.8	58.3	29.4	8.1	1.1
14/11/89	85.2	54.8	11.1	6.8	0.5

Table 4.2b Major ion chemistry from the Bog site at sampling times.

Date	Na^{+} $\mu eq*L^{-1}$	K^{+} $\mu eq*L^{-1}$	Ca^{+2} $\mu eq*L^{-1}$	Mg^{+2} $\mu eq*L^{-1}$	pH
1/01/89	NS	NS	NS	NS	NS
13/3/89	NS	NS	NS	NS	NS
4/24/89	67.9	19.8	14.6	20.1	5.2
15/6/89	109.6	28.9	48.8	41.3	4.9
14/11/89	73.9	20.5	25.0	31.5	5.3

Date	Cl^{-} $\mu eq*L^{-1}$	SO_4^{-2} $\mu eq*L^{-1}$	ANC $\mu eq*L^{-1}$	DOC $mg*L^{-1}$	DIC $mg*L^{-1}$
10/1/89	NS	NS	NS	NS	NS
13/3/89	NS	NS	NS	NS	NS
24/4/89	50.8	11.0	32.9	6.0	3.5
15/6/89	85.2	6.30	52.3	18.8	3.9
14/11/89	63.7	31.1	28.9	3.6	1.1

4.3 Aquatic Enzymes

No replicate samples were collected during the field part of the study, as the enzyme work was very experimental and in this work was simply meant to give an indication of biological activity. This approach had to be taken due to the inaccuracies involved in the measurement technique, as well as because of the enzymes being tested for were not all relevant to environmental conditions. The results shown below are the sum of total measured activities.

No enzyme measurements were done at the January sampling, though activity would not be expected to be very high in freshwaters during that time of the year. This was confirmed by the March enzyme activity which was lower than at any other period. However, activity values were generally at their highest in November than at any other sampling time (Figures 4.5, 4.6, 4.7, 4.8).

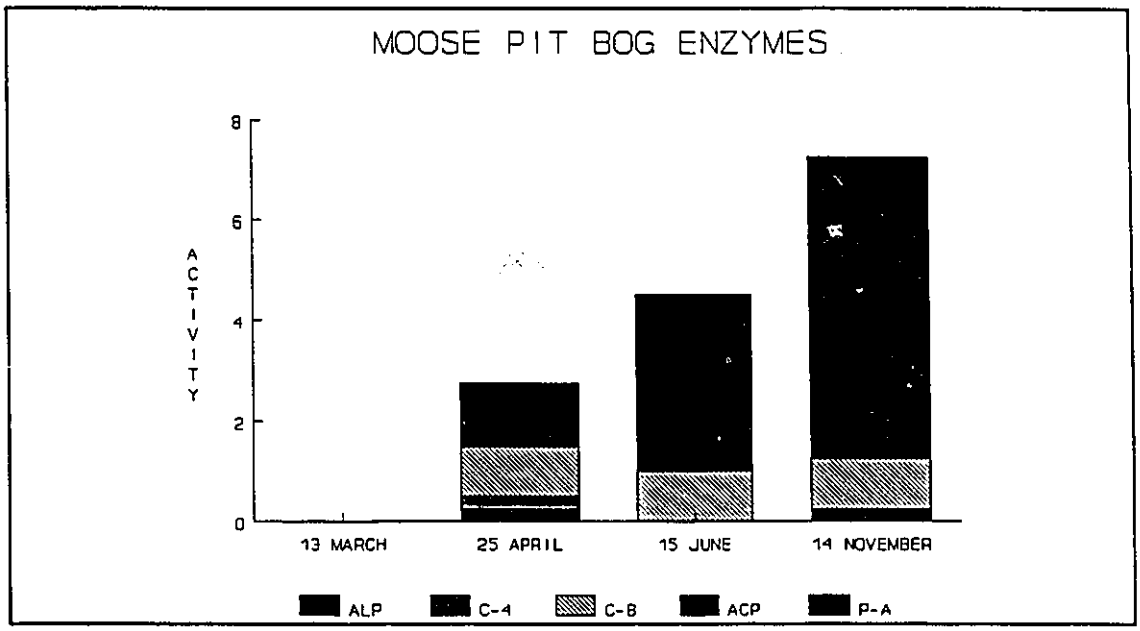

Figure 4.5 Enzyme activity at the sampling times at the Moose Pit Bog site.

This result is somewhat biased by the lack of information during the late summer when activity probably was highest. However, it does show that decomposition of in-stream organic matter was going on at this date, especially in the more confined sites such as the lake and bog. The Moose Pit Brook enzyme values (Figure 4.6) were probably more influenced by the bogs in the basin than was the Mersey River, where the location of soil and lake decomposition was further removed.

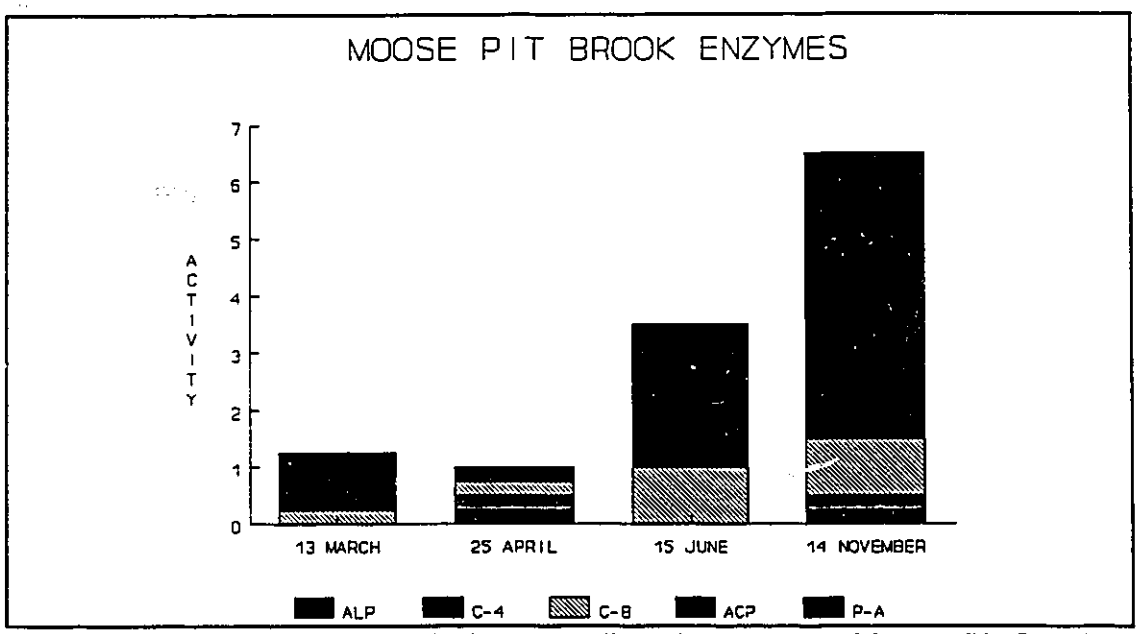

Figure 4.6 Enzyme activity during sampling times at the Moose Pit Brook site.

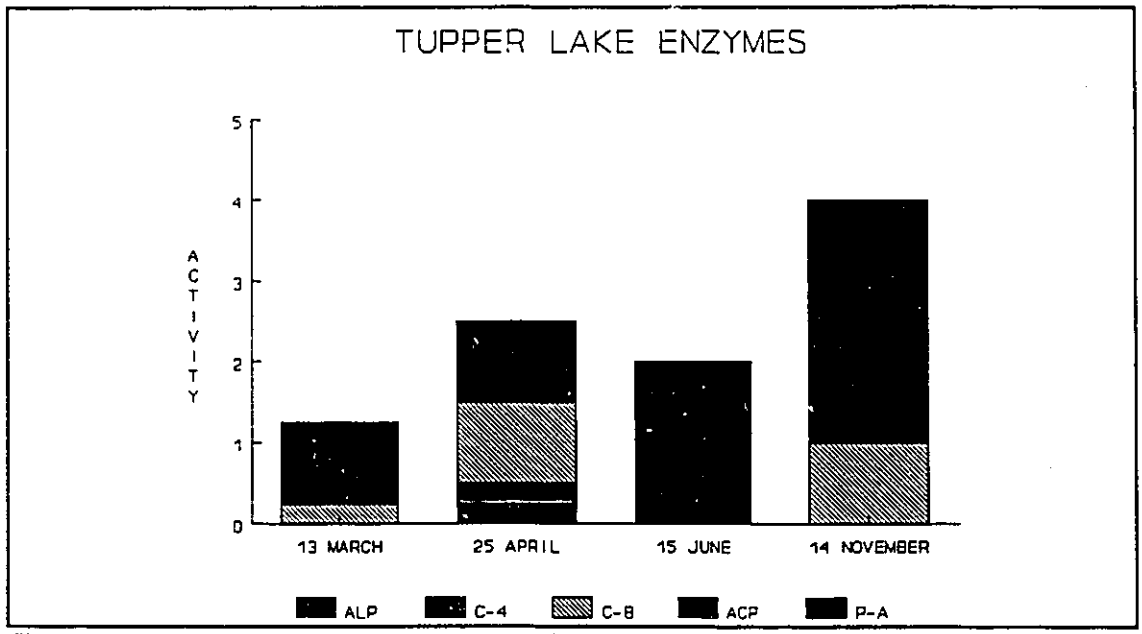

Figure 4.7 Enzyme activity at sampling times at the Tupper Lake site.

Enzyme activity was greatest at the Bog site, followed in order by the Moose Pit Brook, Mersey River and Tupper Lake sites. If it is assumed that the enzymes represent total biological activity, then the low activity in Tupper Lake, compared to Moose Pit Brook was surprising. It may be that the enzymes measured using the API ZYM® method were more selective in the range of biological reactions measured than was initially thought, and that the photosynthetic activity occurring in the lake was not monitored by the test kit. The high values in the Bog are not surprising, and it may be that Moose Pit Brook water was simply runoff from active bogs and soils.

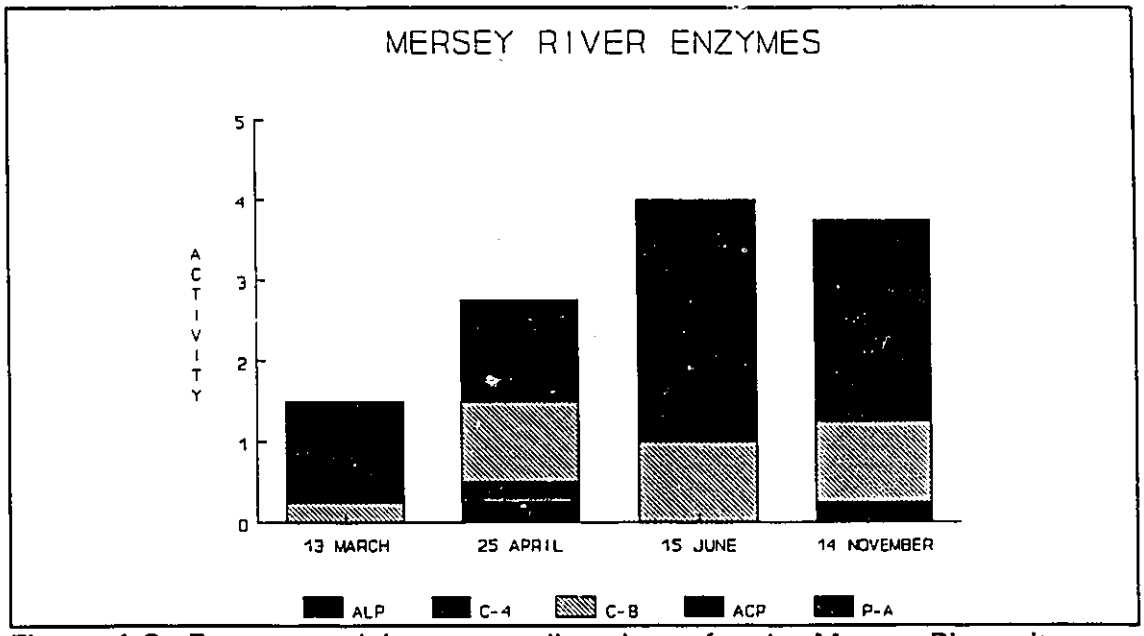

Figure 4.8 Enzyme activity at sampling times for the Mersey River site.

The interpretation of enzyme-freshwater DOM interaction is still in a very primitive state. Enzymes in freshwater are thought to be the product of autolytic decomposition of cells, ie the escape of enzymes from cells breaking down, or from exoenzymatic processes, where bacteria and fungi release

enzymes into surrounding water to breakdown organic matter (Chrost 1989). With the techniques used in this study, there was no guarantee that the enzymes measured could also not be endocellular, as cell material was not filtered out before analysis. As the DOM measured in Kejimkujik area waters is mostly composed of aliphatic material (see Chapter 6), I expected to measure enzymes responsible for the breakdown of these structures, eg. carbohydrases, or glucosidases. However, six of the seven enzymes measured were associated with inorganic phosphate metabolism, i.e. phosphorus cycling, and the seventh, leucine-amino peptidase, which hydrolyzes NH_2-terminal peptide bonds of amino acids, was at very low concentrations. As amino acids only comprise less than 5% of DOM (Thurman 1985), it is difficult to explain these measurements.

The absence of carbohydrate or sugar enzyme results is also difficult to explain, but seem typical for natural environment studies. Chrost (1989) attempted to measure β-glucosidase activity in a German lake, and found no exo-cellular activity in waters over the course of the year, even though endo-cellular enzyme activity was quite high. He also showed that the presence of sugars dissolved in water, ie glucose, hexose, etc.., formed during cell lysis, inhibited the formation of enzymes, a possibility perhaps explaining the results from this work. Other studies have measured estarases in soil, as well as ureases and polysaccharidases, but have had difficulties relating them quantitatively to soil organic composition, texture and use (Skujins 1978 and Kiss et al. 1978).

Because of analytical shortcomings, polysaccharidases were not measured, but may have been present in this study. However, the absence

of glucosidases, galactosidases and other enzymes suggest that modification of acid-base moieties and major structural features of DOM may occur at such low levels, not easily measured without more specialized work. This work has shown however, that phosphate cycling is measurable using simple tests.

4.4 Conclusions

Enzyme tests were never conducted in these waters previous to this study, so that results cannot be compared with other times or sites. Results showed that the enzymes tested for were most active at the Bog and then in Moose Pit Brook, followed by the Mersey River, and closely by the lake site. Enzyme activity was highest overall in November, but this may be more a reflection of sampling frequency than actual importance. The comparative low activity in the lake samples must be either to low biological activity there, or more likely to an inadequate enzyme selection which did not reflect the influence of primary productivity activity. More on the subject of enzymes is presented in Chapter 7.

The results presented in this Chapter show that 1989 was a typical year meteorologically, hydrologically, and in terms of the inorganic chemistry of the sites. The year was somewhat drier than usual due to lower rainfall but the difference from the mean was not significant, as it was well within the normal range of 1250 to 1640 mm total precipitation for the area (Watson 1977).

The same can be said of the discharge from the sites, as the mean annual discharge at the Mersey River sampling site over the study period was

well within the mean value of 22.5 +/-4.6 m^3/sec, as calculated by Ambler (1983). These results showed that more water retention occurred in the Moose Pit Brook basin than in the Mersey which could influence the formation and modification of DOM in basin soils.

The water chemistry results from the two regularly monitored sites, the Mersey River and Moose Pit Brook also fell within the range of normal conditions expected in this region. Again, the assumption can be made that the chemistry from the other two sites, for which there is no background data, also behaved normally. Together, the above pieces of information suggest that the DOM results generated for the 1989 study year were typical for the sites, as there was no reason to believe that the year was unusual compared to others.

CHAPTER 5

ACID BASE CHEMISTRY OF DOM AT THE STUDY SITES

5.0 Introduction

The purpose of this Chapter is to describe the variability in both acid-base and structural chemistry of DOM at the study sites over the study period. Quality control procedures used to verify the affinity spectrum approach are also described. The results of analyses discussed below are from samples collected simultaneously with the major ion and enzyme chemistry which have been characterized in Chapter 4, as well as with the structural chemistry samples described in Chapter 6. Further interpretation of the data discussed here is also carried out in Chapters 8 and 9.

Perdue (1985) defined the general composition of sites according to their pK_a composition. Using his approach, sites in the pK_a range 3.0 to 6.0 were classified as carboxylic sites, 6.5 to 8.5 as mid-range sites, composed mostly of β-carbonyl compounds, enols and alcohols, and 9.0 to 11.0 as being phenolic acid sites. Results from affinity spectrum calculations produce a picture which quantifies the acid functional site concentration at each pK_a from of 3 to 11, at 0.5 unit spacings. Because comparison of samples to each other can be difficult without some integration of results, site concentrations were summed within each category defined by Perdue (1985) to allow for

easier interpretation.

5.1 Quality Control

In order to assess the reproducibility of the affinity spectrum method, triplicate samples from sites chosen at random were collected during the March, June and November samplings. The sample results were compared to provide some idea of the natural and analytical variability which could be expected from samples. The analysis of replicate samples was not to be as simple as was hoped. From Figure 5.1, 5.2, 5.3, the spectra for the three samplings (normalized for carbon concentration), showed considerable scatter between samples.

The three March, Moose Pit Brook samples (Figure 5.1) showed variability in the spectra, especially in the phenolic acid sites. Study of the sample major ion, ANC and DOC concentrations however, showed no differences between the samples, so that this difference in values cannot be easily explained. It should be noted however, that there was no variability in the mid-range and little in the low pK_a ranges of the samples, though Sample #1 shows a low carboxylic value which is difficult to accept based on experience. As it seemed the most reasonable and consistent with theory and in comparison to the other samples collected, Sample #2 was chosen for interpretation. Of the other triplicate samples collected, one Tupper Lake June sample showed an anomalously high phenolic acid concentration, while one November Tupper Lake sample tended to show a somewhat higher carboxylic content than the other two.

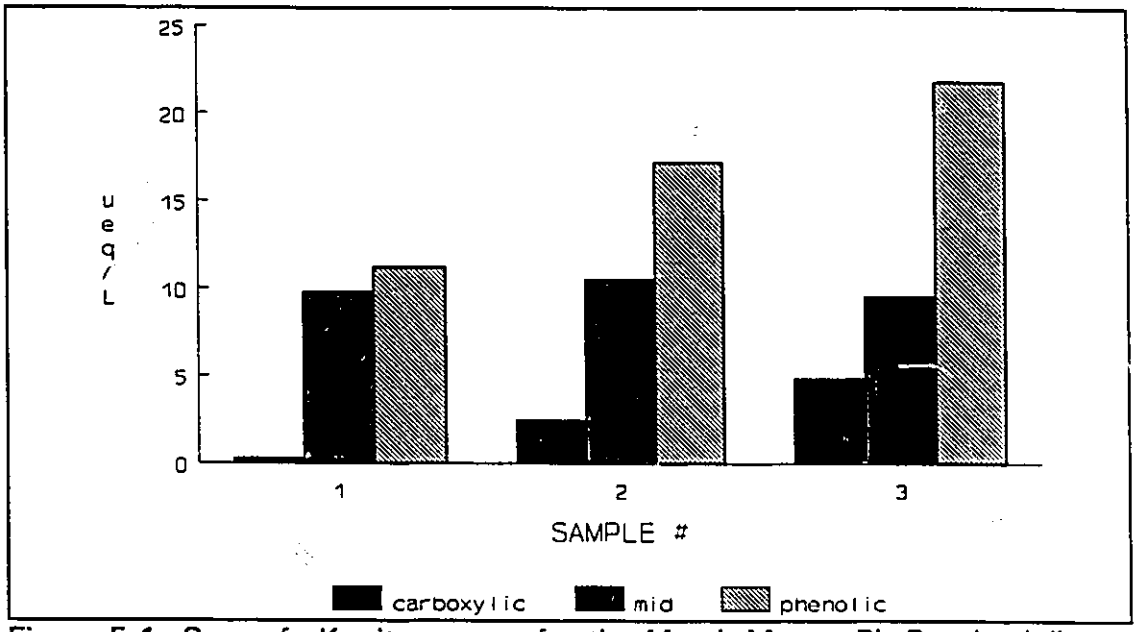

Figure 5.1 Sum of pK_a site groups for the March Moose Pit Brook triplicate samples.

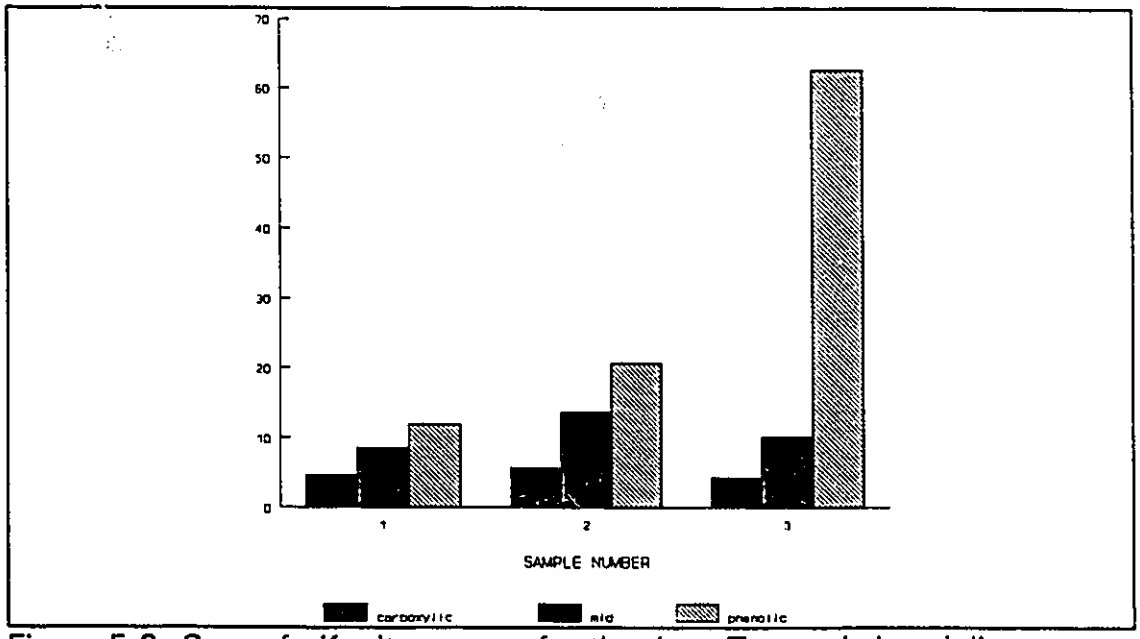

Figure 5.2 Sum of pK_a site groups for the June Tupper Lake triplicate samples.

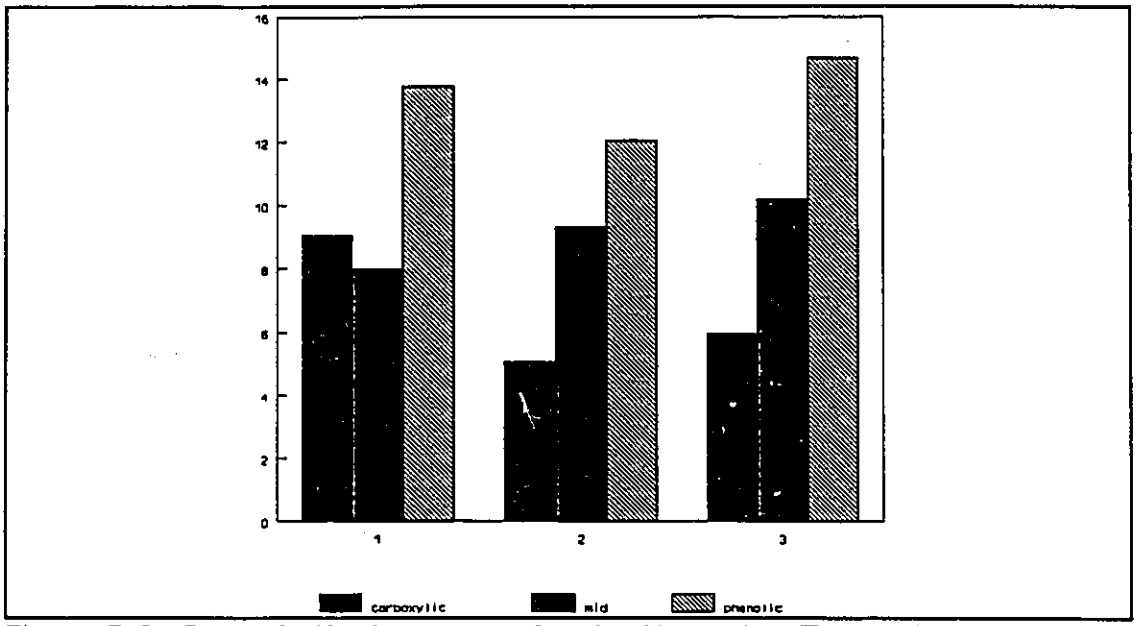

Figure 5.3 Sum of pK_a site groups for the November Tupper Lake triplicate samples.

The causes of these variations are difficult to pinpoint, as is the amount of confidence which can be placed in the data. Laboratory analysts at McMaster believe that at least for the samples collected until June, electrical interferences with the probes and meters caused some variability in readings to occur (P. Collins, pers. com.). The problem was resolved by placing the apparatus in a Faraday cage. Another problem, also discussed above, is that samples can degrade in the laboratory if not analyzed immediately. As the triplicates were usually analyzed within a few days, if not hours, of each other, this explanation is probably not sufficient to explain the variability, as degradation would be expected to occur in an analogous fashion to similar samples kept under similar conditions. A third possibility is that as the DOC is acid titrated, a fraction of it precipitates on the probe surface, reducing proton exchange with the standard solution, and thus giving the false

impression of higher pH which then suggests the (false) presence of high concentrations of carboxylic acids which cause a buffering effect.

Contamination could also be the cause of some of the spectral variation, as acid contamination from bottle washing procedures could precipitate the high pK_a fraction of the DOC. This was not the case with the Moose Pit Brook major ions and pH (Table 5.1)

Table 5.1 Major ion, pH and ANC values for triplicate samples. The Moose Pit samples were collected in March and the other two in June and November. Nitrate was not analyzed.

Sample	pH	DOC	DIC	Ca	Mg	K	Na	Cl	SO_4^{-2}	ANC
		all values in $mg \cdot L^{-1}$								$\mu eq \cdot L^{-1}$
MooseA	4.8	7.0	1.0	0.66	0.9	0.5	2.5	3.6	3.0	14.3
MooseB	4.7	6.9	0.8	0.60	0.47	0.5	2.5	3.6	2.9	0.7
MooseC	4.9	6.9	0.9	0.60	0.47	0.5	2.3	3.6	3.0	6.0
Tup6A	5.1	8.2	1.6	0.83	0.46	0.6	2.4	2.8	2.6	29.4
Tup6B	5.2	8.1	1.1	1.20	0.48	0.5	2.4	3.0	2.8	337.6
Tup6C	4.6	8.1	1.2	1.0	0.47	0.5	2.4	2.9	2.6	155.7
Tup11A	5.4	6.8	0.5	0.72	0.52	0.4	2.3	3.0	2.6	-87.5
Tup11B	5.3	7.0	0.7	0.72	0.51	0.4	2.3	3.0	2.6	-113.7
Tup11C	5.3	7.2	0.5	0.74	0.53	0.4	2.3	3.0	2.6	11.2

In fact, contamination did occur with some of the triplicates, though in sometimes surprising ways. All the major ions, DOC and DIC are generally quite consistent, and testify to the quality of the analyses. The most changeable parameter was ANC, which explains the variability in the spectrum compositions. Surprisingly, the ANC variability was not related to pH, except on one occasion (Tupper June, sample C) where ANC should have been lower, compared to sample B. This last value is quite likely much higher than it should be, but the anomaly does not show up in the affinity spectrum. The

November triplicates show a wide variability in ANC values, but not in pH, or affinity spectrum results, which is surprising.

Some of the causes of the discrepancies in ANC and affinity spectrum results have been discussed above. I discounted differences in major ion chemistry from bottle contamination in all of these the cases, as the one potential case of acid contamination (Tupper June, sample C) has a surprisingly high ANC value. However, one potential source of problems is the fact that the base titrant might be contaminated by contact with CO_2. This would tend to generate peaks in the higher pK_a regions which would be uncorrected if the contamination was unknown. There is no consistency in these results however, so that further study into these problems is warranted.

The most likely reason for the variability in affinity spectra and probably also for some of the large ANC differences reported in Table 5.1 is most likely the degradation of the DOM, while in storage. Proof that DOM acid-base spectra can vary with time will be shown in Chapter 7. As the titration method is often successfully calibrated against known standards, natural sample integrity (or its absence) may be an important problem to be considered. This problem is probably compounded by some of the other contamination factors discussed above, as well as natural variability of the samples, especially if suspended organic matter accidentally is included in the sample.

Moreover, there are two quality control checks which should also be passed before the spectra are accepted. Through experience with the water chemistry from this region (Clair and Freedman 1986, Freedman and Clair 1987), it has been observed that water pH at these sites will range from 7 to

4, and that [ANC] will range from -100 to +100 $\mu eq*L^{-1}$. Measured pH and calculated ANC values which deviate from this range are immediately suspect, and are most likely incorrect due to one of the problems discussed above. All samples generated in this study were subjected to this analysis, and those not conforming to the standard were either rejected, as in the case of the triplicates, or flagged. With common sense and experience, the problem of confidence in the data can be addressed in a logical, consistent, and sound manner. Further discussion on quality control aspects is done in Chapter 7.

Improvements to the sampling and analytical procedure, at least until the nature of all difficulties is understood, should involve titration of samples in-situ to prevent degradation problems, increased rate of replication of samples to insure greater confidence in the results, and daily analysis of titrant to insure against base contamination.

5.2 Field Results

Affinity spectrum results for the four sampling sites are shown in Figure 5.4, 5.5, 5.6. The results from the two stream sites and the Bog suggest that DOM acidity increased over the year, with total carboxylic concentration increasing from 60 to 124 $\mu eq*L^{-1}$ in the Mersey River, from 27.2 to 154.5 at Moose Pit Brook, and from 24.6 $\mu eq*L^{-1}$ in April to 96.5 in the Bog. The increase was not continuous however, as at both stream sites, values decreased somewhat in June for the Mersey, and March for the Moose Pit. Total carboxylic acidity remained stable between 37.1 and 42.7 at Tupper Lake. One anomalous sample was noted from the Mersey River March trip.

Though its pH and ANC are not out of the ordinary (Table 4.1a), the spectrum is unlike any other calculated during the study because of the very high value at pK_a 3.0. This result may be due to surface coating or modification of the electrode junction. It is therefore excluded from the following discussion on trends.

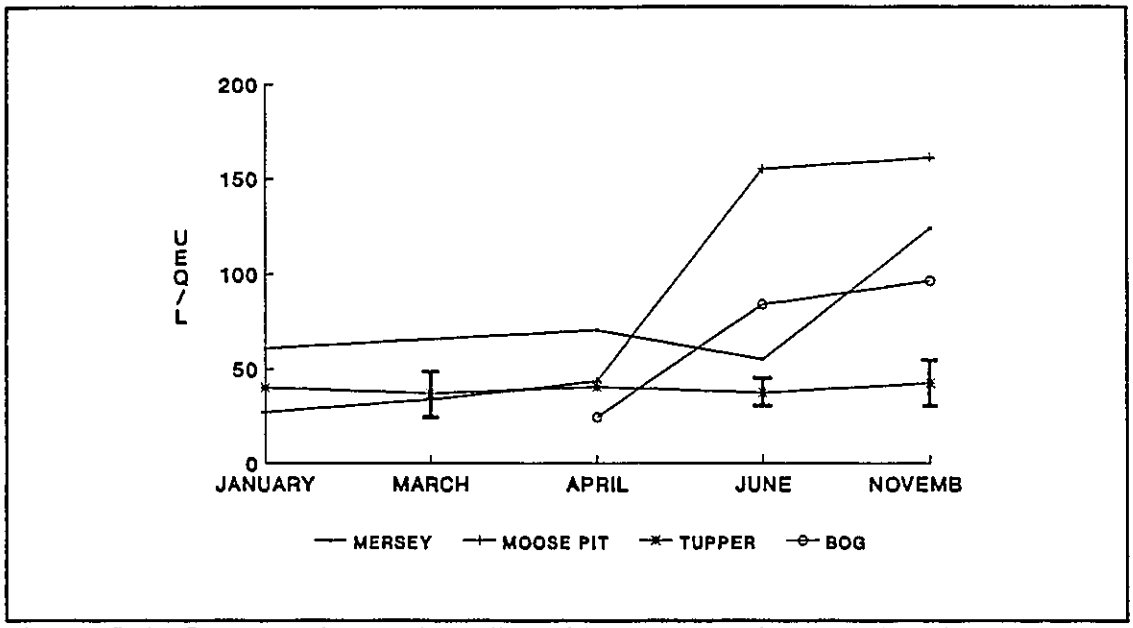

Figure 5.4 Comparative carboxylic acid concentrations at the sites over the study period.

These results suggest that for the stream and bog waters, carboxylic acidity becomes more important as the year progresses because of less dilution in the summer, and the flushing out of freshly formed DOM from soils in the fall. The January and March results suggest that the greater water volumes moving through soils dilute DOM and thus its total effect on water acidity.

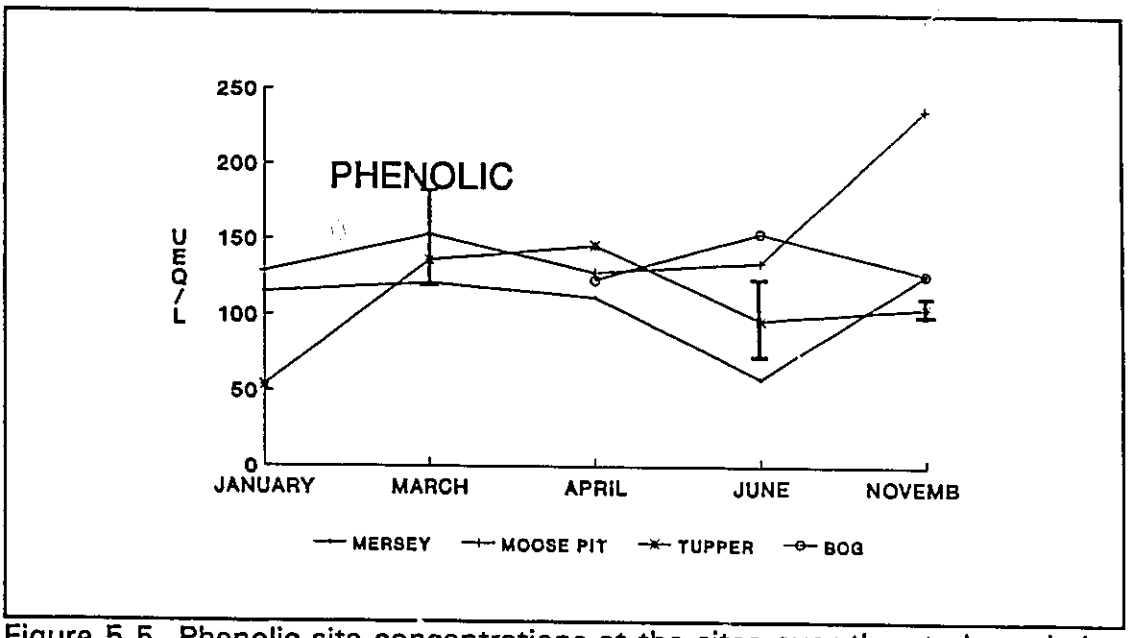

Figure 5.5 Phenolic site concentrations at the sites over the study period.

At Tupper Lake, Moose Pit Brook and the Bog, phenolic acid concentration is at its peak in June, though it is at its minimum at the Mersey River at that time, while it is at its highest in November at that site. Phenolic acidity does not contribute to the acidity of the water, but is an important part of both aquatic and soil organic material. Their relative contribution to organic matter will be discussed further below.

Mid-range pK_a groups reach their peaks in Moose Pit Brook and the Mersey River in November, April in Tupper Lake and June in the Bog. The contribution by this group to water acidity is minimal at the normal pH range found in these waters, and interpretation of their importance to water chemistry has not been much discussed in the literature and is beyond the scope of this dissertation.

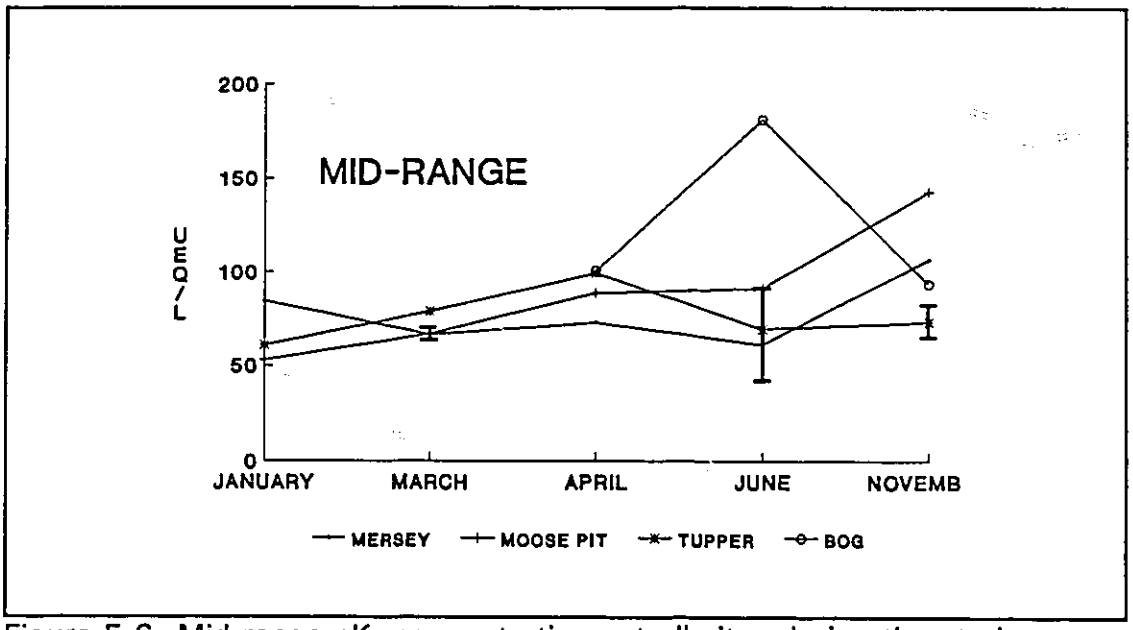

Figure 5.6 Mid-range pK_a concentrations at all sites during the study period.

In order to understand how the quality of the organic material varied between sites and time, the site concentration data were normalized to DOC amount by dividing the sum of sites within the carboxylic, mid-range and phenolic ranges by the water DOC concentration. The results (Figures 5.7, 5.8, 5.9) show that usually, carboxylic site concentrations are much lower than 10 μeq*(mg C)$^{-1}$ suggested by Oliver et al. (1983). However, the amounts vary between sampling periods and between sites. For example, the Tupper Lake site mean concentration remains stable at approximately 4 μeq*(mg C)$^{-1}$ throughout the whole year, though in the Mersey River, carboxyl ranged up to 10 μeq*(mg C)$^{-1}$ in April and November. In Moose Pit Brook, all values were below 10 μeq*(mg C)$^{-1}$.

Study of the Bog samples showed that all three pK_a site group results were closely grouped together, unlike the results from the other sites where

phenolic acid sites were always significantly higher than the mid-range, which were usually higher than the carboxylics.

The phenolic site concentrations at all sites except for the Bog, reach a maximum concentration in March or April, decreasing in June. The mid-range pK_a sites tend to follow the same pattern as the phenolic groups, but at lower concentrations.

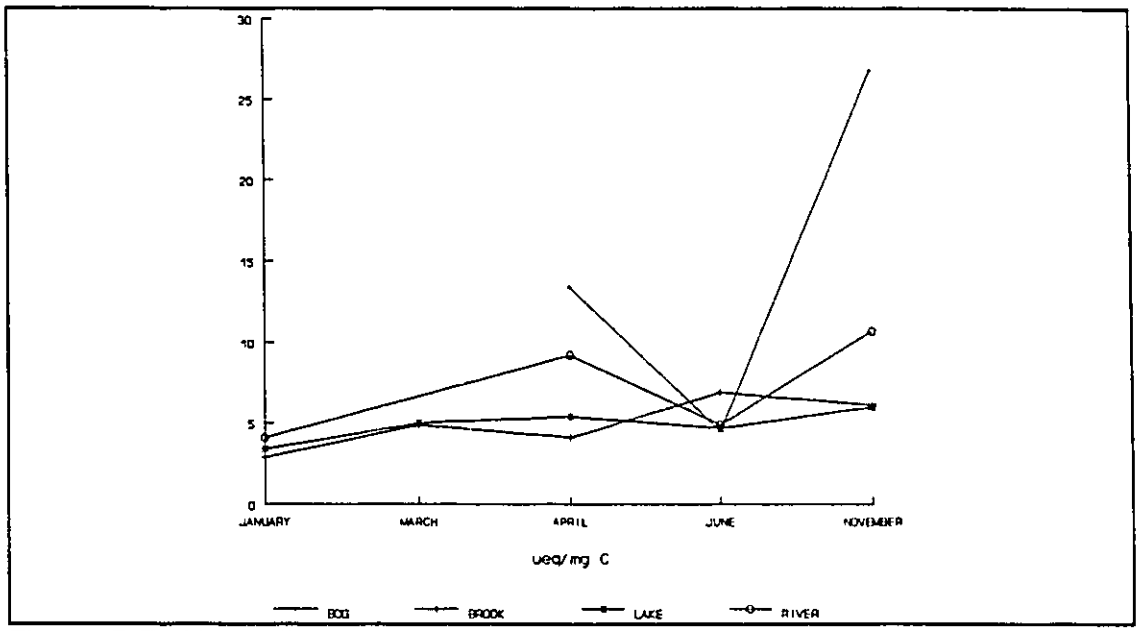

Figure 5.7 Normalized carboxylic site concentrations for each site over the study period. (Mersey March values absent due to bad data).

The results from the bog samples present some difficulty in analysis, as they do not seem to resemble those of the Brook, as could be expected if the Brook was immediately downstream of the site of DOM formation. A number of reasons could be responsible for this, the most important perhaps being the pathway which water would have to follow to get from the Bog into the Brook channel. Selective retention of DOM fractions by soils could be quite important. Another potential problem could be that open pools in bogs may

be subject to chemical interactions not easily understood.

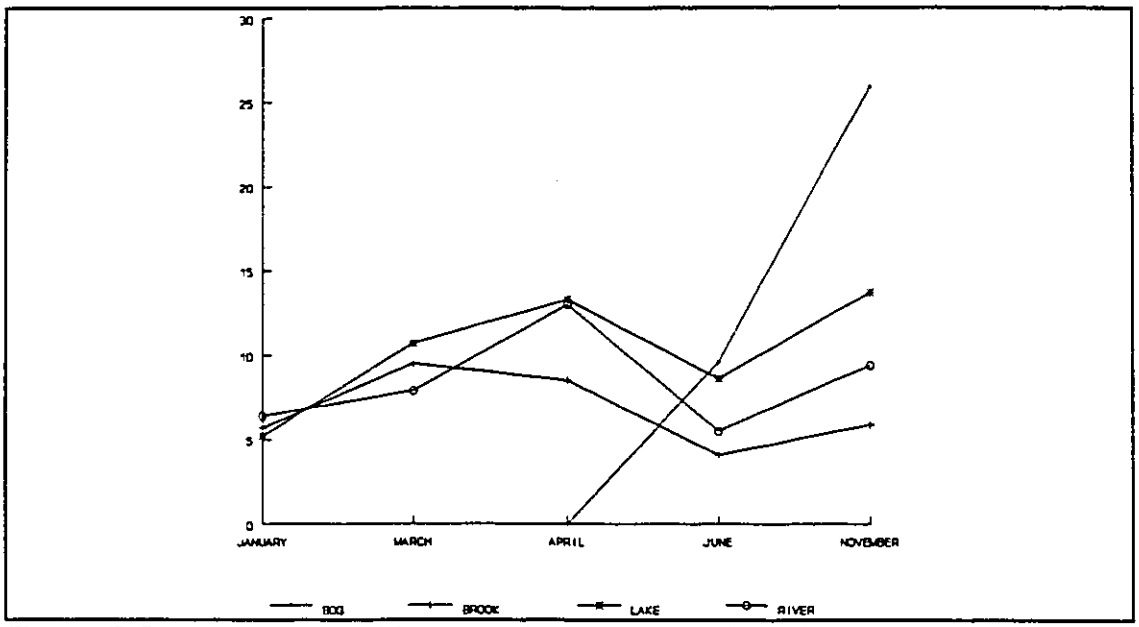

Figure 5.8 Normalized mid-range site concentrations for all sampling sites.

The acid-base chemistry of the lake is quite stable probably because of the biological activity occurring which would generate neutral DOM. Photochemical reactions may also be important in modifying lake DOM, and may be playing an important role in ensuring low carboxylic acid concentrations. The Mersey River results should provide an integrated picture of the results generated in the Brook and Lake as a large number of lakes are found in its headwaters. In fact, the Mersey results tend to be between the lake and Brook results, especially in the mid range and phenolic sites. For the carboxylic sites, the Brook and River tend to be most similar to each other.

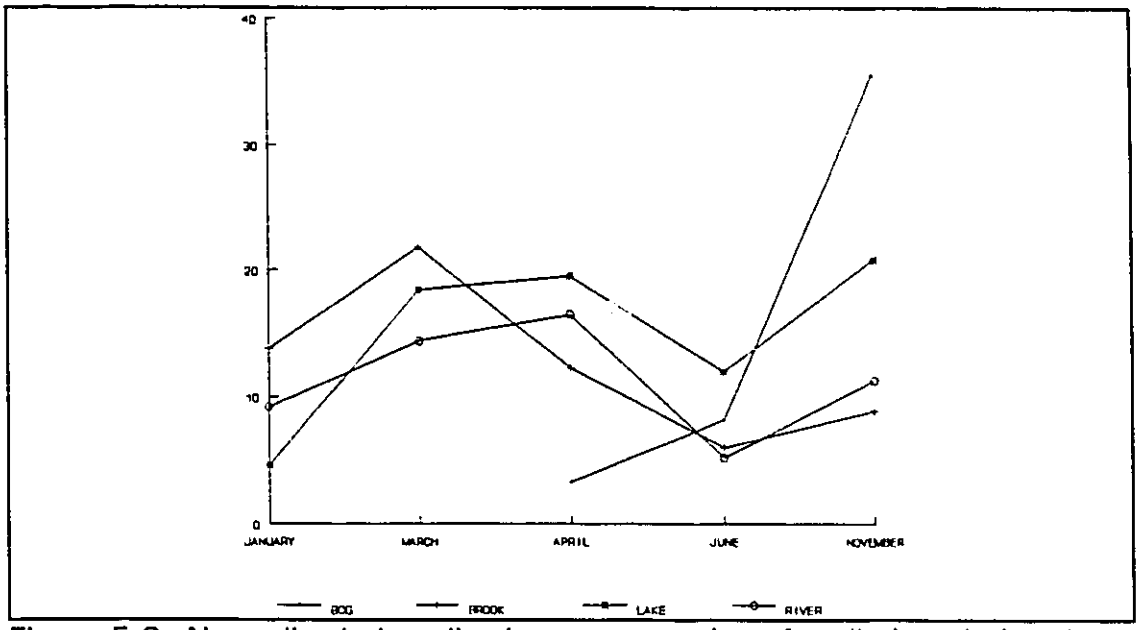

Figure 5.9 Normalized phenolic site concentrations for all sites during the study period.

5.3 Basin generation of organic acids

This section shows how the DOM acidity exports from the two stream sites varied seasonally. In order to calculate exports of the various C acid functional site groups, I calculated monthly DOC exports from Mersey River and Moose Pit Brook from weekly water samples collected by the Water Quality Branch of Environment Canada (unpub. data). Using measured DOM fractional values and functional group composition, as well as water discharge (Chapter 4), export of acid functional sites were calculated.

Calculation of acid functional group exports (Figures 5.10, 5.11) shows that phenolic acids (pK_a 9-11) are the main component exported, with carboxylic (pK_a 3-6) and neutral acid groups (pK_a 6-8) being roughly equal in importance. As carboxylic acids are those which contribute to natural acidity,

and most probably to weathering processes, it is interesting to note that in Moose Pit Brook, its greatest export occurs during late spring, early summer, when it also reaches its maximum structural carboxylic export. The Mersey River, which demonstrates effects further downstream of a first order wetland site, seems to show a surprisingly rapid reaction to spring melts, probably due to the large portion of non-wetland area in it.

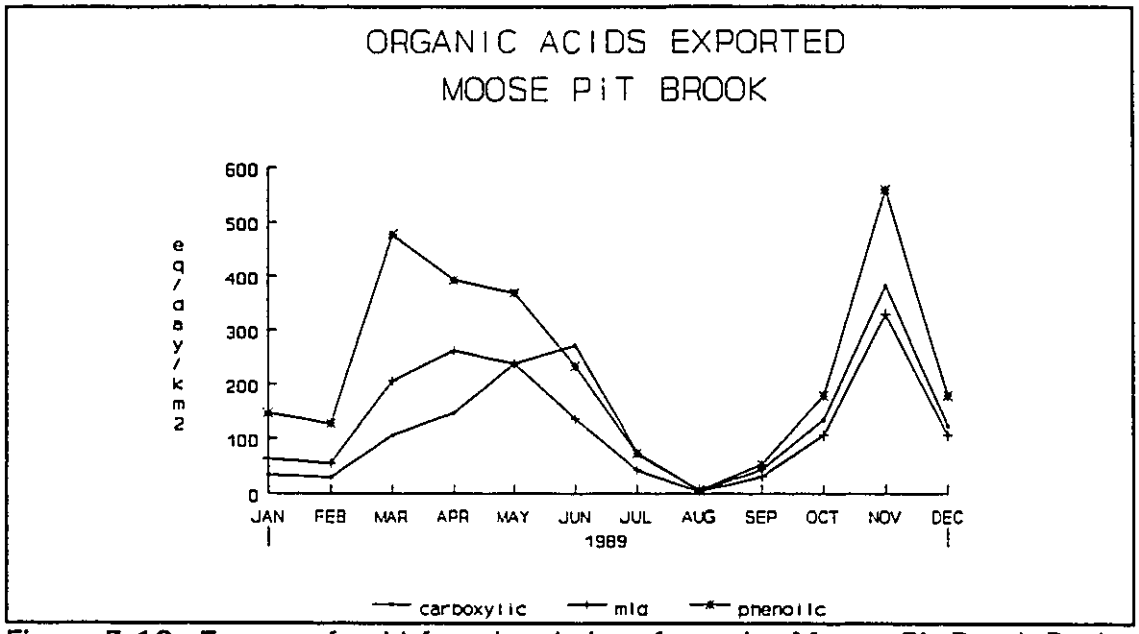

Figure 5.10 Export of acid functional sites from the Moose Pit Brook Basin during the study period.

These data confirm that the DOM effect on water chemistry will vary based on hydrological and basin characteristics. Most DOM transport from basins occurs during the fall to late spring period. In terms of basin effects, the summer export of DOM is trivial, but it is during the summer that biological activities in soils produces the material which will be exported during the fall discharge period.

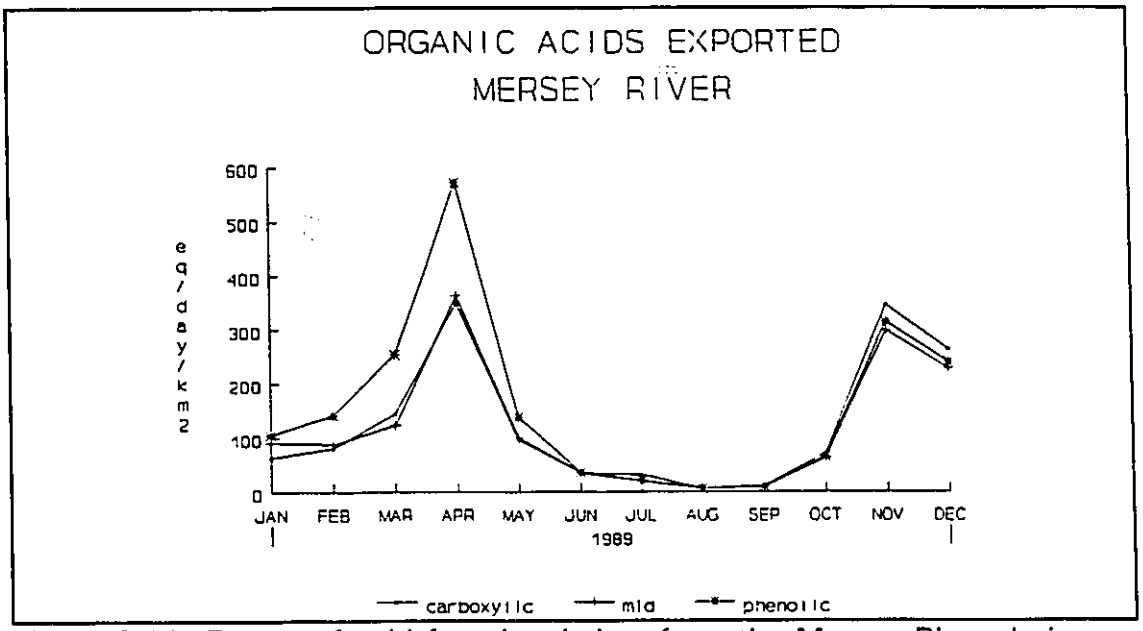

Figure 5.11 Export of acid functional sites from the Mersey River during the study period.

5.4 Summary

Results from the quality control portion of the study show that care must be taken when inte· ·ting affinity spectrum results, as the titration of organic waters especially with acids can produce surprising effects. As the titration method is regularly calibrated against known standards, the variabilities encountered were probably due to problems of sample integrity. Affinity spectra must be interpreted with major ion and ANC results at hand in order to ensure that all results point in the same direction. As well, great care must be taken from the collection of the sample, to its analysis, to ensure that the DOM is not modified by contamination or biological or chemical factors which would tend to change its character.

The acid-base chemistry of the DOM tended to show some slight tendencies over the year studied. DOM carboxylic acidity was near 5 μeq * $mg^{-1}C$ at the Brook and Lake sites, though it sometime reached values near 10 in the Mersey River. Values from the Bog were usually higher than 10 μeq * $mg^{-1}C$. These results tend to complicate the picture presented by Oliver et al. (1983) and also Driscoll et al. who estimated purified fulvic acids to contain approximately 10 μeq*$mg^{-1}C$. However, as these were direct measurements and not statistical approximations, the results tend to paint a more complicated picture than is often assumed.

There was a general tendency for mid-range and phenolic acids to show a peak in the Spring and one in the Fall at all sites except for the Bog. This result is not explainable with the data generated in this study, but remains another one of the further paths which should be pursued in the future. This result is probably due to a combination of DOM formation in soils and the impact of water flow through soils and wetlands during the fall runoff period.

The DOM acidity results from the Bog site compared to the water bodies also suggest that once produced in submerged soils, it is subject to a number of factors, such as photochemical and biochemical degradation, flocculation, as well as filtration through the soils which change its nature. More on this topic will be presented in Chapter 9.

CHAPTER 6

DOM STRUCTURAL VARIABILITY IN FIELD SAMPLES

6.0 Introduction

There exist few published CP/MAS ^{13}C NMR spectra of spatial and temporal changes in stream and lake fulvic or humic acids, and none for stream and lake DOM. Malcolm (1989) summarized work from three studies and showed roughly similar patterns of both fulvic and humic composition at the same site between seasons, as well as between river sites. From this, he hypothesized that there is little variability in material from similar environments over the year. He also found that fulvic and humic acids from a lake resembled those from streams into the lake.

This section displays the ^{13}C NMR results of DOM collected at the five samplings made at the study sites over the study periods. These are then compared to each other and to those reported by Malcolm (1989) to see if the patterns he reports for humic and fulvic acids are also applicable to unfractionated DOM. I also compare and discuss the differences and similarities between the structural and acid-base results to see if trends noticed in one analysis are complementary to those from the other.

6.1 Field Results

As with water chemistry and affinity spectrum, no sampling could be done at the Moose Pit Bog site in January and March due to the bog being frozen. All other sites were sampled five times. The percent carbon composition of the samples at each site over the year is shown in Figures 6.2, 6.3, 6.4 and 6.5 (individual spectra in Appendix B). In all samples, the carbonyl fraction for the samples ranged from 2.8 to 4.5% and was low compared to the other fractions and will not be considered further here due to its small and unvarying contribution to the C budget. It should be pointed out that the values calculated are fairly robust as the spectral peaks tended to fall within certain natural patterns, and that these patterns also tended to follow the shift range assignments of Preston (1988). To demonstrate this, a typical spectrum measured is shown in Figure 6.1, along with the range assignments.

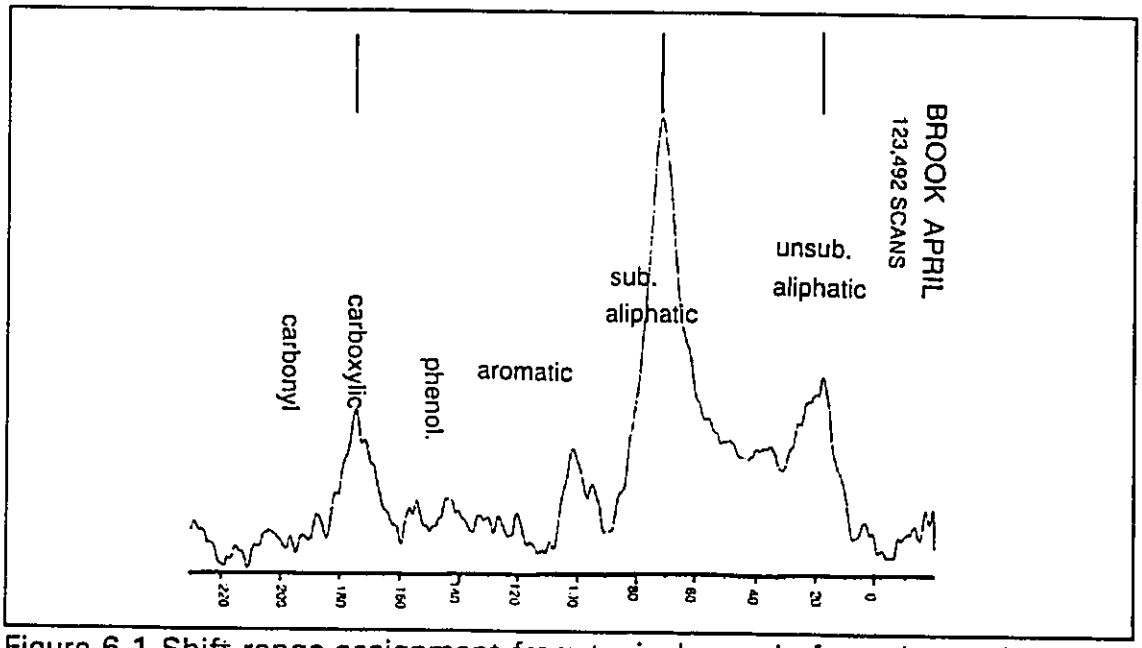

Figure 6.1 Shift range assignment from typical sample from the study.

Results from all sites show somewhat similar patterns of abundance, though differences were detected, especially at the Bog site, where values theoretically represented those of environments of formation. Results for most carbon fractions did not show large differences between dates at this site, except in the June sample. In this case, substituted aliphatics (SUB) were replaced by unsubstituted (UNSUB) (Fig. 6.2). There was little variability at this site compared to the others, probably because only three samples were taken, giving a lesser basis for comparison. It may also be due to the fact there is not much movement of water at this site compared to the others, or it may be that a consistent source of DOM was being generated by primary producers which tended to influence the overall structure.

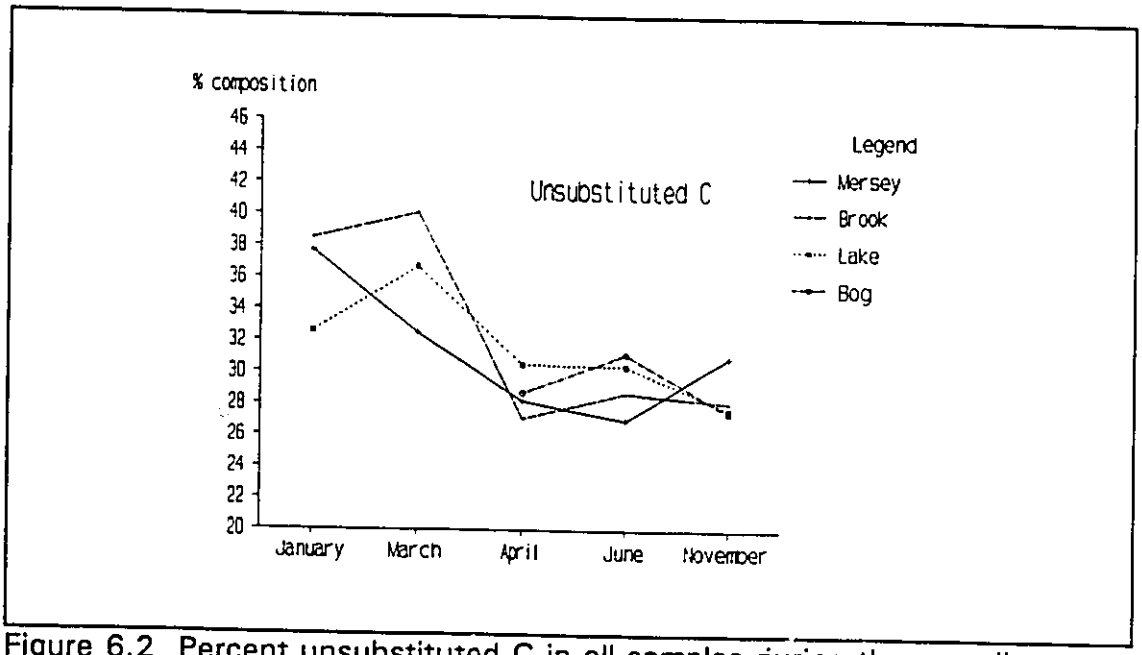

Figure 6.2 Percent unsubstituted C in all samples during the sampling period.

The Bog site also provided a shallow, open environment which was always exposed to sunlight and it may be that higher water temperatures allowed different biological and chemical reactions to occur.

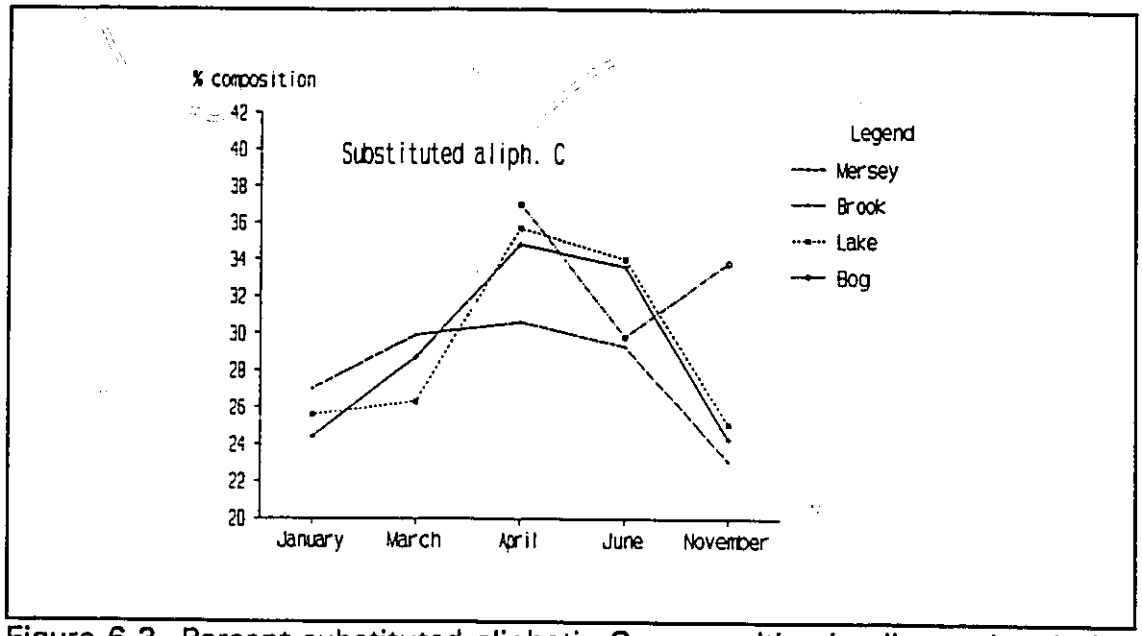

Figure 6.3 Percent substituted aliphatic C composition in all samples during the study period.

The unsubstituted aliphatic C pattern was quite similar at all sites (Figure 6.2) and was characterized by a continuous loss of importance as the year progressed, except at the Bog site where it did not vary greatly. The substituted aliphatic C (Figure 6.1) showed an increase in composition from winter to spring, with a decrease thereafter.

Interestingly, the total aromatic fraction (Fig. 6.4) increased continually at all sites over the course of the study, suggesting that the DOM was getting more and more complex with time. It could be suggested that continuous biological degradation of the stream and soil organic matter by bacteria was resulting in structurally more complex matter.

The pattern shown by the substituted aliphatic fraction was mirrored by the carboxylic C fraction (Figure 6.5). It showed a decreasing importance at all site in April, followed by a return to winter proportions in November.

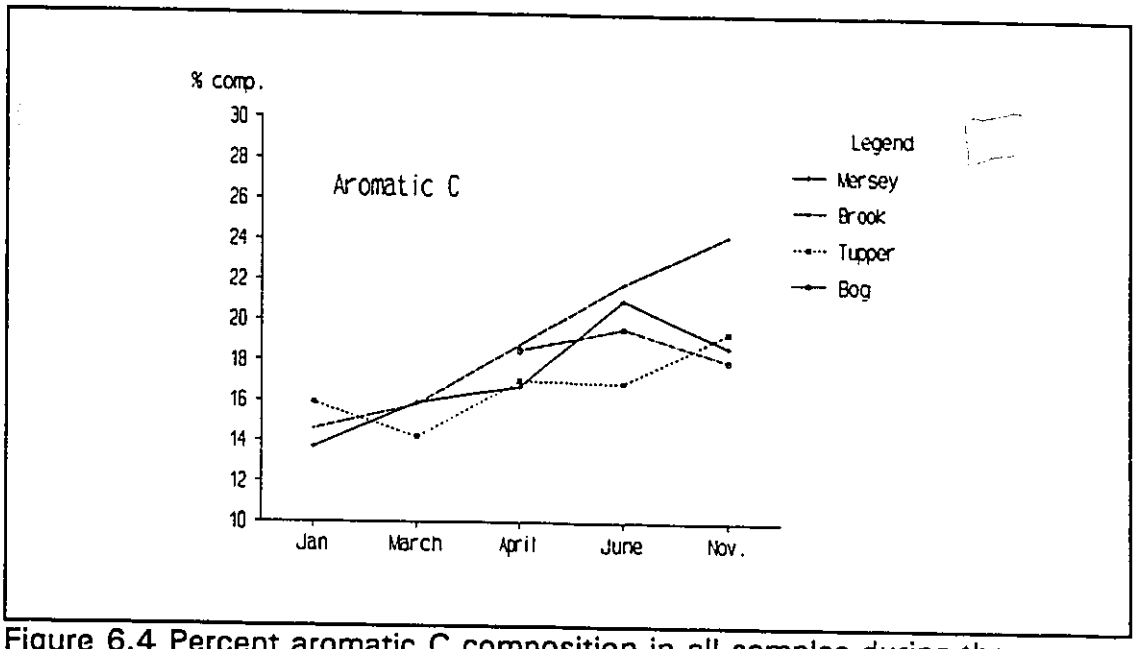

Figure 6.4 Percent aromatic C composition in all samples during the sampling period.

This relationship seems to suggest that the carboxylic C is perhaps linked to the unsubstituted aliphatic C fraction. Based on the evidence available, it is difficult to generalize further than this, but it seems obvious that based on general chemical principles, the carboxylic fraction is more likely to be associated with aliphatic than aromatic structures, and that from the results shown here, they are thus more likely to be related to the unsubstituted fraction.

In comparing these results site by site, it can be seen that the Moose Pit Brook results show the sharpest pattern of all sites. At that station, unsubstituted aliphatics decreased significantly in April, to be replaced by increases in the aromatic (AROM) and phenolic fractions (PHENOL). Substituted aliphatics and carboxylics showed opposite patterns, with the first increasing, and the second decreasing to its minimum in April. These patterns

were repeated in Tupper Lake and Mersey samples.

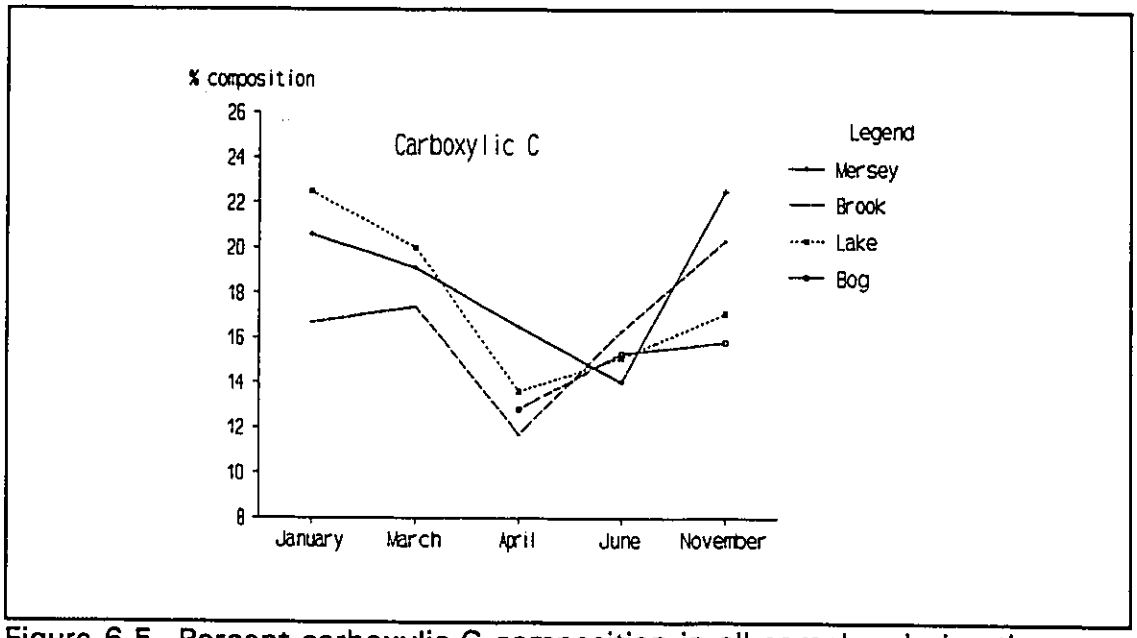

Figure 6.5 Percent carboxylic C composition in all samples during the sampling period.

Surprisingly, the site which seemed most unlike the others was not Tupper Lake as was expected. Tupper Lake was thought to be different from the other sites because of the influence of phytoplankton exudates and diagenesis of DOM in standing waters. The colour of the raw water, liquid concentrate, as well as the freeze dried powder supported this theory, as Tupper Lake samples were generally lighter in colour than the samples from the other sites. The lack of strong difference of these with samples from the other sites, suggests that the ^{13}C NMR spectra were not measuring all the differences that existed. Assuming that the lighter colour indicated a lower concentration of chromophores than in darker samples, it can be suggested that the differences seen could give an indication of measurable structural differences. Schnitzer and Khan (1972) identified conjugated double bonds,

aromatic rings and phenolic groups serving as colour centres in humic materials. As the fractions did not vary significantly between Tupper Lake and the other samples, it must be that O, N and other substituted carbons are less common in Tupper Lake samples, replaced by algal polysaccharides.

What these data also show is that DOC structural chemistry in freshwaters of the Kejimkujik National Park area, and probably in much of temperate Canada varies over much of the year. Seasonally, the main trend discerned was that DOM is generally less complex chemically in the winter when little soil biological activity occurs. This changes with increases in soil and water biological and chemical activity as seen by the replacement of unsubstituted aliphatics by substituted, the increasing importance of aromatics, and the decreased presence of carboxylic C in the spring and summer.

The results from this work were compared to those from four rivers and a lake reported by Malcolm (1989). He did not calculate percent C composition for his humic and fulvic fractions, but examination of his figures suggests that the material he studied did not vary much between sites, or with season. The data collected here shows more variability between closely located sites than those he reported, and my spectra seem to show lower aromatic fractions, though it is difficult to directly compare spectra due to insufficient information. Although it is difficult to compare humic fractions to whole DOM, their study clearly demonstrates seasonal and spatial differences.

The reasons for the difference between studies may be in the nature of the waters sampled. Malcolm studied materials from the Missouri, Ogeechee, Ohio and Yampa Rivers, all of which are larger, thus integrating more local

environments, located in more temperate environments than that of central Nova Scotia. Moreover, it can be hypothesized that the larger rivers tend to homogenize the materials produced from all types of small basins, and thus produce samples which are not truly indicative of basin processes. Further discussion about these problems is found in Section 9.2.

The field structural chemistry data reported were also compared to those collected from other workers. Table 6.1 shows the few results reported from solid state ^{13}C NMR characterization from the studies which were found.

Table 6.1 Structural distribution of aquatic DOM from a number of studies. UNSUB is unsubstituted Aliphatic C, SUB - substituted, AROM - aromatic, PHENOL - phenolic, CARBOX - carboxylic, CARBON - carbonyl, and %AROM - AROM + PHENOL.

study	UNSUB	SUB	AROM	PHENOL	CARBOX	CARBON	%AROM
1a	31	19	17	6	18	9	23
1b	23	19	27	10	14	8	37
2a	49	18	-	-	14	-	20
2b	49	15	-	-	15	-	22
2c	25	42	-	-	21	-	13
3	38	24	9.4	4.3	21	3.5	14
4	28	23	16	8.4	20	4.3	24

1a IHSS Suwannee fulvic standard
1b IHSS Suwannee humic standard
2a Wilson et al. (1983) coastal seawater
2b Wilson et al. (1983) extracellular phytoplankton cell extracts
2c Wilson et al. (1983) intracellular phytoplankton cell extracts
3 This study January Mersey sample (least aromatic collected)
4 This study November Moose Pit Brook sample (most aromatic).

The values in Table 6.1 should be interpreted realizing that they were all acquired using different types of equipment, with different instrument settings, and from material which was collected using various concentration schemes, so that few comparisons can be concluded with any degree of confidence. Nevertheless, contrasts of this study's results with the tabulated

data should give some idea of the range of values which should be expected in nature.

When this work's results were compared to those in Table 6.1, it was seen that the substituted aliphatic and carboxylic C in other studies are usually lower than my values. These results suggest that the RO approach to DOM concentration retains more of the uncharged fractions which would usually be lost when using the adsorption chromatography approach used by other researchers. It thus suggests that a reappraisal of the importance of the aliphatic components of DOM from both a biological and geochemical standpoint should carried out.

6.2 Basin exports of DOM structural fractions

Using the export calculation approach discussed in Section 5.3, the structural carbon exported from the basins was calculated. The results are in Figures 6.6 and 6.7 and show that most C exported from both basins is in the aliphatic form, and that the aromatic fraction usually is a relatively small part of the total. The data also show that most C is exported during high flow periods, and that it is mostly in aliphatic form, thus potentially more easily biologically degraded. The two sites differ in that DOM from Moose Pit Brook, which has a longer water retention time as shown in Chapter 4, has a greater aromatic component than the material from the Mersey River in both the spring and late fall. This could be due to the greater contact time between soil water and organic material which must occur, allowing greater diagenesis of DOM in Moose Pit Brook soils. It could also be due to the different nature

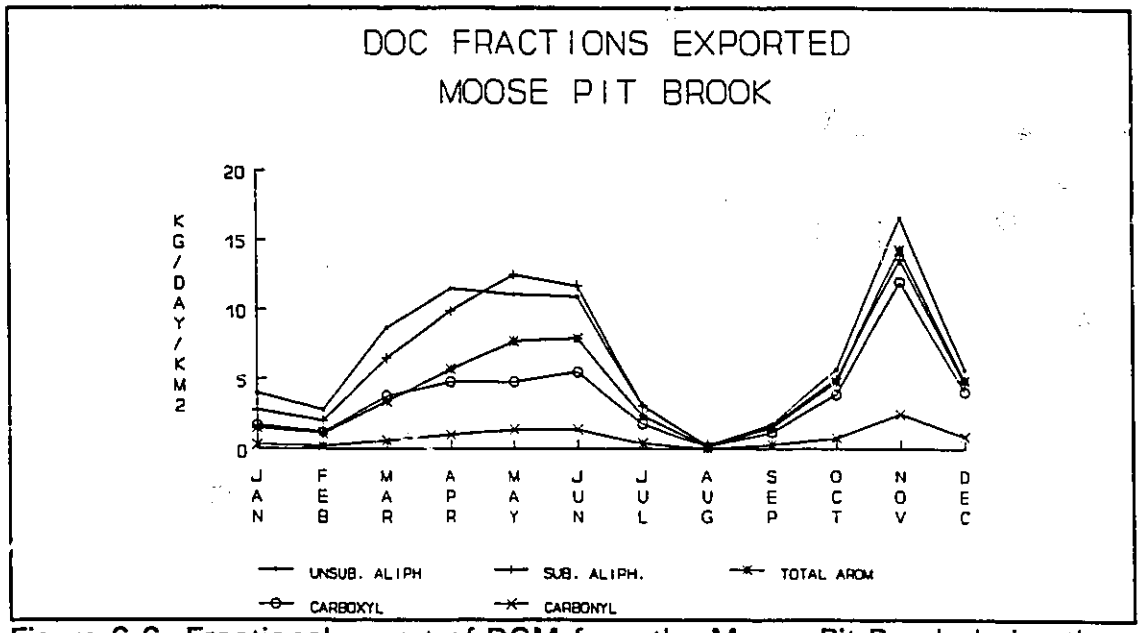

Figure 6.6 Fractional export of DOM from the Moose Pit Brook during the study period.

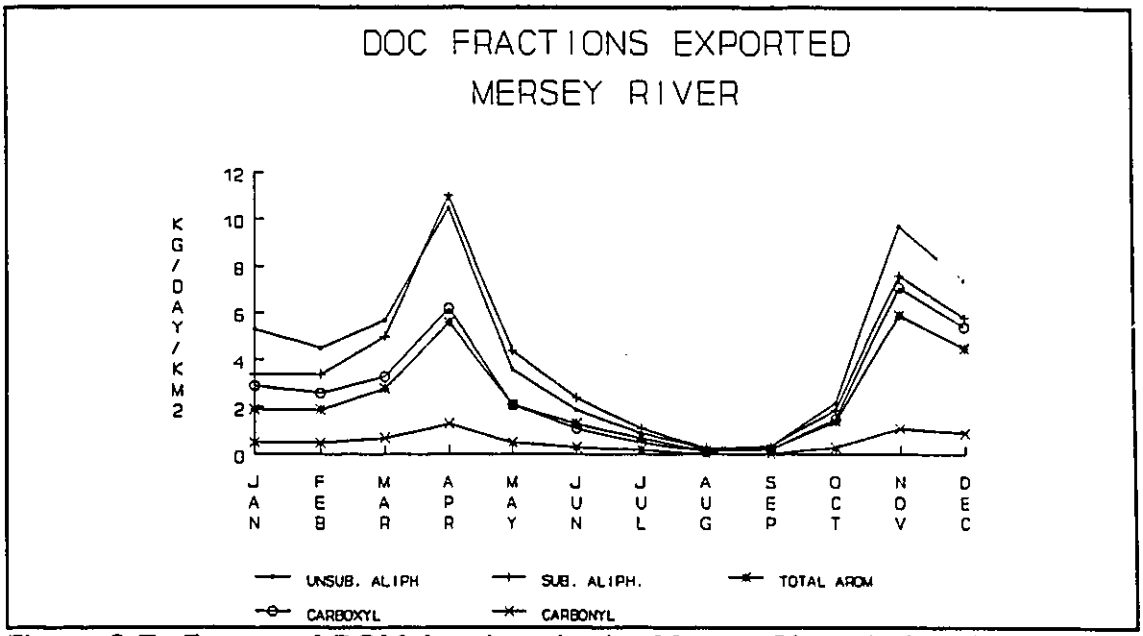

Figure 6.7 Export of DOM fractions in the Mersey River during the study period.

of soil organic material in the two basins, or to a combination of the two factors.

These data show that freshwater DOM is more labile than is usually thought, because of its low aromaticity compared to the soil humic fraction. These data also show that the DOM becomes less labile over the course of the year, with the unsubstituted aliphatic fraction decreasing from the winter into the summer, while the structurally more complex substituted aliphatic and aromatic carbons increase over this period. This pattern was consistent at both stream sites, as well as in bog waters and in a lake which were also sampled. It is generally assumed that the more aromatic the DOM, the less chemically reactive it is. The more aliphatic on the other hand, the more reactive it is and the more easily degradable.

6.3 Conclusions

The ^{13}C NMR results showed results somewhat similar to the acid-base chemistry, though with certain differences. The structural carboxylic fraction generally tended to track the acid-base carboxylics, with the lowest values measured using both approaches being in June at the Mersey, Bog, and at the Tupper sites. Results from Mocse Pit Brook showed the lowest structural carboxylic C in April, which was also the lowest acid-base sample. The highest structural carboxylics were measured in November, which also happened to be the time with maximum carboxylic acid concentrations for all sites except for the Moose Pit Brook, though it was also higher than usual there.

The similarities in DOM structural and acidity results over the length of the study could not be quantitatively correlated, but the trends between the two types of results were generally consistent throughout all samples. Though the structural work shows an increase in aromatic and phenolic C in March-April, peak values in aromatic and phenolic C occur in June when the acid-base chemistry shows the aromaticity to be at its lowest. Aromaticity values are at their highest in November, when high pK_a values are also at or near their highest values at all sites except for Moose Pit Brook.

No correlation was noted between the neutral sites and the aliphatic DOM fractions (UNSUB + SUB), and it seems that the large fraction of the DOM which falls within this structural component is relatively unimportant in the acid-base chemistry of the solution, though obviously not in terms of biological and hydrophobic interactions.

In summary, though similarities exist when comparing the results generated by the two techniques, these could not be quantitatively correlated to each other. This could have been due to differences in formation, diagenesis, or even interaction with the inorganic fraction of the solution, complexing phenolic and carboxylic sites, thus making them unavailable to the titration method. Moreover, samples analyzed by nmr were dried, which could have some effect on the measured structures. The most likely way of definitely quantifying the relationships between the two methods is probably by methylating the concentrated DOM, and analyzing the treated material using ^{13}C NMR (Schnitzer 1974, Thorn et al. 1987). In this way, the differences in quality of DOM which are evident with the results seen here, could be better understood.

This work also shows that the chemistry of DOC, as well as its chemical reactivity varies seasonally. During the winter and spring, hydrological factors seem to control DOM movement. DOM fractional composition at the two stream sites is similar, and the largest fraction is unsubstituted C. During summer and fall, basin storage and biological oxidation become significant. Thus the more complex substituted C and aromatic C fractions become more important, especially in Moose Pit Brook. The carboxyl fraction also decreases, especially in the Mersey River samples.

The results of this study indicate that the quality and quantity of DOC generated in poorly drained basins varies with hydrological, biological and soil effects which are poorly understood. These changes show that DOM is more liable to biological and chemical modification than was initially thought. These results also show that in temperate basins, more attention should be spent on the chemistry of DOC during high flow periods, compared to the summer when carbon exports are low, even though concentrations are high.

In summary, the study of the structure of DOM from three of the four sites showed that there were noticeable differences in its chemistry, but that the differences were more important seasonally than between sites. The exception to this generalization occurred in Bog waters, though the lentic nature of the environment, as well its environmentally exposed situation made it too different from the other sites to allow useful comparisons. Based on this reasoning, it was also expected that the lake DOM would be different from that in the brook and stream. It was not the case, though the reasons for the similarities are not understood.

An attempt was made to attempt to correlate the results of these analyses to the acid-base results of the previous Chapter. it met with lack of statistical success, probably for a number of reasons. The first is that in concentrating DOM, the method may have modified some of its structural components. Secondly, carboxylic carbons on DOM are not necessarily accessible for proton exchange because of involvement in other complexation reactions or because they are physically inaccessible.

CHAPTER 7

INCUBATION STUDIES

7.0 Introduction

As seen in previous sections, the acid-base chemistry and structure of DOM varied between sites and with time. As statistical studies have been unable to closely explain DOC-pH relationships, (Gorham et al., 1987) it was hypothesized that DOM contribution to water acidity varied with differences in source material, complexation of acid sites by dissolved metals, as well as in-stream diagenesis.

In order to understand some of the differences in acid-base and structural chemistry of DOM measured in the field, I conducted three laboratory studies (described in Chapter 2) to attempt to understand the variability contributed by source material and in-stream diagenesis. The first study was conceived to show the structural and acid-base nature of DOM generated from the decomposition of plant material. The second experiment was set up to show how the acid-base characteristics of DOM in stream water changed by biological (both autotrophic and photosynthetic) and chemical agents. This section details results from both studies and presents a preliminary discussion of what the data mean in nature. Further interpretation is presented in Chapter 9, where the results will be placed in the

context of the formation and modification of DOM.

7.1 Parent Material Incubation Study.

The object of the Sphagnum and Spruce incubation in well water, was to simulate conditions existing in wetlands where DOM is formed. It was hoped that the acid-base and structural chemistry of DOM formed in the well water would give an indication of what DOM formation pathway was followed.

It was expected that the parent material incubation would produce affinity spectra similar to those found in the treed mire samples as this environment was thought to be one of the main sources of the organic material found in brooks and rivers. This hypothesis was not supported by the data from the incubation work (Figures 7.1, 7.2). The incubation water affinity spectra showed phenolic acid concentrations which were very high compared to stream values for the Sphagnum and Spruce respectively (160 and 70 μeq*(mg C)$^{-1}$) , compared to roughly 20 μeq*(mg C)$^{-1}$ for stream material.

Surprisingly, the Sphagnum produced higher concentrations of phenolic acids than the Spruce even though in theory, mosses are devoid of structural aromatic compounds which are usually associated with phenolic acid sites. The Sphagnum sample also contained higher carboxylic and neutral sites and the Spruce. It may simply be that mosses, are more easily biologically degradable to aromatic form through the phenolic aldehydes from non-lignin material pathway (Stevenson 1982) than are the Spruce twigs.

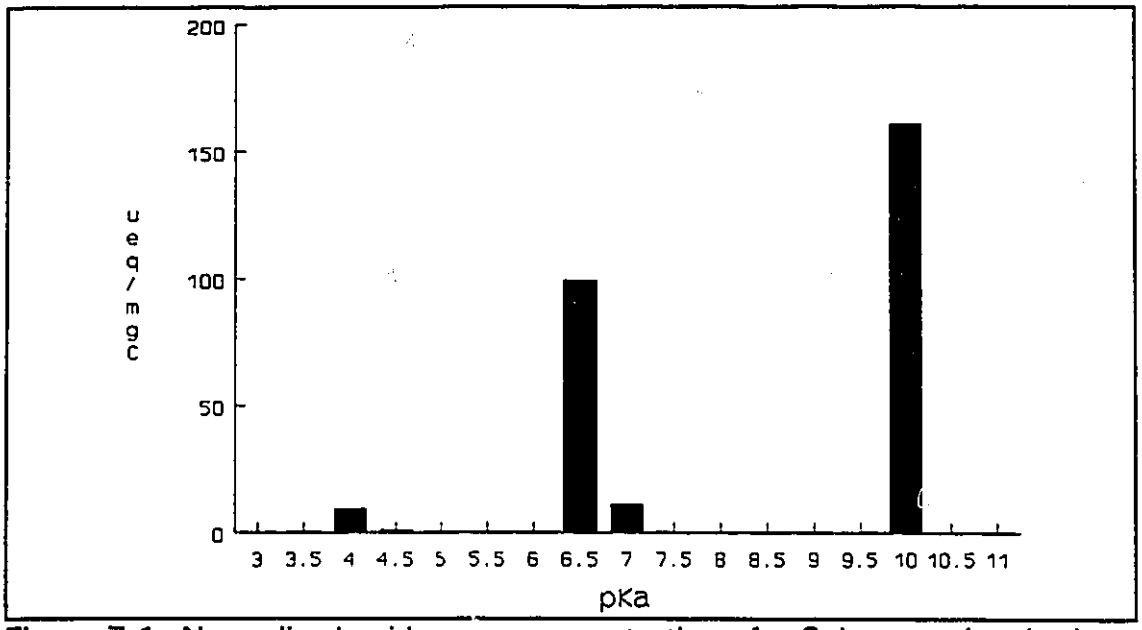

Figure 7.1 Normalized acid group concentrations for Sphagnum incubation.

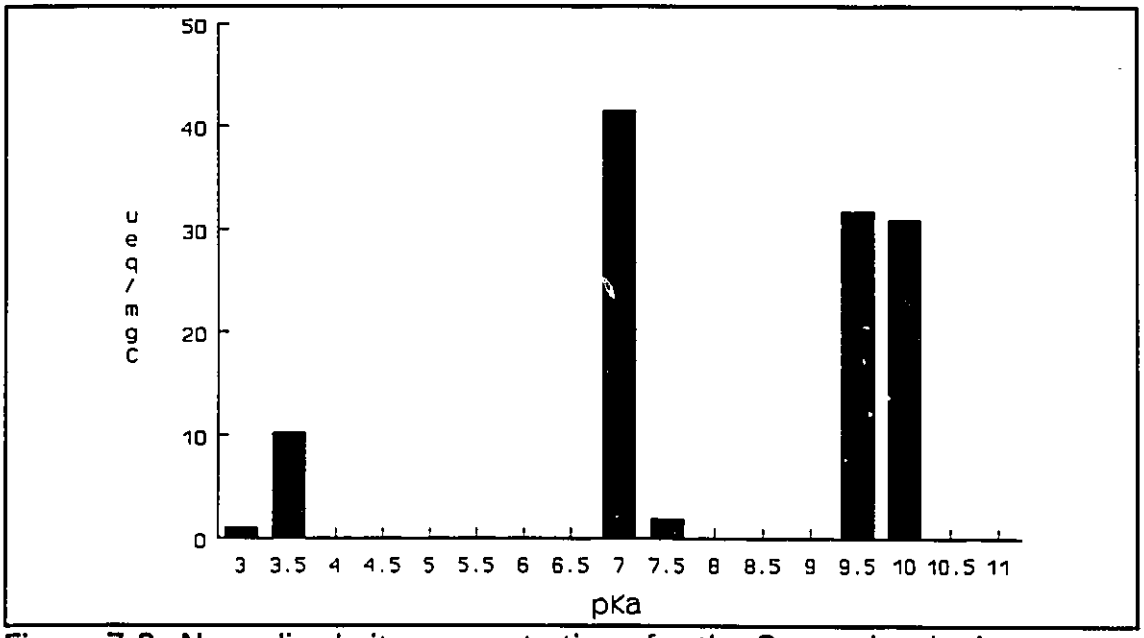

Figure 7.2 Normalized site concentrations for the Spruce incubation.

Another possibility is that mosses already contain non-structural aromatic material which then leaches out of the parent material when incubated.

The ^{13}C NMR spectra from the spruce and the Sphagnum are quite similar to each other (Figure 7.3). Though some difference was expected between treatments, the actual results were unexpected, as the Sphagnum sample again showed a higher aromatic composition than the Spruce. As mosses are mostly carbohydrate, little aromatic C should have been measured from this sample. The reverse occurred, as the moss incubation not only produced a higher aromatic fraction than any measured in the field samples, but also produced a greater fraction than the Spruce. This result is similar to that from the affinity spectra. It thus seems that the higher aromatic fraction in the Sphagnum is also producing higher phenolic site concentrations. The reasons for this will be discussed further in Chapter 9.

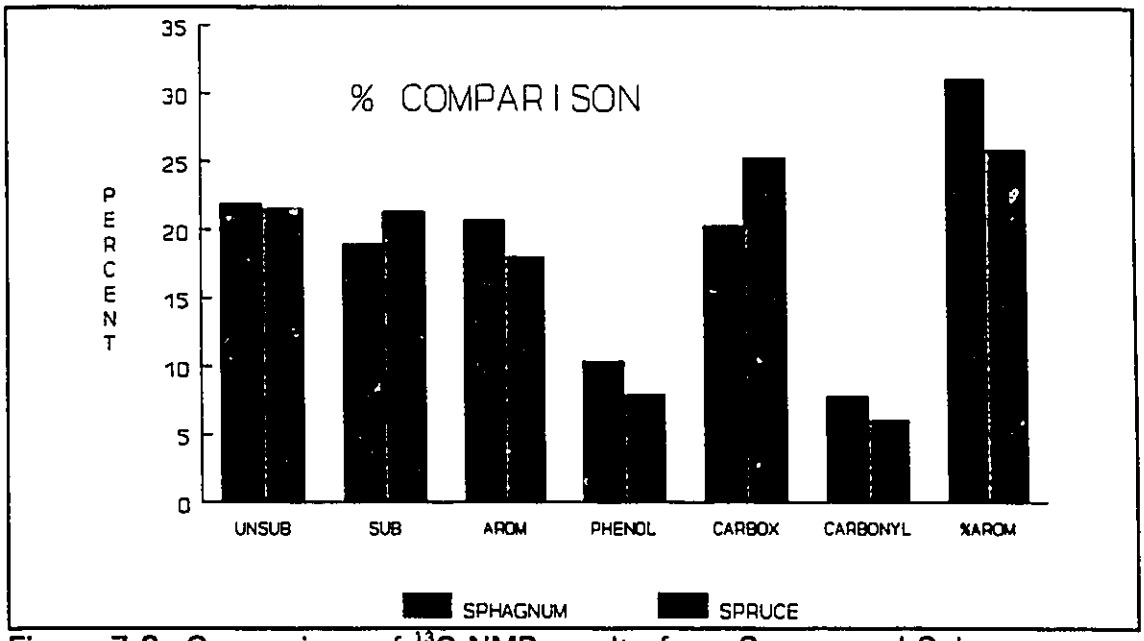

Figure 7.3 Comparison of ^{13}C NMR results from Spruce and Sphagnum incubation experiment.

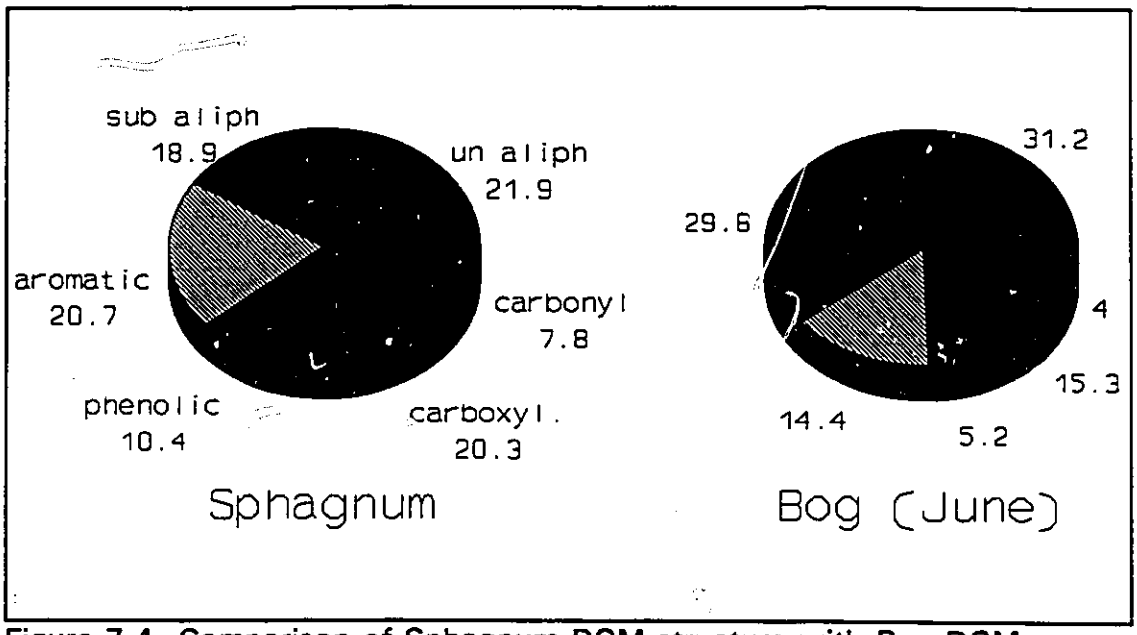

Figure 7.4 Comparison of Sphagnum DOM structure with Bog DOM.

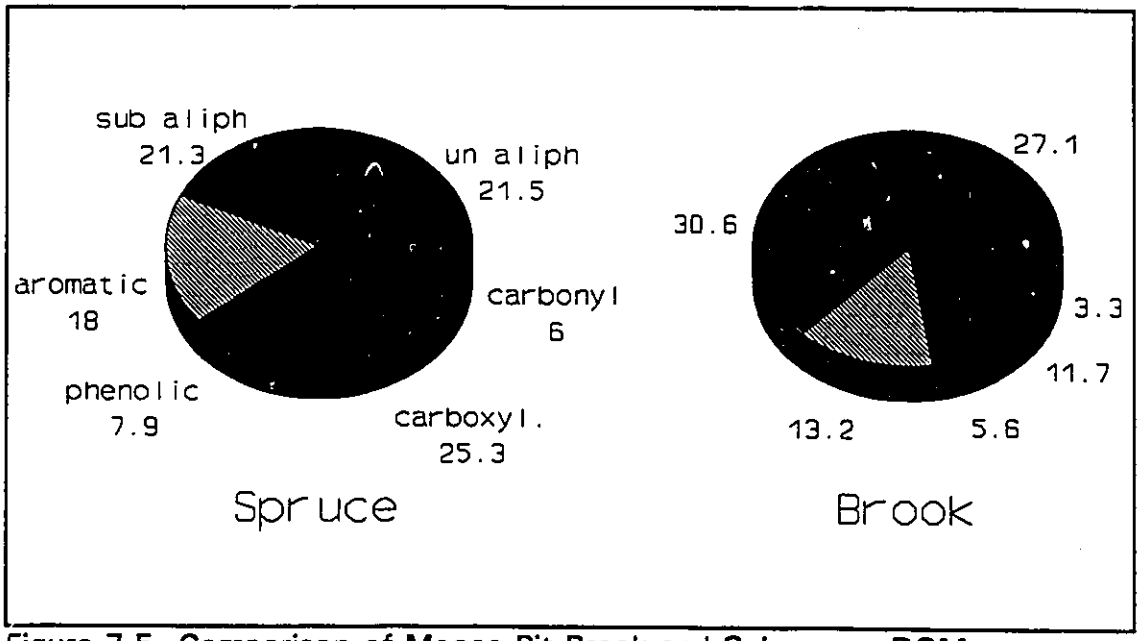

Figure 7.5 Comparison of Moose Pit Brook and Sphagnum DOM.

Results from the incubation experiment were also studied to see if the structural composition of the DOM produced could be related to that extracted from the treed mire (Bog). The samples did not resemble each other at all (Figure 7.4), suggesting that the water in the Bog was probably subject to a number of natural influences, such as photochemical effects, coagulation and precipitation with cations and temperature driven chemical reactions which did not occur in the laboratory incubation. The water sample which most closely resembled that of the incubation material was from the November Moose Pit Brook sample (Figure 7.5). This occurred during a high runoff period, when organic matter was being washed out of basin bogs. This sample may represent the most likely result of organic matter decomposition in the basin making its way to a first order stream.

7.2 Streamwater Incubations

This section reports results from the three experiments (described in Table 2.2) which were conducted to determine how and why the acid-base properties of freshwater DOM seem to change while in streams and lakes. The purpose of the work is thus to attempt to modify the acid-base characteristics of DOM by simulating field situations under controlled laboratory conditions. In order to better understand the potential changes which could occur to organic matter, biological activity was also monitored as measured using enzymes, as was major ion chemistry.

7.2.1 Water Biological Activity

Enzyme activities of the water in sterile controls were measured in Experiments # 1 and 3. No activity was detectable in the sterile samples throughout Experiment 1, and only slight differences in activities were measured in water between non-sterile aquaria which did or did not contain sediments. Of the 19 enzymes tested for using the API ZYM ® system, only seven were positive, most of them esterase/lipase and phosphatase. None of the eight carbohydrases and only one of the peptidases were positive. The activity of most enzymes increased rapidly during the first part of the dark experiment, reached a peak on Day 42 in Experiment 1 and Day 50 for Exp. 3 (Figures 7.6, 7.7) and declined thereafter, while the peak occurred on Day 101 in the light treatment (Figure 7.8).

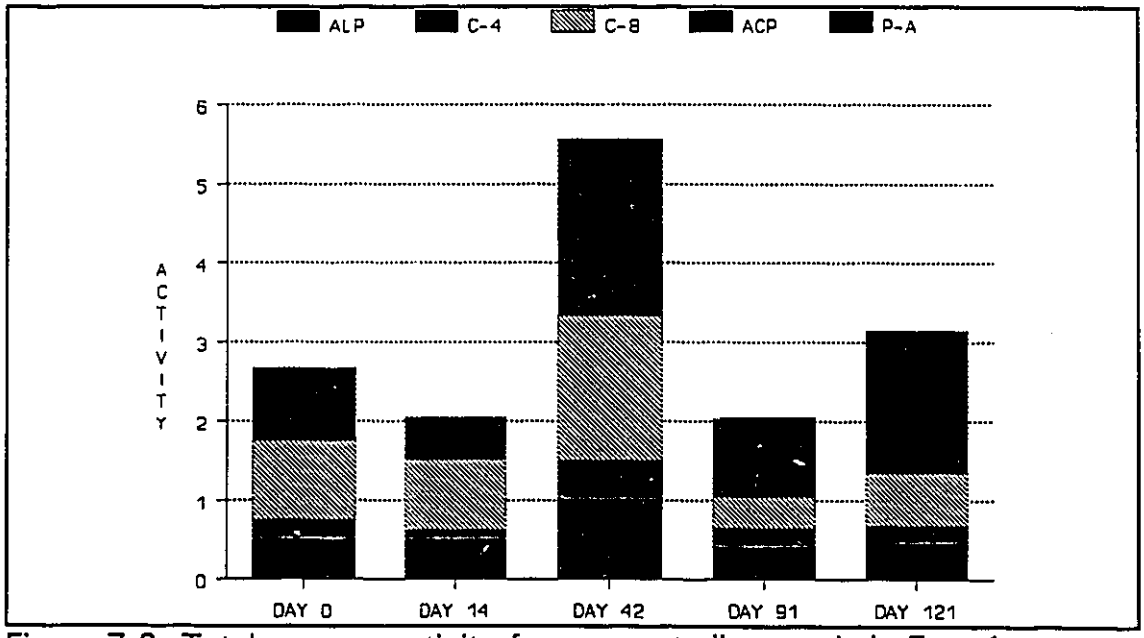

Figure 7.6 Total enzyme activity from non-sterile aquaria in Exp. 1.

The flasks containing sterilized water in Exp. 3 also became biologically active over the course of the study. Enzyme activity in both sterile and non-sterile flasks were almost identical on Days 50 and 131, for both light and dark conditions, causing me to believe that the 0.2 μm filtered samples were not sterilized. The cause of this problem could lie in inadequate care in the preparation of the flasks or filtration of the samples, or else in the presence of very small bacteria (nanoplankton) not removed by the filtration process (W. Li, Bedford Inst. Oceanography, pers. com.). As the phenomenon occurred with all the samples, and not simply just a few, I think that the latter explanation is most likely. This result casts some doubt on the interpretation of the results from the sterile aquaria during Exp. 2, as no enzyme measurements were made.

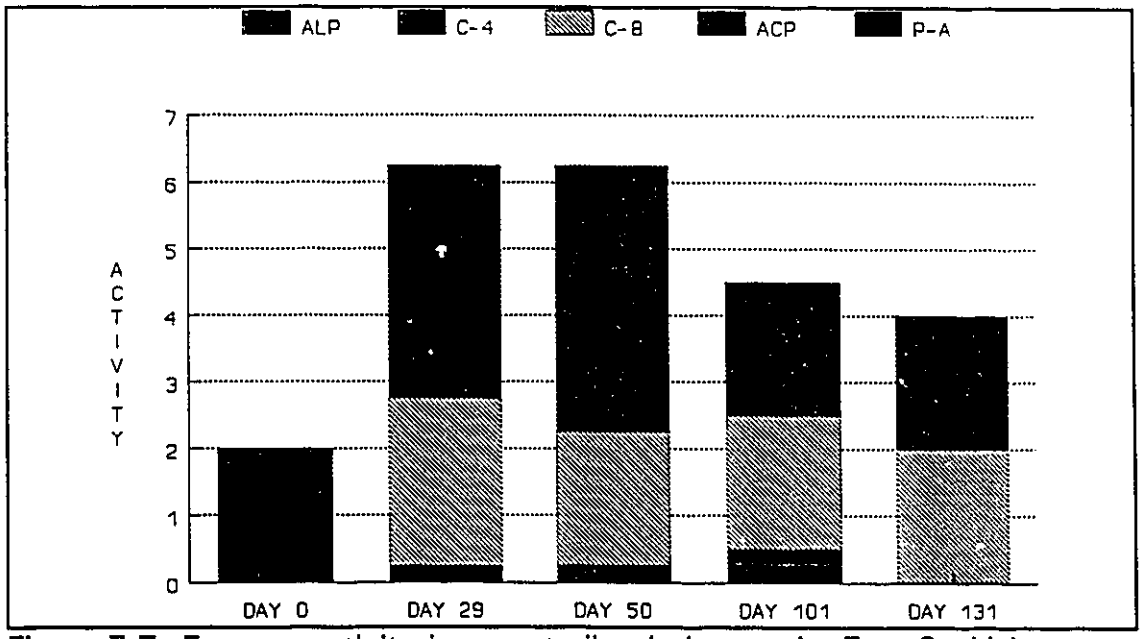

Figure 7.7 Enzyme activity in non-sterile, dark aquaria, Exp. 3. Values shown are totals of the two replicates.

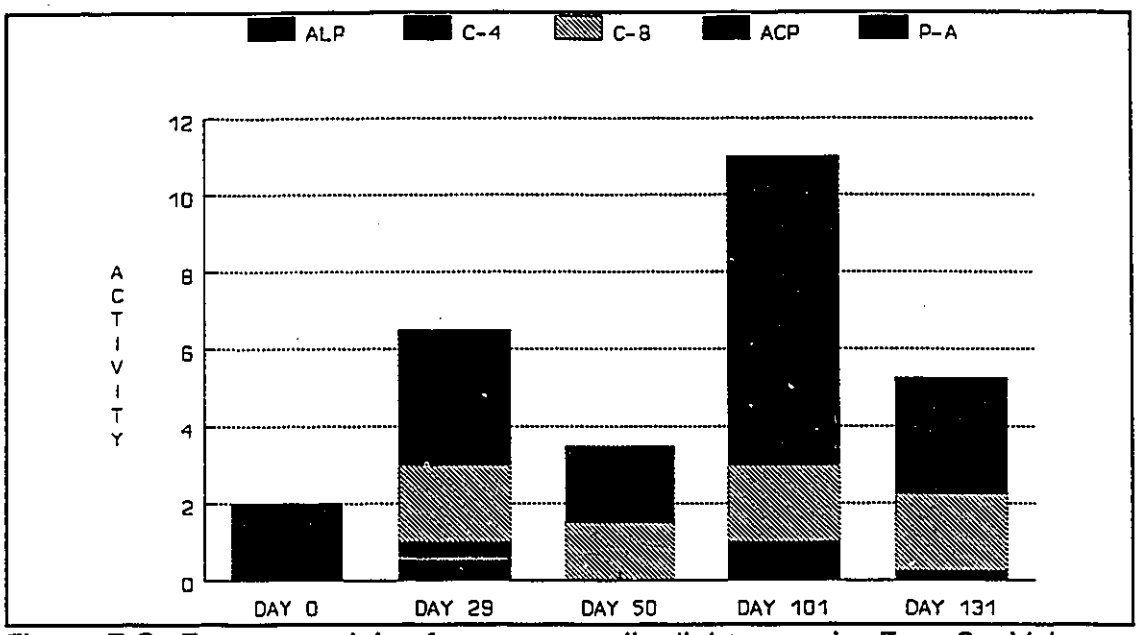

Figure 7.8 Enzyme activity from non-sterile, light aquaria, Exp. 3. Values shown are totals of the two replicates.

There was surprisingly little difference in enzyme activity and composition between the dark and light non-sterile flasks from Exp. 3 (Figures 7.7, 7.8), though Enzyme 12 (phospho-hydrolase) was slightly higher in the dark samples than in the light, and measured enzyme activity peaked later in the lighted flasks than in the dark ones. These results suggest that similar microbial processes were active in both light and dark flasks. I expected that photosynthesis would generate different enzymes than biological oxidation, however the enzymes measured did not differ between the treatments. The main reason for this may simply be that the enzymes measured by the API-ZYM® kit did not include those that would be implicated in environmental situations, as the kit was developed mostly for hospital use.

The enzyme activity thus presented a picture of increased microbial activity in the water samples peaking within 40 to 50 days in the dark

aquaria, and near 100 days in the light ones. It was then expected that water chemistry and DOM affinity spectra changes would follow similar patterns as enzymes.

7.2.2 Major Ion Chemistry

The water chemistry results of the initial samples for the two incubations where DOM affinity spectra were calculated are displayed in Table 7.1. These show values typical for the Moose Pit Brook site. pH is low and DOC is high in Exp. 2, while DOC is somewhat lower in Exp. 3 perhaps due to a dilution effect from spring meltwater.

Table 7.1 Selected water chemistry values for initial water samples from Experiments 2 and 3. ANC values are in μeq/L and other parameters in mg/L.

Exp. #	pH	ANC	DOC	DIC	SO_4^{-2}	Cl^-
2	4.8	-43.0	9.0	0.3	3.8	4.4
3	4.9	58.3	7.4	0.6	2.9	3.6

As some of the waters were sterilized by filtration, chemical analysis of the 0.2 μm filtered water at the beginning of the experiment revealed no differences in any chemical parameter between raw and filtered water, so that chemically, though not biologically, the water quality at the start of the experiment was similar for both sterile and non-sterile microcosms.

Changes in pH and ANC over time for both Exp. 1 and 2 in the sediment-water aquaria were reported by Clair et al. (1989), and followed the same increasing trend. The pH of the non-sterile microcosms increased rapidly

within the first month of the experiment, matched by increases in ANC. The sterile water-sediment samples on the other hand, showed little change in both cases, suggesting in Experiment 1 and 2 that microbiological activity, was the determining factor in changing these parameters. Further analysis of the experimental set-up however, revealed that the line used to aerate the aquaria was contaminated with marine salts, and thus the increases in cations and bicarbonate measured were probably in error.

Major ion results from Exp. 3 (Table 7.2), were stable except for DIC, an indicator of CO_2 production by bacteria. It should also be pointed out that ANC values from Day 29 are suspect due to problems of titration acid and base calibrations which could not be resolved in discussions with the laboratory staff. One value from Day 50 and one from Day 101 of the dark non-sterile, were discarded (along with the matching pH values) due to obvious contamination problems probably caused by dirty glassware.

The pH of the dark non-sterile samples of Exp. 3 (Table 7.2a) did not increase significantly, nor did that of the dark "sterile" pH (Table 7.2b), suggesting again that contamination from the aeration system may indeed have been the cause of the pH increase noted in Exp. 1 and 2.

In both Exp. 3 light set-ups (Tables 7.2c, d), pH increased slightly from a mean value of 4.9 to 5.11 and from 4.9 to 5.5 in the non-sterile and "sterile" respectively. The pH changes were not matched by changes in ANC (Figure 7.9), as this parameter varied greatly over the experiment. It can be seen however that there may be some contamination in Day 50 samples in the "sterile" samples, as a number of parameters increased in value, so that few solid conclusions can be drawn with this particular set of samples.

ANC in all microcosms decreased with time, perhaps due to a reduction in the acidity of the organic material. From Table 7.2, it seems obvious that the decrease was not due to decreases in bicarbonate buffering, as DIC was relatively stable over the course of the study.

Table 7.2a Major ion chemistry from dark microcosms, Exp. 3

Day	Na	K	Ca	Mg	pH	Cl	SO4	ANC	DOC	DIC
0	2.37	0.61	0.66	0.48	4.9	3.58	2.83	66.8	7.6	0.5
0	2.4	0.47	0.63	0.47	4.8	3.55	2.89	49.8	7.2	0.6
29	2.45	0.51	0.66	0.51	4.7	3.58	3.13	-68#	6.9	0.2
29	2.48	0.48	0.64	0.51	4.8	3.46	2.75	- 199#	7.0	0.3
50	2.39	0.45	0.68	0.51	5.0	3.56	2.7		6.1*	1.0
50	2.68	0.48	0.64	0.51	6.8*	3.61	3.22	13.6 635*	7.6	7.63*
101	3.01	0.62	1.67*	0.74*	5.9*	7.53*	3.97		7.4	0.94
101	2.79	0.48	1.08	0.51	4.9	3.46	3.3	80.1* 30.8	7.6	0.65
131	nd	nd	nd	nd	4.9	nd	nd		nd	nd
131	nd	nd	nd	nd	4.9	nd	nd	8.8 18.3	nd	nd

* - contaminated sample. ANC values are in μeq/L and all others in mg/L.
# - titration base problems

Table 7.2b Major ion chemistry from sterile dark microcosms, Exp. 3

Day	Na	K	Ca	Mg	pH	Cl	SO4	ANC	DOC	DIC
0	2.37	0.61	0.66	0.48	4.9	3.58	2.83	66.8	7.6	0.5
0	2.4	0.47	0.63	0.47	4.8	3.55	2.89	49.8	7.2	0.6
50	2.68	0.45	0.68	0.53	5.0	3.72	3.05	15.5	7.9	0.39
50	2.6	0.44	0.68	0.52	4.9	3.58	2.79	15.1	7.9	0.45
131	nd	nd	nd	nd	6.0*	nd	nd	383	nd	nd
131	nd	nd	nd	nd	4.7	nd	nd	*15.7	nd	nd

* - contaminated sample

Table 7.2c Major ion chemistry from light microcosms, Exp. 3

Day	Na	K	Ca	Mg	pH	Cl	SO4	ANC	DOC	DIC
0	2.37	0.61	0.66	0.48	4.9	3.58	2.83	66.8	7.6	0.5
0	2.4	0.47	0.63	0.47	4.8	3.55	2.89	49.8	7.2	0.6
29	2.65	0.89	0.71	0.50	5.0	1.76*	1.56*	-159	6.5	0.2
29	2.53	0.54	0.66	0.56	4.9	3.65	2.75	-90	6.6	0.2
50	2.55	0.48	0.68	0.53	5.0	nd	nd	17.2	6.6	0.61
50	2.71	0.41	0.66	0.52	5.2	3.68	2.62	26.3	6.6	0.43
101	2.86	0.5	0.73	0.50	5.1	3.84	3.07	25.1	6.2	0.65
101	3.5	0.39	0.77	0.48	5.1	4.43	2.99	30.8	7.6	0.88
131	nd	nd	nd	nd	5.1	nd	nd	47.5	nd	nd
131	nd	nd	nd	nd	5.1	nd	nd	42.4	nd	nd

* - contaminated sample, nd - not done

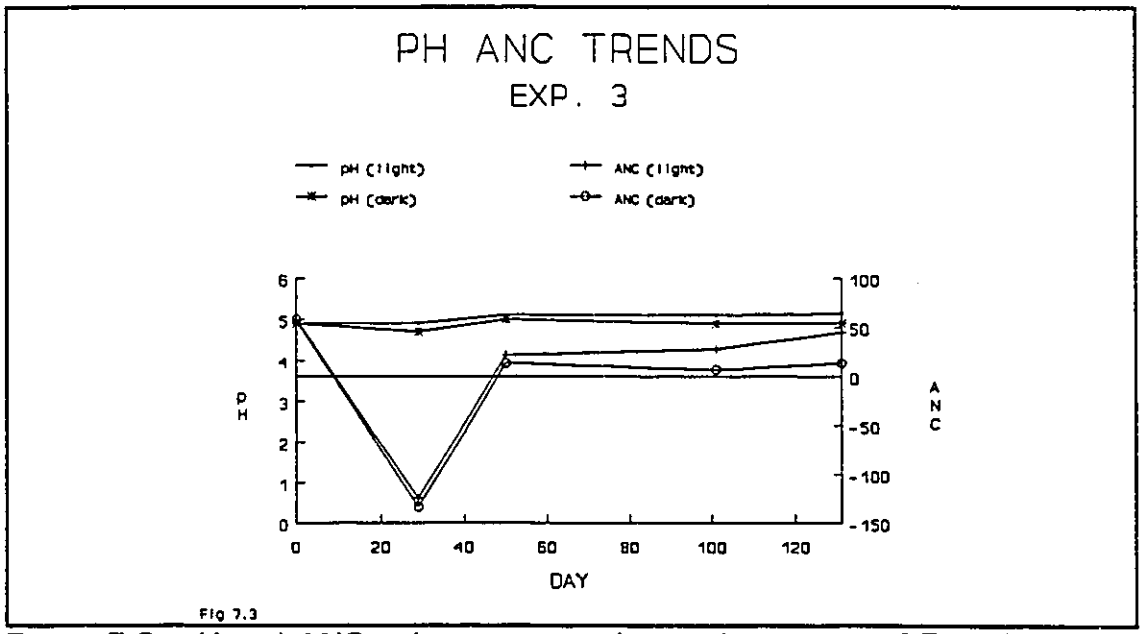

Figure 7.9 pH and ANC values measured over the course of Experiment 3.

Table 7.2d Major ion chemistry from sterile light microcosms, Exp. 3.

Day	Na	K	Ca	Mg	pH	Cl	SO4	ANC	DOC	DIC
0	2.37	0.61	0.66	0.48	4.9	3.58	2.83	66.8	7.6	0.5
0	2.40	0.47	0.63	0.47	4.8	3.55	2.89	49.8	7.2	0.6
50	2.61	0.44	0.83	0.51	5.1	3.69	3.09	19.6	7.0	0.8
50	3.02	0.44	0.71	0.55	5.4	3.85	3.04	28.7	6.6	0.46
131	nd	nd	nd	nd	5.7	nd	nd	43.8	nd	nd
131	nd	nd	nd	nd	5.3	nd	nd	49.3	nd	nd

nd - not done

DOC was also monitored over the experiment, except for the last sampling day when no Carbon Analyzer was available. Overall no DOC concentration trend could be detected. The lack of major ion trends in Exp.3 thus confirmed that those measured in Exp. 1 and 2 were due to contamination from the aeration system. Uncontaminated samples showed no increase in any parameter over the course of the study, except for pH. Its change was slight and gradual, and did not seem to match that of the enzyme activity.

7.2.3 Affinity Spectra

Affinity spectrum calculations from both non-sterile and sterile aquaria during Experiment 2 (Clair et al. 1989), where all samples were under dark conditions, showed that the carboxylic acid sites disappear within 2 months to be replaced by phenolic sites (Figures 7.10, 7.11). Midway through the experiment on Day 51, a mixture of carboxyls, mid-range groups and phenolic acids appeared which eventually led to DOM which contained on Day 124, mostly phenolic groups.

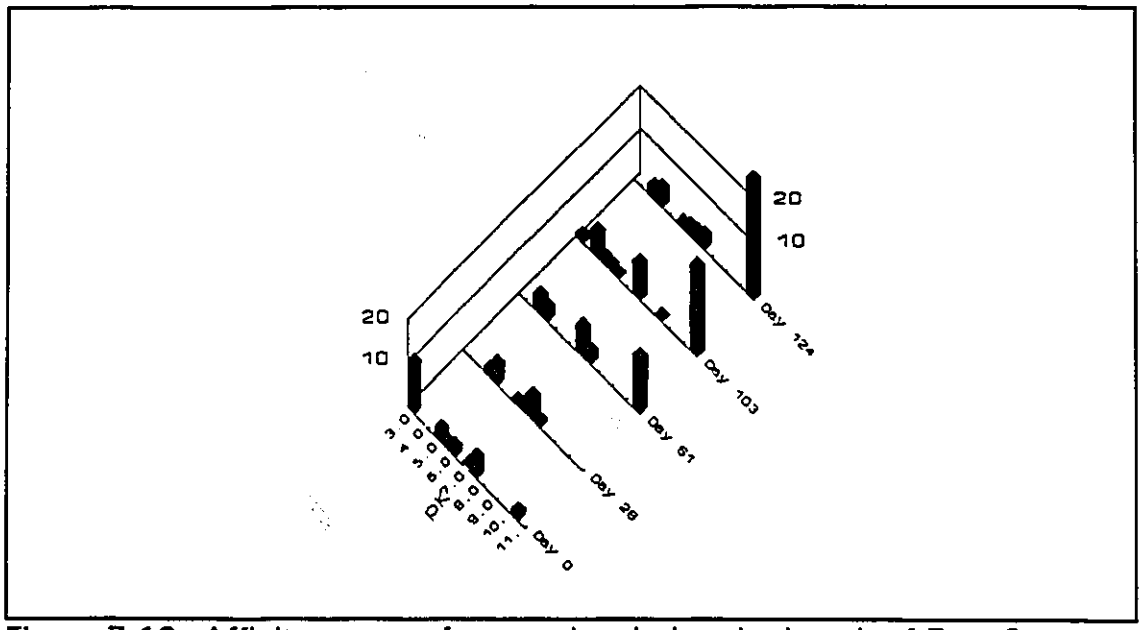

Figure 7.10 Affinity spectra for samples during the length of Exp. 2. Values are normalized for DOC concentration.

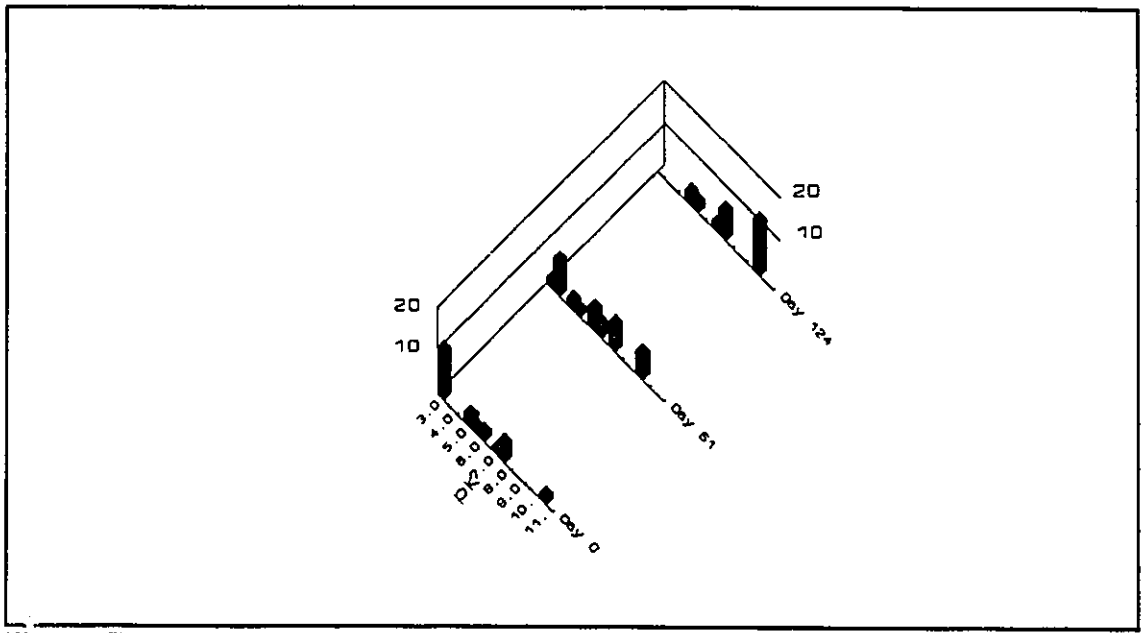

Figure 7.11 Affinity spectra for sterile samples from Exp. 2. Values are normalized for DOC concentration.

The affinity spectrum results from Exp. 3 revealed different results. First, except for the two obviously contaminated samples, duplicate spectra were generally similar, so that the results of duplicates were averaged to produce the Figures. Secondly, the DOM used on Day 0 of Exp. 3 had lower carboxylic and higher phenolic content than the DOM from Exp. 2, and also showed approximately 8 $\mu eq*mg^{-1}C^{-1}$ of sites at pK_a 7-7.5, compared to approximately half this value in Exp. 2.

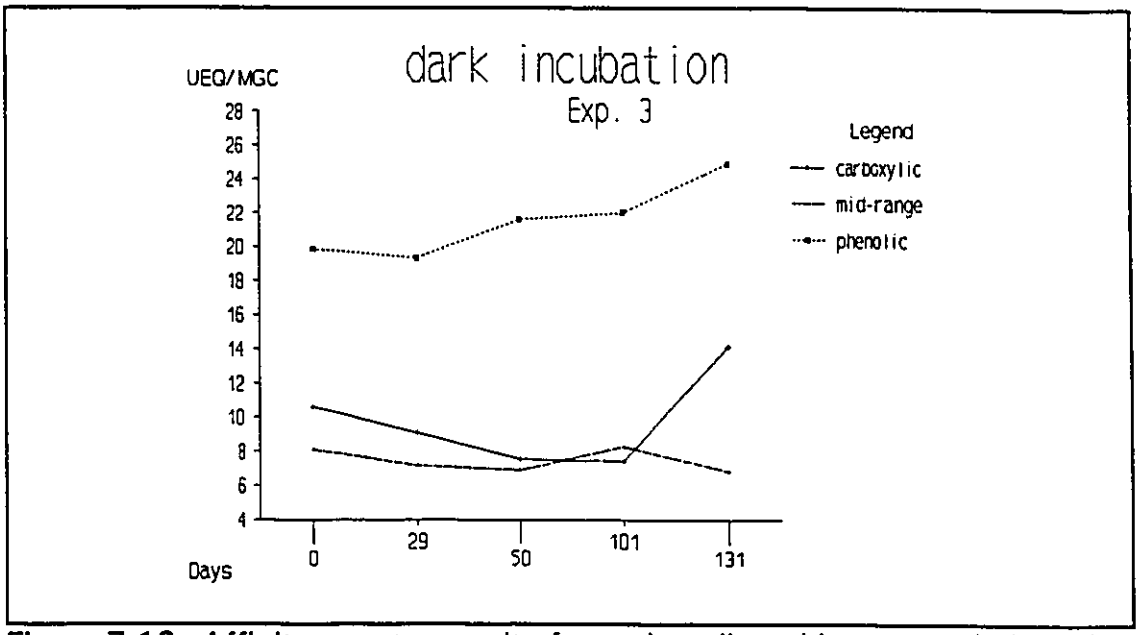

Figure 7.12 Affinity spectra results for carboxylic, mid-range and phenolic acids in the dark non-sterile microcosms in Exp. 3.

The dark non-sterile experiment (Figure 7.12) followed a trend different from that in Exp. 2, as carboxylic sites decreased over most of the experiment, only to increase at the last sampling. As both duplicates followed this trend, it is quite difficult to explain. Middle pK_a groups decreased slightly or remained stable, while higher pK_a site concentrations increased over time, but not as dramatically as with the previous experiment. The difference

between Experiments 2 and 3 was perhaps due to the differing starting acid-base conditions of the DOM, or to differences in microfauna which was acting on it.

The Exp. 3 light samples showed different results from the dark. Carboxylic acid concentration decreased with time for both the "sterile" and non-sterile treatments (Figures 7.13, 7.15), as did mid-range values. Phenolic groups however, decreased unlike the increase seen in the dark aquaria in both Experiments 2 and 3. This decrease of phenolic sites could be due to an inhibition effect from photosynthetic processes, or it could be that oxidized DOM is reused by phytoplankton to produce released organic carbon (ROC) (Feuillade et al. 1988). Much of the ROC is thought to be due to the lysis of cells in water and is thought to be quickly taken up by bacterioplankton (Chrost and Faust 1983). The fraction not taken up, which would have been measured in the lighted treatment, seems to be acid neutral, as shown by the decrease in phenolic site concentrations. It can also be that breakdown of cells occurred in the 0.5 μm filtering of samples before titration, thus releasing cellular organic material into the water, which were then analyzed using the affinity spectrum method.

The dark "sterile" treatment (Figure 7.13) which was determined not to have been sterile after all, showed a different pattern from the dark non-sterile sample, as carboxyl and phenolic groups decreased and mid-pK_a groups increased. The so-called light sterile samples also did not show the decrease in phenolics.

Ignoring the "sterile" samples, an interesting pattern emerges which, based on both Exp. 2 and 3 dark microcosms, suggests that bacterial and

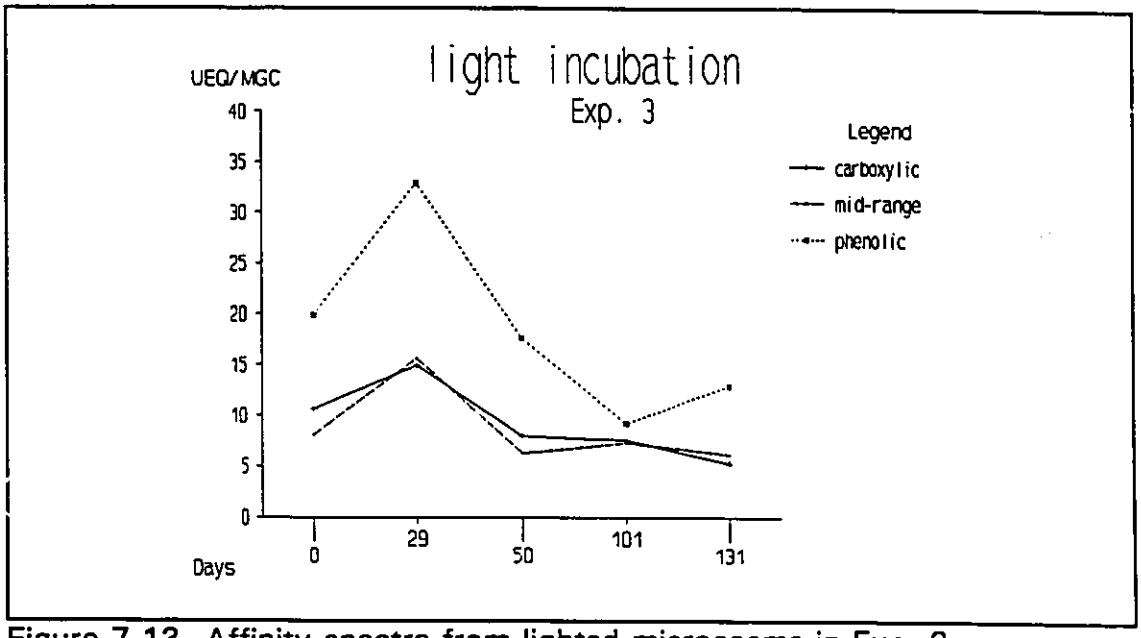

Figure 7.13 Affinity spectra from lighted microcosms in Exp. 3.

fungal activity on DOM, generally tends to reduce its acidity by oxidizing carboxylic acid sites. The increase in carboxyl groups shown in the dark non-sterile samples does not fit in this generalization, but seems to be an unexplainable anomaly. As carboxyls are lost, phenolic sites are formed, though more phenolic sites are generated than carboxylics are lost though two separate activities are probably occurring. First, carboxylic sites are often removed by microbial action. The phenolic sites are simultaneously produced as the DOM becomes more aromatic. The first step is suggested by the data, while the second is in accordance with the general theory of humic material formation suggested by Stevenson (1982).

An analysis of the "sterile" sample complicates the picture described above, as it became obvious that biological activity was present in the flasks of Exp. 3. The light "sterile" samples behaved almost identically to the dark

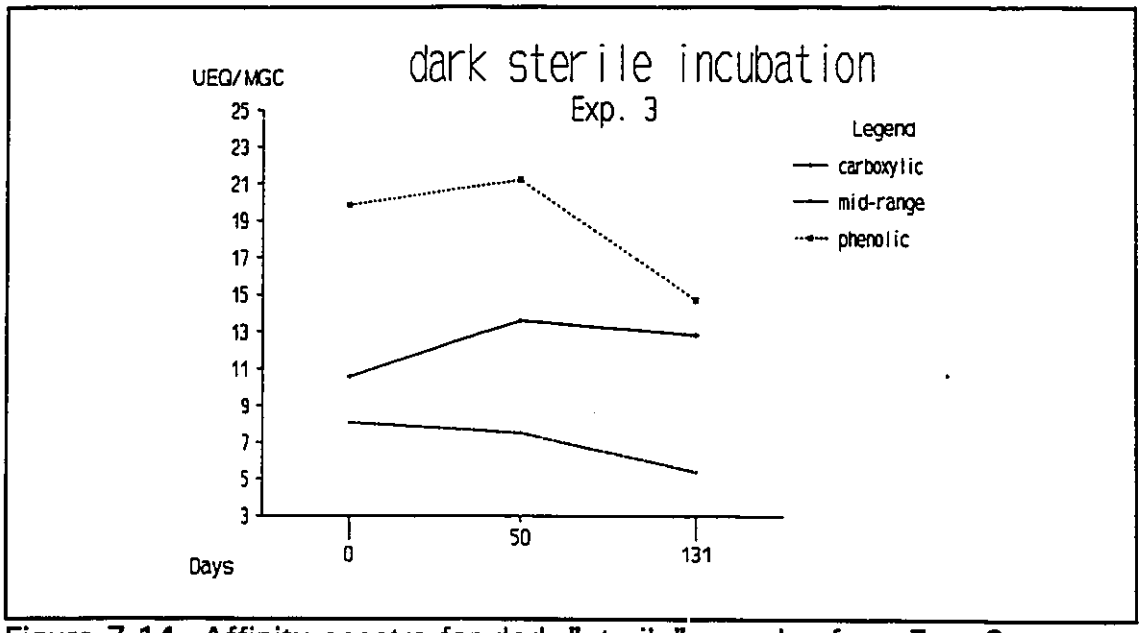

Figure 7.14 Affinity spectra for dark "sterile" samples from Exp. 3.

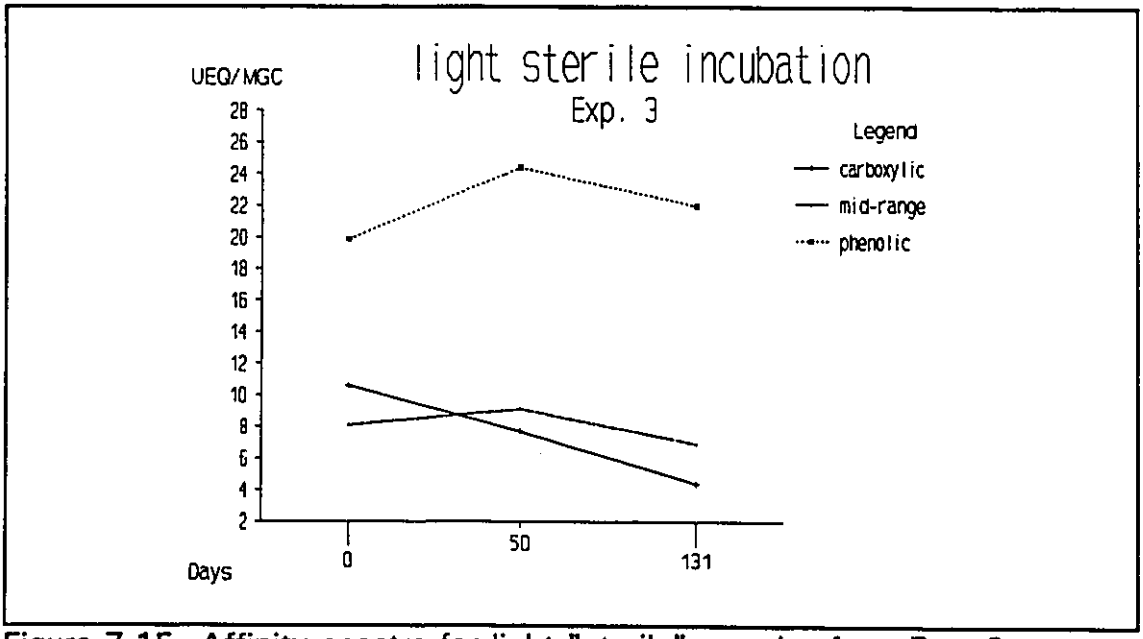

Figure 7.15 Affinity spectra for light "sterile" samples from Exp. 3.

non-sterile, with decreases in carboxyl groups, increases in phenolic halfway through the experiment, with a slight decrease after, and little change in the mid-range groups. It seems that photooxidation, as well as bacteria had some effect in reducing the carboxylic site concentrations. No specific tests were conducted to confirm this idea. However, the 0.2 μm filtering probably removed all phytoplankton, but not decomposers, thus ensuring differences with the non-sterile samples.

Because of measured enzyme activities, the dark "sterile" samples were also known to be biologically active. This suggested that the pattern of stable or decreasing carboxylic acid sites, and increasing phenolic sites would be found. Indeed, this was the case, while mid-range groups increased.

To conclude, the affinity spectra results present a mixed bag of results. The basic trend in DOM modification by microbes seems to be a decrease in carboxylic acids and an increase in phenolic sites as long as primary productivity is not important. This would tend to decrease the acidity of DOM, as predicted by Kramer et al. (1990) who show that as DOM moves from headwater streams to larger rivers, its acidity decreases. When primary productivity becomes more important, carboxylic acids again tend to decrease in importance, as do phenolic sites. Again DOM becomes less acidic, as the effects of biological activity come into play.

The enzyme results showed that no correlation could be drawn between DOM modification and enzyme activities. It may be that the level of the biological breakdown of acid-base sites, or of structural modification is too low to be easily followed using the API ZYM® tests, or that the activity is so low as to be not observable using even specialized techniques. The work of

Chrost (1989) seems to suggest that the latter condition exists.

7.3 Summary of Results and Conclusions

The parent material incubation study demonstrated that the structural and acid-base properties of DOM generated by the biological decomposition of plant matter does not resemble that found in the streams and lakes. Either the processes followed in the laboratory did not accurately simulate those in the field, or the filtration of newly created DOM through soils in basins changes its overall composition. This will be further discussed in Chapter 9.

Despite some differences between streamwater incubation set-ups, the results show similarities between experiments as the same enzymes were measured in all experiments, including the supposedly sterile microcosms in Exp. 3. Though this work provides interesting insights into phosphorus cycling of brown waters, enzymes do not seem to provide information on the modification of acid-base or structural chemistry of DOM.

Little other work has been done to determine the change in acid-base properties that could occur to DOM as a factor of in-stream diagenesis and autochthonous production. Visser (1982,1983) showed that differences in C and O concentration existed between organic materials collected in rivers and lakes, with material from the former richer in O and poorer in C than the latter. He related this difference to higher carboxylic acid concentrations in river waters, suggesting that organic matter was more acidic closer to its terrestrial source of production. This work supports that hypothesis.

These results also probably explain the source of some of the problems

identified in the replicate study. The changes in affinity spectrum distributions over time demonstrate that DOM is not an unvarying substance in solution. Any changes in conditions, such as exposure to light, differences in bacterial populations, or contamination from either unclean bottles or inadvertent matter in a sample may result in unpredictable changes of affinity spectra. A number of precautions, such as rapid filtering of samples, and titration immediately after collection would ensure a reduction of interpretation problems.

CHAPTER 8

DOM AND WATER CHEMISTRY

8.0 Introduction

The results from the field and theoretical work cast new light on the acid-base chemistry of DOM found in freshwaters. In this chapter I discuss how DOM in solution controls the acid-base characteristics of the natural solvent, and how its acidity compares with that contributed by acid precipitation.

Three approaches are used to achieve this goal. The first is to compare the results of the affinity spectrum estimates to those calculated using the Oliver et al. (1983) method. Secondly, the affinity spectrum results are compared to those calculated by difference using the electroneutrality assumptions described in Chapter 3. Finally, the organic acidity calculated using the various approaches is compared to the seasalt corrected SO_4^{-2} values measured in the water to see which of the components was most important in controlling water acidity.

8.1 Natural acidity of DOM

In order to study the first hypothesis, the total and dissociated sample

carboxyl fractions (C_t and A^-) were calculated for all samples using the method of Oliver et al. (1983). It assumes that each mg of DOC contains 10 μeq of carboxyl acid sites (C_t). The mass action dissociation quotient for the ambient proton condition is calculated using the empirical equation:

$$pK_{or} = 0.96 + 0.9pH - 0.039(pH)^2 \quad \text{(Eq. 8.1)}$$

Thus, A^- is calculated by multiplying C_t with the dissociation fraction (α_{or}) described in Equations 5 and 6 in Chapter 3.

The whole approach described above was determined empirically from a number of samples collected from in North America. Because of this, the approach provides an "average" carboxylic site value which could vary somewhat from site to site. Moreover, the approach was calculated mostly from fulvic acids which are an operationally defined fraction of the total DOM.

The total carboxylic acid concentration of a water sample (Carb) was determined using the affinity spectrum calculation, and summing all the sites with pK_{or} values between 3.0 and 6.0 (Perdue 1985). The dissociated fraction (Car-) was calculated by multiplying each site concentration by the dissociation fraction at the ambient pH, and summing the ionic sites in the range described above. Also estimated, was the organic anion concentration (Or^-) calculated by difference using the approach described in Equation 7 of Chapter 3.

The affinity spectrum calculation estimates the total number of carboxylic acid sites in the sample (Carb), and should be equivalent to the Oliver et al. (1983) value for C_t. A^-, Car- and the calculated (Or^-) were thought to produce roughly similar numbers, as they are all estimators of the

dissociated carboxylic acid concentrations. The values for all five variables at the four sites are shown in Figures 8.1, 8.2, 8.3 and 8.4. Table 8.1 summarizes the origin and calculation procedure for each symbol used.

Table 8.1 Summary of calculation approaches used to quantify anion concentrations from the sites.

Symbol	Calculation	Reference
Ct	Total organic anion sites: DOC*10	Oliver et al. 1983
A-	$Ct*\alpha$	Oliver et al. 1983
Carb	Affinity Spectrum calculated value for total carboxylic acids: $\Sigma Carb_i$	Brassard et al. 1990
Car-	Dissociated carboxylic acids: $\Sigma Carb_i*\alpha_i$	Brassard et al. 1990
O- (Or^-)	Organic anion conc. calculated by diff.	Equation 7, Chapter 3
SO4	Seasalt corrected sulfate	Watt et al. 1979

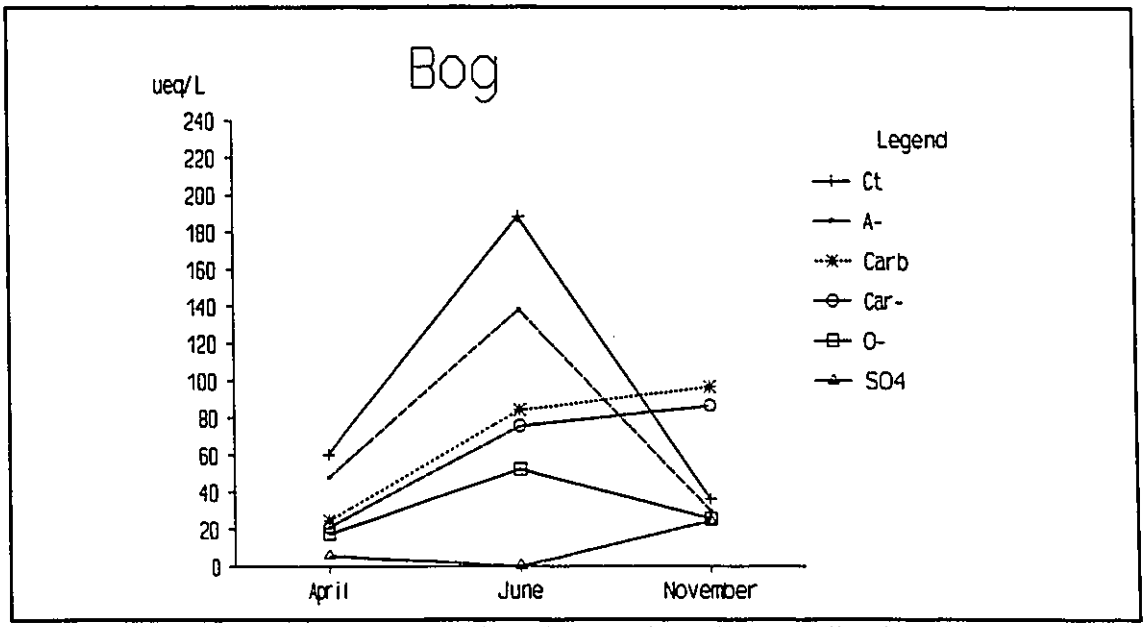

Figure 8.1 Comparative mineral and organic acid contributions at the Bog site.

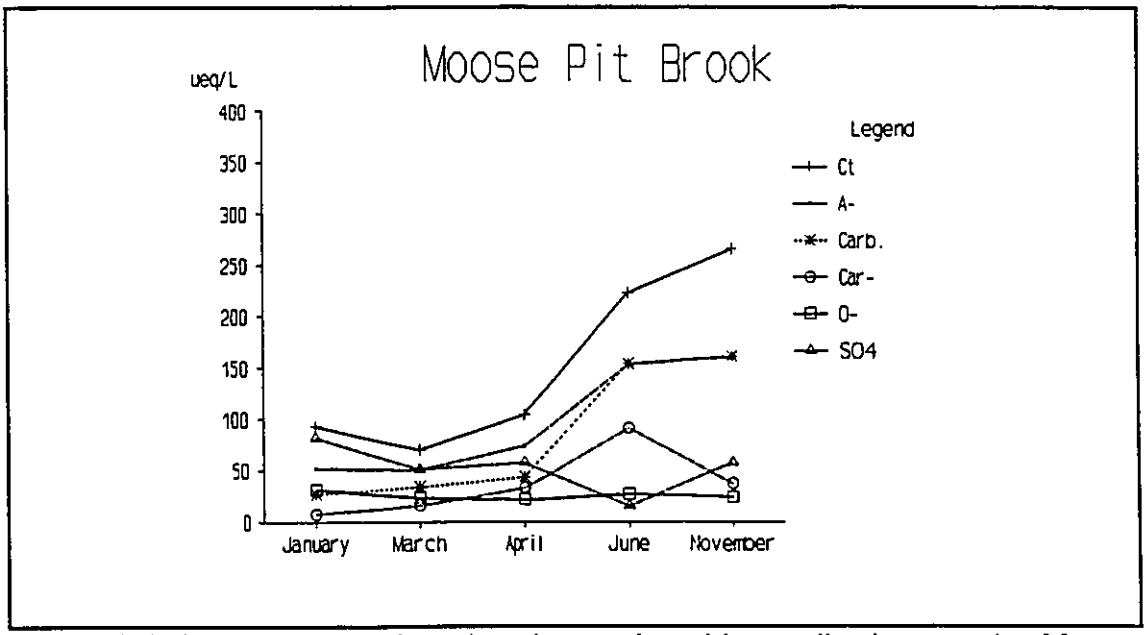

Figure 8.2 Comparative mineral and organic acid contributions at the Moose Pit Brook site.

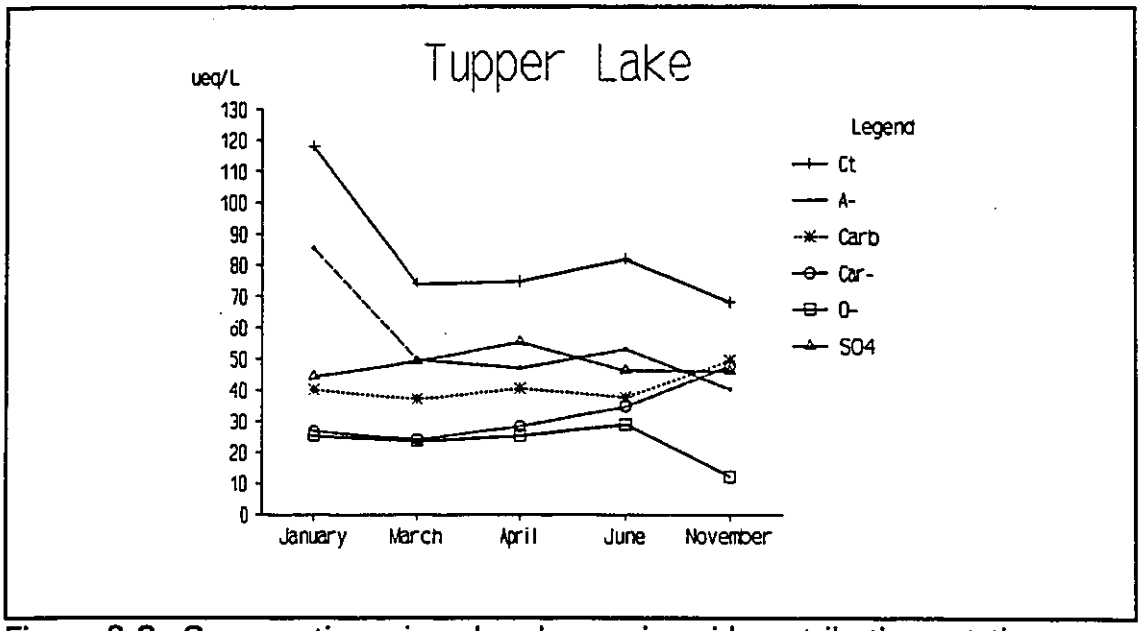

Figure 8.3 Comparative mineral and organic acid contributions at the Tupper Lake site.

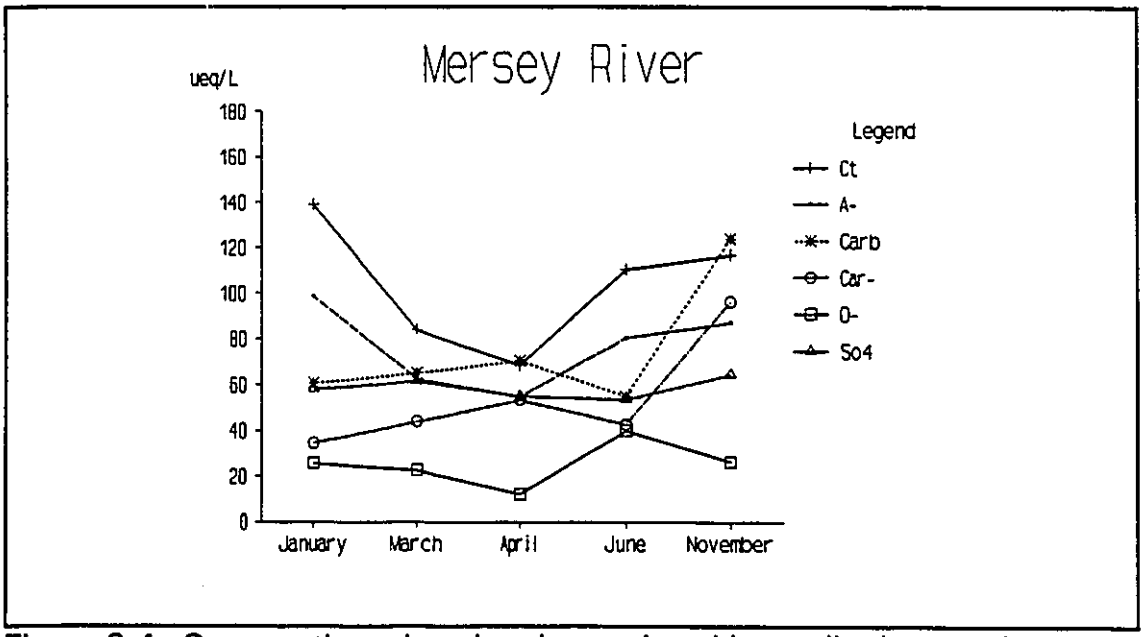

Figure 8.4 Comparative mineral and organic acid contributions at the Mersey River.

As can be seen from Figures 8.1 to 8.4, in almost every case the total carboxylic concentration (C_t) was greater than the affinity spectrum total (Carb) and (A^-) was greater than Car-. Exceptions to this trend occurred occasionally at all sites in, especially in November. Table 8.2 shows the mean difference between the two estimators, as well as the range of values at each site. The differences are graphically shown in Figure 8.5.

As Carb and Car- are directly measured, this shows that the Oliver et al. estimator, which is simply an average value calculated from a number of samples, usually overestimates the total organic anion concentration during most of the year at this site.

The difference between the estimated and measured carboxylic values are not consistent from site to site, nor from time to time, making it difficult to predict carboxylic site concentration in samples. These results also show that the acid-base quality of DOM at the sites sampled, and most likely at most other sites, seems to vary a great deal. This suggests that soil and water processes as well as hydrological fluxes which vary throughout the year, especially in temperate environments, continually modify the impact of the DOM found in water. The DOM itself did not change as much, as could be seen by the carboxylic acid concentration normalized for weight (Figure 5.7). Those results showed great variability in Bog samples, but were generally more stable, especially at the lake and Moose Pit Brook sites. In all non-bog cases though, the main trend detected in Carb and thus Car- was of lower values in the winter, increasing in the fall.

Table 8.2 Data summary of the percent difference between total carboxylic concentrations (Col 2), dissociated carboxylic acids (Col 3 and Col 4) at the sites.

Site	% diff. C_t - Carb	% diff. A^- - Car-	% diff. Car- - Or^-
Bog			
Mean	-17.0	-30.0	39.7
Range	-168 to 59	-191 to 45.3	17 to 70
Brook			
Mean	50.2	24.4	-42
Range	30.8 to 70.8	0 to 47	-302 to 70
Tupper			
Mean	48.5	35.2	21.8
Range	27 to 65	-18 to 68.6	2.5 to 74
Mersey			
Mean	24.1	26.7	46.1
Range	-2 to 56	-10 to 65	2.8 to 77

These results lead to a number of points which must be taken into consideration when assessing the importance of organic anions in freshwater solutions. First is the validity of the Oliver et al. (1983) approach. Neglecting results from the Bog where DOM was subject to a different environment than the surface waters, A^- overestimated the dissociated organic anion concentration by roughly 30%, though this figure varied throughout the year. Generally, samples collected in the fall of the year had similar values for A^- and Car-, while those from the winter and spring were more different. Amongst sites, the Tupper Lake samples showed the least variability, while those from the Mersey showed the most.

The reasons for the differences are difficult to understand. The affinity spectrum approach calculates total carboxylic anion concentration on undifferentiated DOM. If the Oliver et al. approach was correct, the carboxyl anion calculated using the affinity spectrum would be nearly equal to the DOC concentration X 10. In fact, A^- is often greater than Car- (Figure 8.5) for most of the year, except for the November samples at all sites excluding the Bog. The difficulty in estimating A^- resides in the way that it was originally calculated. Oliver et al. used purified fulvic acids from different sources which they titrated to determine an "average" number of carboxylic sites. There are a number of problems with this approach which must be addressed. First, fulvic acids are not very representative of total DOM. P. Takats (McMaster Univ. pers. com.) has shown that when fulvic acid is produced by acidifying solutions and XAD extraction, acid functional sites not found on the original material are generated, thus increasing C_t. This is probably due to breaking larger molecules into smaller, and by uncomplexing metals from carboxylic sites on the organic matrix. As fulvic acids are the most reactive portion of the DOM, it is then not surprising that a discrepancy has appeared. The Oliver et al. (1983) estimation obviously does not describe actual conditions.

The Or^- (O^- in Figure 8.1 to 8.4) calculated by difference should have provided roughly the same value as Car-, as it estimates the "average" undissociated carboxylic acid activity in solution. In most cases, the Or^- value is somewhat smaller than the Car-. The only exception to this occurs in Moose Pit Brook in the winter and early spring, when Car- is greater. Despite the percent difference between them (Figure 8.6, Col 4 of Table 8.2), the

absolute values are not very different from each other at these two dates (Figure 8.2). An examination of the differences between these two parameters, shows them to vary between sites, and usually being greater in November than during the rest of the year. In fact, despite Car- increasing at all sites in November, Or^- concentration decreased everywhere, showing that some solution factors are not taken into account when simply describing the DOM itself.

The difference often measured between Car- and Or^- values points out a subtle distinction which must be made in understanding the acid-base effect of DOM in freshwaters. The affinity spectrum carboxylic acid calculation results describe the chemistry of the DOM found in the sample, but not its influence in acid-base chemistry of natural samples. In order to measure Carb and Car-, water samples are acid titrated to pH 3.0 or lower and then base titrated to pH 11. In the acid titration, cations complexed with the carboxylic acids in nature are released, thus exposing sites which were not available in water at normal environmental pH. The Or^- value on the other hand is calculated from the ANC acid titration whose measured inflection point is usually greater than pH 3.5 and thus is on samples which have not necessarily been completely protonated, and thus which have different acid-base properties than could be predicted from knowing the samples' DOM chemistry.

This means that though the acid-base chemistry of the DOM itself can be described, its behaviour in a natural solution of different ionic strength and composition cannot be easily predicted. This latter property can more easily be estimated using the Or^- calculation. Except for the bog site, Or^- remained relatively stable over the study period, though tended to decrease at the

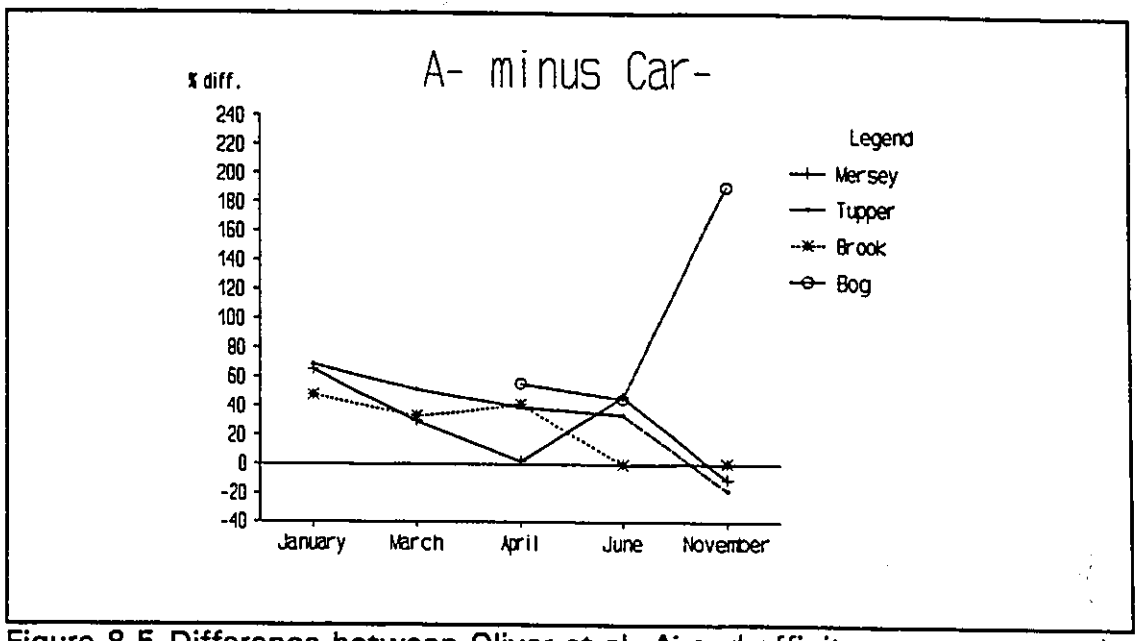

Figure 8.5 Difference between Oliver et al. A^- and affinity spectrum organic anions. The % difference is calculated by dividing the difference by A^- and multiplying by 100.

November sampling even at sites which showed large variabilities in the calculated carboxylic fraction.

The above results thus show that organic anion estimation using the Oliver et al. approach usually overstates the carboxylic acid concentration of a sample, though the exact values are dependant on natural sample variability. The affinity spectrum calculation provides a much better assessment of organic acidity of samples, but though important in describing the nature of the DOM, this value cannot be used to predict organic acid effects in a natural solution. This value is best estimated using Or^-.

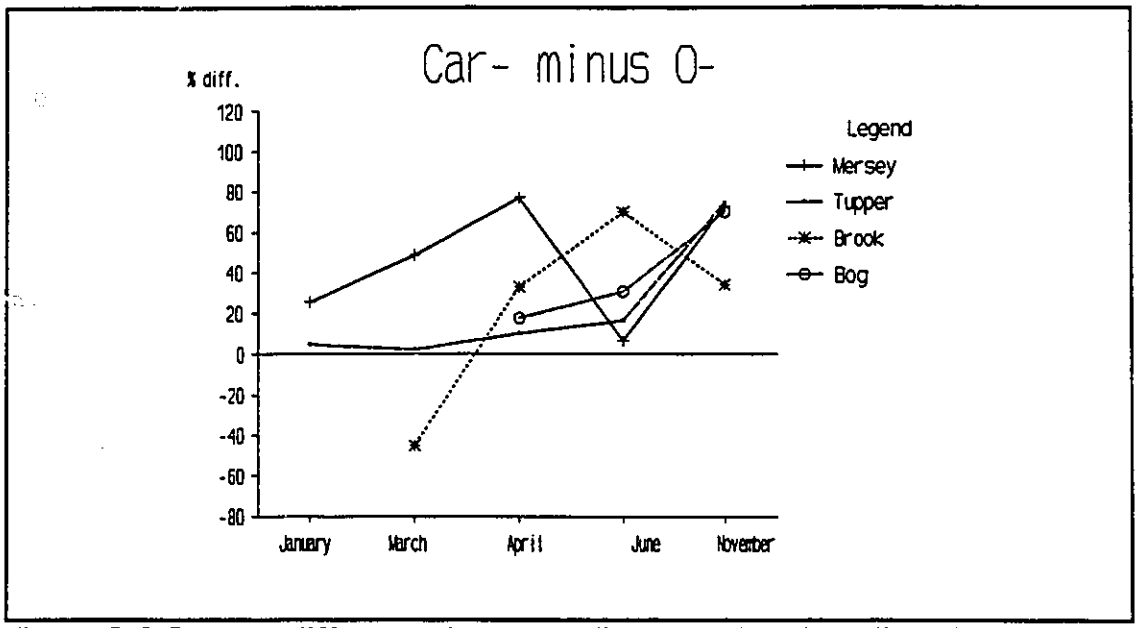

Figure 8.6 Percent difference between dissociated carboxylic acid concentration (Car-) and Or⁻. The January Moose Pit Brook value was -302%.

8.2 Mineral Versus Organic Acidity

In attempting to understand the relative importance of mineral and organic conjugate bases in the freshwater solutions, SO_4^{-2} concentrations (corrected for seasalt) were compared to organic anion concentrations (Or^- value) at the four sampling sites (Fig. 8.1 to 8.4). The seasalt correction was done using the method of Watt et al. (1979). Using stable isotopic techniques, Clair et al. (1989) showed that seasalt corrected SO_4^{-2} realistically described both the seasalt and anthropogenic components in most basins of the region. An exception to this generalization was shown to occur at Roger's Brook, located near the Mersey River, but the bedrock SO_4^{-2} measured in water was also detected by mass balance calculations. At both the Mersey and

Moose Pit Brook sites, mass balances did not indicate that bedrock sources were present (Freedman and Clair 1987).

Except for at the Bog site, there is almost always a consistent pattern of higher dissolved SO_4^{-2} than Or or Car-. The exceptions occurred in the Bog samples where SO_4^{-2} was very low, and in June at Moose Pit Brook and the Mersey river. It may be that sulfur in the bog is incorporated into vegetation or is chemically reduced, thus decreasing its importance in the acidity balance.

It can thus be shown that the dissociated organic acid fraction of these waters is generally lower than the mineral acidity (as represented by SO_4^{-2}) throughout most of the year. This pattern is reversed in the June sample, and probably also during most of the summer, when organic acidity predominates because of greater DOC concentrations. This means that despite the presence of high levels of organic acids, SO_4^{-2} originating from acid precipitation is still the main component of water acidity during winter, spring and late fall. Organic acids are more important mostly during the summer when DOC concentrations are at their highest.

8.3 Conclusions

These results demonstrate the difficulties of generalizing about organic acidity of freshwaters. As could be seen in Chapter 7, the carboxylic acid concentration of a sample will vary over time, as biological and chemical reactions modify it. Because of this, the Oliver et al. (1983) approach cannot produce consistently useful information in describing the organic acidity of freshwaters.

The affinity spectrum approach (Brassard et al. 1990) on the other hand, provides a good description of the acid-base chemistry of DOM, allowing an accurate picture of material's chemical composition. The patterns shown here indicate that DOM organic acidity at the sites was relatively stable though it increased at all sites in the fall, due to higher DOC concentrations in the water. With further work along the lines of the experiments described in Chapter 7, and more work correlating hydrological to geochemical parameters, a better idea of the origins of this material should be made.

Describing the acid-base chemistry of DOM in a water sample does not necessarily allow a description of its behaviour in solution. This is because of the complexation of cations with carboxylic sites and solution activity effects. A rough estimate of organic anion importance in water can be calculated from electroneutrality considerations, as was shown in this Chapter.

CHAPTER 9

DIAGENESIS OF DOM

9.0 Introduction

In this dissertation, acid-base and structural variability of DOM in natural environments is described, as well as acid-base changes in "fresh" DOM from the incubation of stream waters. Moreover, incubation of land based organic parent material in water was done to determine the chemical characteristics of DOM generated. The purpose of this Chapter is to study the results generated by the four types of work, to see if a picture of DOM formation, variability and modification in freshwaters can be done. In particular, the validity of three of the questions which were suggested in Chapter 1 were tested.

First, the results of the structural incubation work were studied to see if pathways of DOM formation could be elucidated, at least for the experimental conditions. Secondly, the structure of the DOM measured from field samples was compared to that reported from other studies and from the incubation experiment to see if pathways of DOM modification and transport from terrestrial to aquatic environments could be deduced. Third, the acid-base chemistry of the DOM collected at the sites was compared to the results of the stream water laboratory incubations to see if the field results could be

explained in the light of processes elaborated under controlled conditions.

9.1 Formation of DOM

The structural chemistry results from the parent material incubation experiment were studied to see if they could help explain the pathway of DOM formation. Though differences were expected between Spruce and Sphagnum DOM spectra, the actual results were surprising, as the Sphagnum sample showed a higher aromatic composition than the Spruce (Figure 9.1).

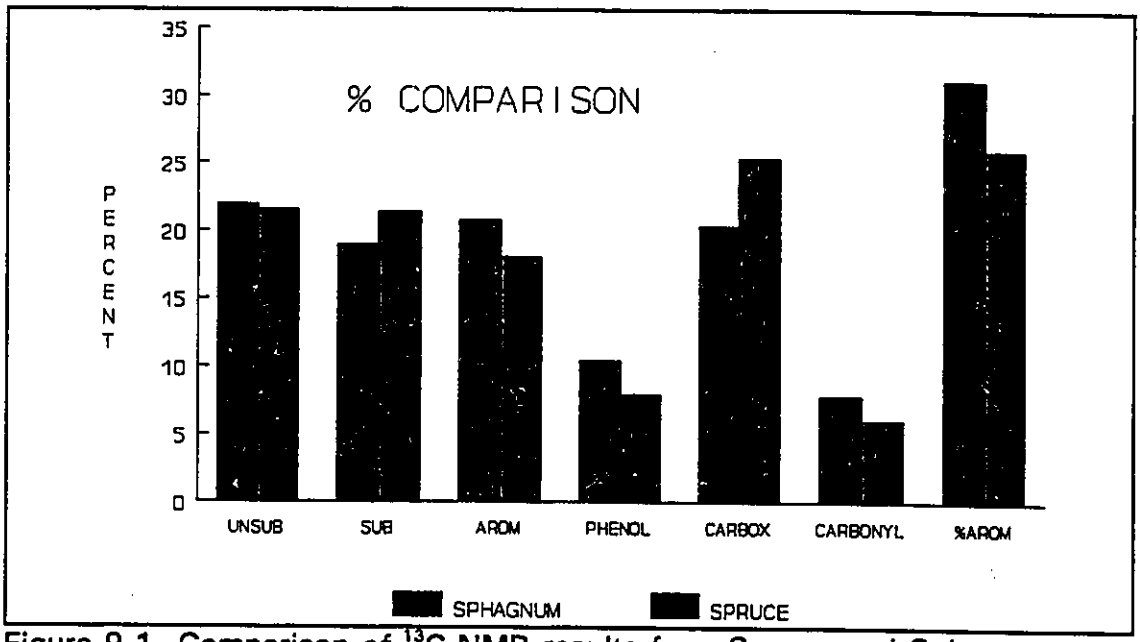

Figure 9.1 Comparison of ^{13}C NMR results from Spruce and Sphagnum incubation experiment.

As mosses are mostly carbohydrate, little aromatic C should have been measured from this sample if the humic material from lignin theory described in Section 1.2 (Stevenson 1982) was correct. This was not the case as the moss incubation not only produced a higher aromatic fraction than any

measured in the field samples, but also a greater fraction than the Spruce incubation. This may be due to non-structural phenolic compounds incorporated into Sphagnum, or it may be because the DOM formation followed a different pathway.

The most likely explanation for the measured structural composition of the incubation DOM is that Sphagnum material is more easily broken down by bacterial action than Spruce. This allows the production of semi-quinone, and then quinone which lead eventually to the aromatic compounds measured. Should this reasoning be correct, the first two theories of DOM formation described in Section 1.2, which require the initial breakdown of lignin into smaller aromatic molecules which are then recombined into larger structures by polycondensation reactions, was not as likely to occur. It is also easier to visualize the formation of aromatics from semi-quinones and quinones than from more complicated chemical pathways, such as the "browning" reaction. The study of some of the organic material collected in this study using Electron Spin Resonance also supports the quinone pathway (D. Eaton, McMaster University, Chemistry pers. com.) as it shows the presence of the free radicals which are necessary for this reaction to occur.

The incubation study thus tends to support the case that organic parent material is completely broken down into aliphatic components in soils and wetlands. These portions are then recombined to form highly aromatic compounds which were measured in the incubation water. If this is the case, why was the stream aromatic DOM fraction low in its aromatic composition? The first possibility is that DOM was moved from soils by hydrological processes before recombination into more complex molecules had occurred.

It would be expected that for at least some part of the year, especially in the late fall when the DOM formed in the summer is flushed out, that its composition in streams would be at its most aromatic. This was the case only with Moose Pit Brook, as its November affinity spectrum was the most aromatic of all aquatic samples measured. The explanation however, did not hold for the other sites. The next section provides further clues in explaining the problem.

9.2 Transport of DOM from soils to water courses

A number of other questions remain about the values generated in the parent material incubation study. First, how similar was the incubation DOM to that produced in soils? Secondly, if it is a true representation of that formed in soils, how and where was it modified to the composition found in the streams and lakes? Was it soil adsorptive processes or in-stream diagenesis? Could the changes in carbon structural composition be related to the site sequence design illustrated in Figure 2.2?

The first question is difficult to answer, but the incubation DOM structures were compared to the soil humic substances collected by other workers in order to give an idea of the range of results which could be expected. The data are listed in Table 9.1. As with Table 6.1, contrasts must be made cautiously, but results should give some indication of the relative importance of the various fractions.

Table 9.1 Soil organic material structural composition from various studies. UNSUB - unsubstituted Aliphatic C, SUB - substituted, AROM - aromatic, PHENOL - phenolic, CARBOX - carboxylic, CARBON - carbonyl, and %AROM - AROM+PHENOL.

study	UNSUB	SUB	AROM	PHENOL	CARBOX	CARBON	%AROM
1	46.2		41.0	-	12.8	-	47.3
2	27.0	-	-	-	3.0	-	30.0
2	38.0	-	-	-	5.0	-	20.0
2	32.0	-	-	-	7.0	-	24.0
3a	15.2	21.1	25.0	9.5	21.1	7.6	34.5
3b	20.5	23.5	27.4	7.7	15.8	5.3	35.1
4	-	-	-	-	-	-	60.0
5a	21.1	53.7	-	-	15.4	-	10.0
5b	20.1	45.5	-	-	19.4	-	14.9
6	21.5	21.3	18.0	7.9	25.3	6.0	25.9
7	21.9	18.9	20.7	10.4	20.3	7.8	31.1

1) Schnitzer and Preston (1986) from 18 humic acids.
2) Gonzalez-Vila et al. (1983) from 3 humic acids.
3) Preston and Schnitzer (1987) from a fulvic (a) and humic (b) acid.
4) Wilson and Hatcher (1988) from a bark sample.
5) Fründ et al. (1988) humic acid from two soils (average of high and low molecular weight fractions)
6) Spruce (this study)
7) Sphagnum (this study)

When this work's results are compared to those from other studies where humic and fulvic acids were extracted from soils, it can be seen that the percent aromaticity of the incubation experiment resembles that from most other work reported in the literature. This study's values are also compared to those of Wilson and Hatcher (1988) for a bark sample. Their spectrum shows a significantly higher aromaticity (%AROM) compared to all other results due to the presence of lignin in wood. The carboxylic fraction (CARBOX) is higher in the incubated material than for all humic acids, and for most fulvic acids tabulated, while the aromatic fraction (AROM) is somewhat lower than the values reported elsewhere, except for Study 5. The latter values were averaged from fractions separated by dialysis into high and low

molecular weight fractions.

Generally, these results show that the DOM generated in the incubations tended to resemble those of soil humic and fulvic acids reported elsewhere. This suggests that it is quite likely that the incubation DOM reasonably represents the quality of organic carbon produced in soils and wetlands.

An explanation for the difference in composition between the stream and incubation values could reside in the fact that some soil constituents are positively charged and that negatively charged material such as DOM which is being transported to water courses by runoff or interflow, may selectively complex with the clays and other minerals in soils. The results from this study suggest that if this were the case, the aromatic fraction would be the fraction complexing, as the Spruce and Sphagnum aromatic fractions measured in the incubation (25.9, 31.1% respectively) change from the stream values shown in Figures 6.1 to 6.4. Carboxylic values on the other hand, do not vary significantly.

The idea that aromatics are complexed in the soil matrix is also supported by the fact that the November Moose Pit Brook sample was found to be most similar to the Spruce (Figure S.1). This result suggests an interesting connection between the runoff of DOM from soils and stream DOM quality. As seen from Figure 4.3, the fall rains increased water flow in the streams at the time of sampling. It is also quite likely that the DOM formed in soils during the summer could be washed into the first order streams, explaining the good comparison between the incubation DOM and the Moose Pit Brook DOM structure. As lakes tend to absorb and integrate basin

water chemistry influences and also introduce the autotrophic production complication, it may explain why both Tupper Lake and the larger Mersey River which contains a number of lakes in its headwaters do not show similar structural results.

There is still the fact that aliphatic C is higher in the streams and lakes than in the soils during the remainder of the year. It may be that both loss of aromatics and gain of aliphatics are combining to produce the gain in importance of the aliphatic fraction. The fact that the high fall runoff sample is the one resembling the incubation sample most, once again suggests that soil retention of the aromatic fraction is the most likely explanation.

It was originally thought that the bog site would produce the material most similar to the incubation DOM. This did not turn out to be the case, as bog DOM pattern at the sampling times, most resembled those of the Mersey River. It may be that a number of processes, such as precipitation, microbial breakdown, photosynthesis and photochemistry, so modify the water collected from the bog pool as to make it very different from the soil material that most likely supplies DOM to streams. This argument could also be made for the samples collected in the streams, at least for the April and June samplings.

These results suggest that without the benefit of in-depth hydrological studies of water routing through bogs and soils, only the first order stream DOM will probably be useful in explaining the sources of DOM formation. The presence of lakes and large rivers where integration of chemical influences occur and where primary productivity also complicates the picture make these sites less informative, at least for this purpose.

9.3 Acid-Base Changes

The main trend in DOM acid-base modification noted in the experiments described in Chapter 7 was that as the organic matter "aged", its acidity as measured by the carboxylic acid fraction, decreased. This trend was observed in almost all replicates of the experiments conducted, regardless of phytoplankton activity. The question which remains to be asked is whether this trend could be shown to occur in the field.

If water temperature or enzyme activity is used as an indicator of diagenetic activity, the June samples should show the lowest carboxylic concentration, as enzyme activity and thus biological decomposition, was then at its highest. All sites except Moose Pit Brook did show their lowest carboxylic acid concentrations then, somewhat supporting the hypothesis. As discussed above, the shorter water retention time in Moose Pit Brook, compared to the Mersey River and Tupper Lake may explain the lack of correlation at the headwater stream. Nevertheless, the field results seem to parallel the laboratory incubation and suggest that DOM acidity is modified by in-stream diagenetic processes.

The modification is more accentuated in the larger water bodies than in the smaller ones, as lake DOM acidity was usually lower than in the streams. This result confirms the theory of Kramer et al. (1990) who suggest a decrease in acidity of DOM with increasing stream order, and increasing size of water bodies.

Another tendency noted in the incubation experiments was that the samples subject to photosynthetic activity showed lower phenolic acid

concentrations than the water kept dark. This was not the case in the field, as Tupper Lake samples usually had higher phenolic acid content than the other sites. This result may be due to constant inputs of fresh stream DOM into the lake, and to the interactions between lake waters and sediments or to biological activities not simulated in the incubations.

The overall suggestion which analysis of the data suggests, is that DOM from soils seems to be generally acidic, with approximately 10 μeq*mg C^{-1} being produced in the parent material incubations (Figure 7.1, 7,2). This material then tends to become less acidic with increased biological and chemical activity. Generally, this tends to be complemented by increases in phenolic acid sites which indicate an increasing aromaticity in the material.

9.4 Conclusions

The above discussion emphasizes the interrelationship between biological and chemical processes which form and modify DOM with the physical characteristics of basins which further modify it on its way to streams and lakes.

All the above discussions show that laboratory experiments can only suggest fundamental mechanisms of behaviour which are then masked by field conditions. Because of these complications, it may be that attempts to understand specific formation and modification pathways of DOM are irrelevant, as no such pure situations exist in nature. However, if such work is to be conducted, small first order streams in basins with well defined soils and hydrological pathways are probably the only sites where success in better understanding the creation and modification of DOM is possible.

CHAPTER 10

CONCLUSIONS

The results from this work confirm that freshwater DOM changes chemically with time and location even within the confines of small watersheds. The differences measured seem dependent on interactions between hydrology, biology and environmental characteristics such basin size and presence of lakes. These attributes affect the diagenetic processes occurring in soils and water, as well as the mixing of DOM from various sources which produced the samples which were collected.

These differences in quality may have repercussions on the interactions between mineral and organic acids, with the formation of trihalomethanes in municipal water supplies, interactions with pesticides applied near lakes and streams and DOM removal from industrial or drinking water supplies. The main specific contributions that this work has made to the study of aquatic dissolved organic matter are described below.

The study of DOM has been improved by perfecting and calibrating new concentration and characterization methods. Chapter 3 discusses the use of reverse osmosis for isolating DOM from water. This technique has reduced some of the problems associated with traditional isolation and measuring methods by simplifying the methodology needed to extract DOM, as well as by reducing the operational constraints on interpretation of results.

The use of the affinity spectrum titration method also described in Chapter 3, allowed the direct measurement of the DOM's acid-base characteristics. The results from this analysis were shown to be an important refinement over the indirect organic acidity measurements which have been used until now. Results reported in Chapters 5, 7 and 8 show that the Oliver et al. method (1983) consistently overestimated the contribution of organic acids to freshwaters, and that this contribution varied due to biological and chemical actions, some of which are poorly understood.

This work also showed that DOM found in first order streams changed with time, losing its carboxyl acidity regardless of photosynthetic activity with approximately 50% of the carboxyl sites lost within 60 days. This result is important in understanding that not all natural organic acids are equal in their impact in water chemistry, and that rough approximations, such as the Oliver et al. (1983) method should only be used in a qualitative fashion. The effect of photosynthesis under laboratory conditions was to suppress the formation of phenolic acid sites and to promote the formation of neutral sites, though this could not be shown to occur in field samples.

I developed an alternate chemical model based on electroneutrality and mass balance principles (described in Section 3.1.2) which allows a calculation of the DOM contribution to brownwaters. Its results, described in Chapter 8, were complementary to those of the affinity spectrum calculation when acid dissociation factors were taken into account.

The samples collected during this study also showed that the relative DOM structural composition varied with time and space and that its aromatic composition was less than usually assumed in the literature. These results

could be easily seen because of the improved concentration methods developed, as well as the selection of a small watershed which proved suitable for showing natural variabilities. Chapter 6 summarized results which showed an increasing molecular complexity of DOM which could be generally correlated to increased biological activity in the summer time.

In various parts of this work, a number of shortcomings in knowledge became obvious as a result of some of the studies. The most important questions raised include the need for better understanding of the linkages between soils, hydrology and stream DOM, and how these modify the physical and biological conditions which affect DOM chemistry. It was also shown that more work should be done to understand the factors and pathways which control the formation of DOM in soils and bogs.

The first question is of primary interest to all soil and water scientists interested in the origins of DOM. A number of clues were drawn from both the incubation and field studies which suggested possible interactions between basin properties and DOM chemistry. These hypotheses should lead to improved field experimental designs to allow a better understanding of DOM controlling factors. The use of lysimeters or drainage tile to collect interflow and groundwater at various times of the year should permit a better interpretation not only of the quality of the DOM transported to streams by hydrological processes, but also might shed more light on the processes forming DOM in soils and wetlands.

The DOM formation pathways could only be speculated upon with the information collected here. However, the parent material incubation approach, once refined to simulate more realistic conditions, along with improved field

collection techniques may provide important information on formation pathways. Better biochemical and enzyme approaches should also be used to reach this goal.

All in all, the results of this study to provide more questions than answers. The most important one is how representative are these results of conditions elsewhere in Atlantic Canada, or even in the northern hemisphere? Can the acid-base results be extrapolated to other regions? Can predictive models of DOM chemistry be produced within basins, based on knowledge of hydrology and diagenetic processes? The study of dissolved humic substances in freshwaters is only in its infancy.

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APPENDIX A

Geography, Geology and Soils of the Study Region:

The study area chosen is located on the Southern Uplands of Nova Scotia (Goldthwait 1924), which ranges in elevation from sea level to approximately 300 meters. The basins selected range from 100 to 170 m. in altitude for the Moose Pit - Tupper Lake basin, and 100 to 240 m. for the Mersey. Drainage is mostly towards the south for both areas. The entire area forms part of the Atlantic peneplain surface, uplifted in the Triassic.

The surface geology of the area consists mostly of thin glacial tills, drumlin and eskers composed of locally derived material. Outcrops are often found in both basins. Shallow lakes and depressions which cause poor drainage, and thus boggy conditions in the study area are also common.

The study basins are underlain by two major geological formations, the Ordovician Halifax slates, and Devonian granites. These have been described by Taylor (1969) whose work is summarized here. Granites occupy the upper 65% of the Mersey River basin at the sampling site, and approximately 80% of the Moose Pit - Tupper Lake basin, with the contact between the two formations clearly visible in the Moose Pit Brook basin.

Soils in both study basins are dominated by two humo - podzolic types, the Bridgewater and Gibraltar Series. The former is developed from medium textured, shaly till, while the latter originates from moderately coarse granite tills. In both cases, shallow, well aerated sandy loams are produced, though they are too stony and nutrient poor to support agriculture (MacDougall et al.

1969). When not located in hollows or in other areas where water flow is impeded, these are well drained, with the Gibraltar series producing stonier soils on steeper slopes.

Sphagnum bogs and poorly drained soils occupy roughly 20% of the study area's surface. Despite their lack of overall importance in total soil types, these tend to have a major impact on water chemistry, as they seem to often drain directly into streams and rivers.

The meteorology of the study area was recently summarized by Freedman et al. (1985) and Gates (1982) from data collected at or near the study area between 1951 and 1980. The frost-free season lasts between 93 and 140 days, with a mean annual temperature of 6.5°C. Windspeeds averaged 17km/hr, generally from the west, with a tendency towards lower speeds during summer. Average precipitation in the area is approximately 1400 mm, of which snowfall can range from 8 to 23% of the total. Generally, precipitation tends to be lower in the warm months compared to the colder. The first snow usually occurs in November. Snow cover is rarely continuous over the winter months due to frequent winter rainstorms. Total snowfalls usually range from 170 to 340 cm, with an annual average of 250.

The hydrology of the Mersey River at the Kejimkujik National Park was described by Ambler (1983) from data collected between 1968 and 1982. Though this analysis was not done for Moose Pit Brook, the similarity of soils, climate and topography suggests that the results from one basin are applicable to the other, though the higher proportion of peatlands in Moose Pit Brook basin may change storage in an unknown way. This can be seen by comparing calculated water exports from the two basins (Figure 3.1).

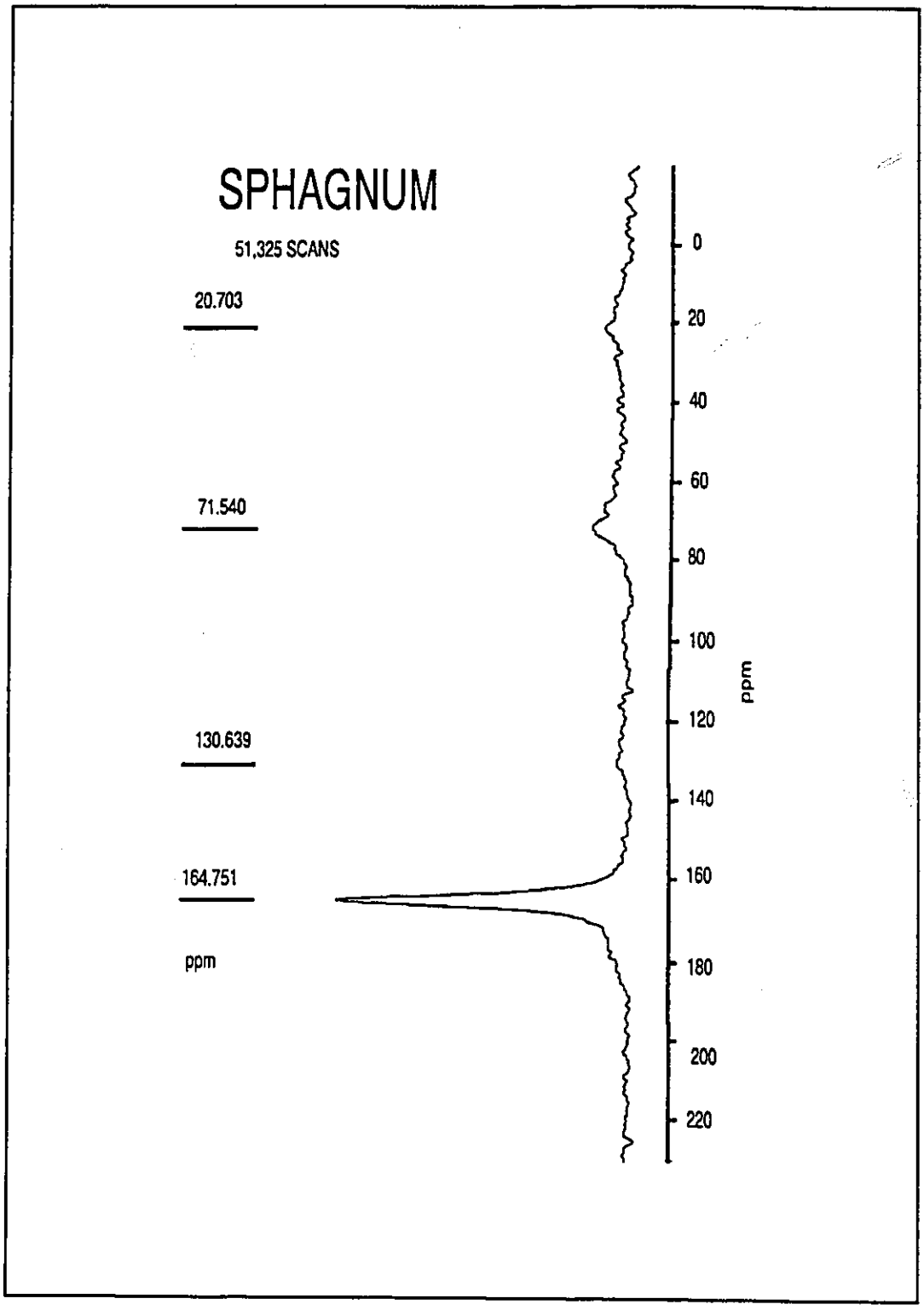
Freedman and Clair (1987) report that Mersey River water exports are 8300 $m^3*ha^{-1}*yr^{-1}$, calculated from the period January 1 1983, to January 1, 1985. During the same period, Moose Pit Brook which received similar inputs of precipitation (11,640 $m^3*ha^{-1}*yr^{-1}$) had measured water exports of 9600 $m^3*ha^{-1}*yr^{-1}$. The differences between theoretically similar basins could be due to the difficulty involved in measuring the Moose Pit Brook basin area, or to differences in basin water storage capacities and evaporation potential. It should also be pointed out that peak seasonal discharges are usually reached more quickly in the smaller basin than in the Mersey where peaks and troughs in discharge are delayed by several weeks. Ambler (1983), following accepted practice, divided Mersey River basin hydrological processes into flow and storage. Flow processes include precipitation, infiltration, evaporation and runoff, while storage involves interception, soil moisture, groundwater and snow. Over the annual cycle, he showed that 68% of total precipitation is measured as runoff, 5% was measured as storage, while the remaining 27% was accounted by evaporation. Inputs to storage occurred from October to March, with loss from this compartment from April to September.

Assuming that the Moose Pit Brook basin size is accurately measured, its greater discharge per unit area compared to the Mersey River suggests that a lower evaporative loss due to differences in vegetation, greater runoff due to shallower soils or a steeper topography, or a mixture of both could be responsible for the differences. Regardless of the reason for the differences in water routing, the discharge data suggests that the water chemistry of the Moose Pit Brook basin will be a more immediate reflection of water-soil interactions than the Mersey River situation, where a larger basin with more

retention time will allow for more modification and mixing of organic materials. The discharge patterns shown by streams and rivers in this area are quite different from those found in more continental locations of Canada which tend to have low discharges in the colder months (Hynes 1972), unlike the high values found in Nova Scotia during the winter months.

APPENDIX B

CP/MAS ^{13}C NMR Spectra from Collected Samples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51,325 SCANS
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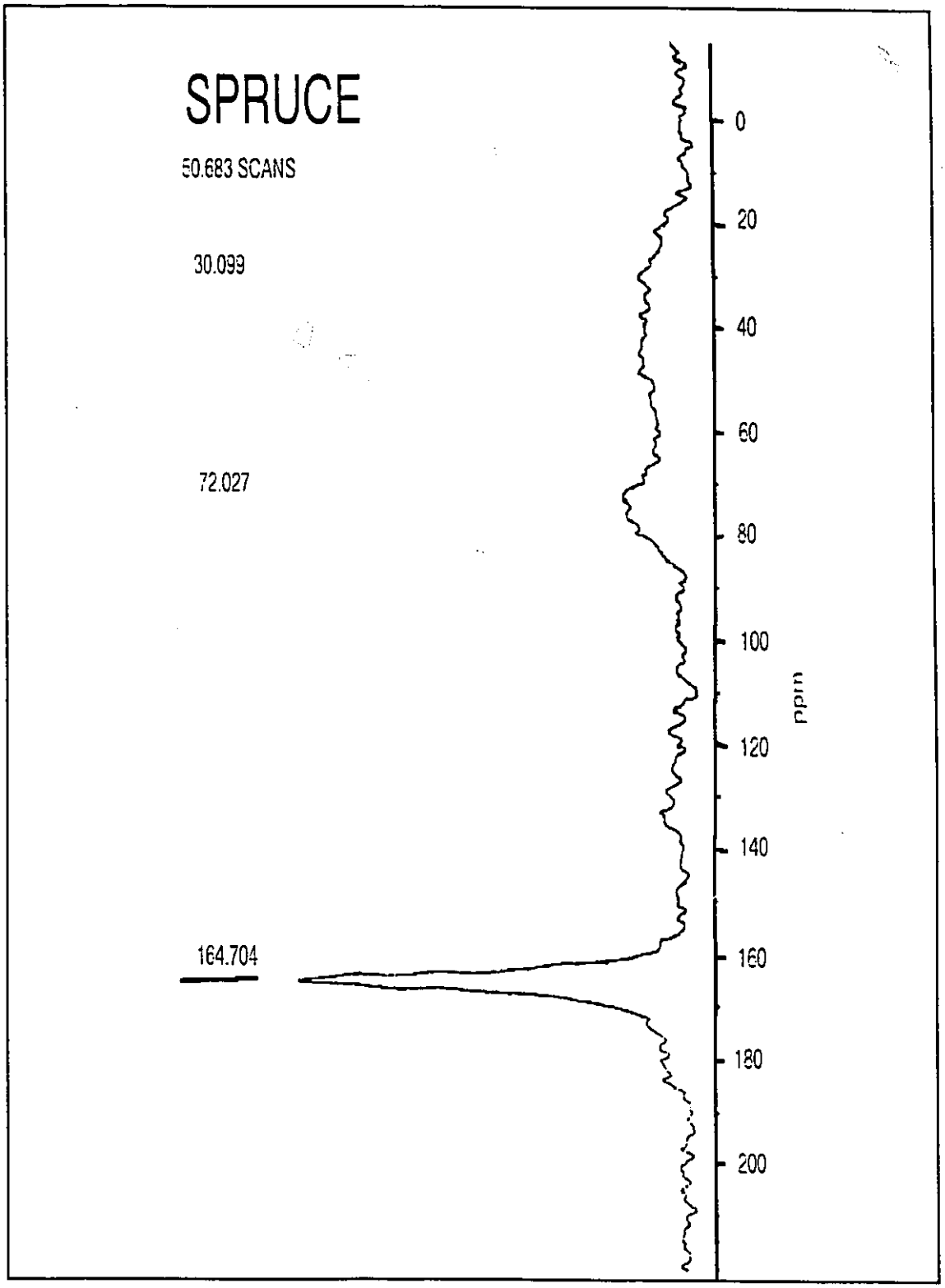
130.639
164.751
ppm
0
20
40
60
80
100
120
140
160
180
200
220
ppm


SPRUCE
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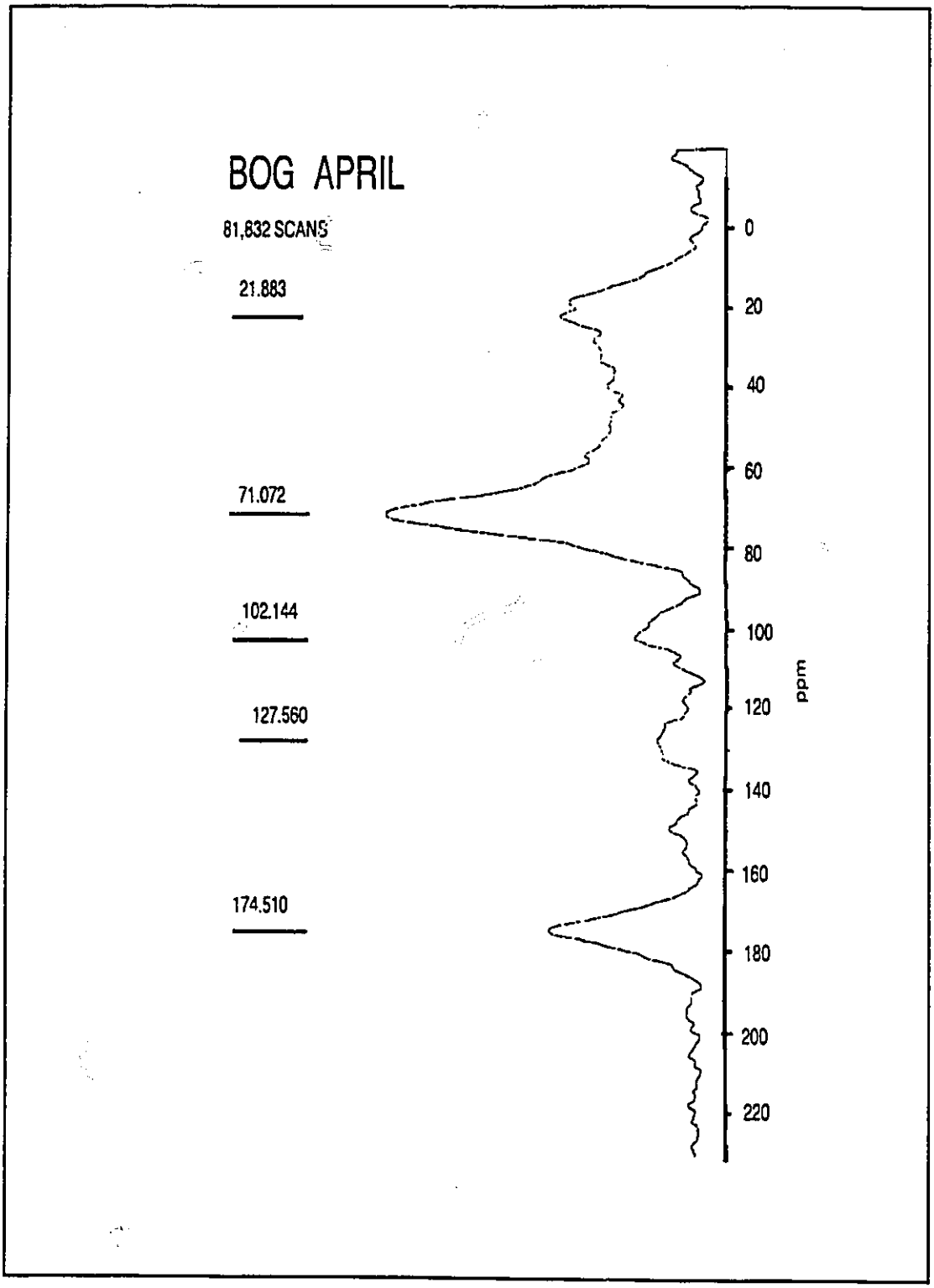
40
60
80
100
120
140
160
180
200
ppm


BOG APRIL
81,832 SCANS
21.883
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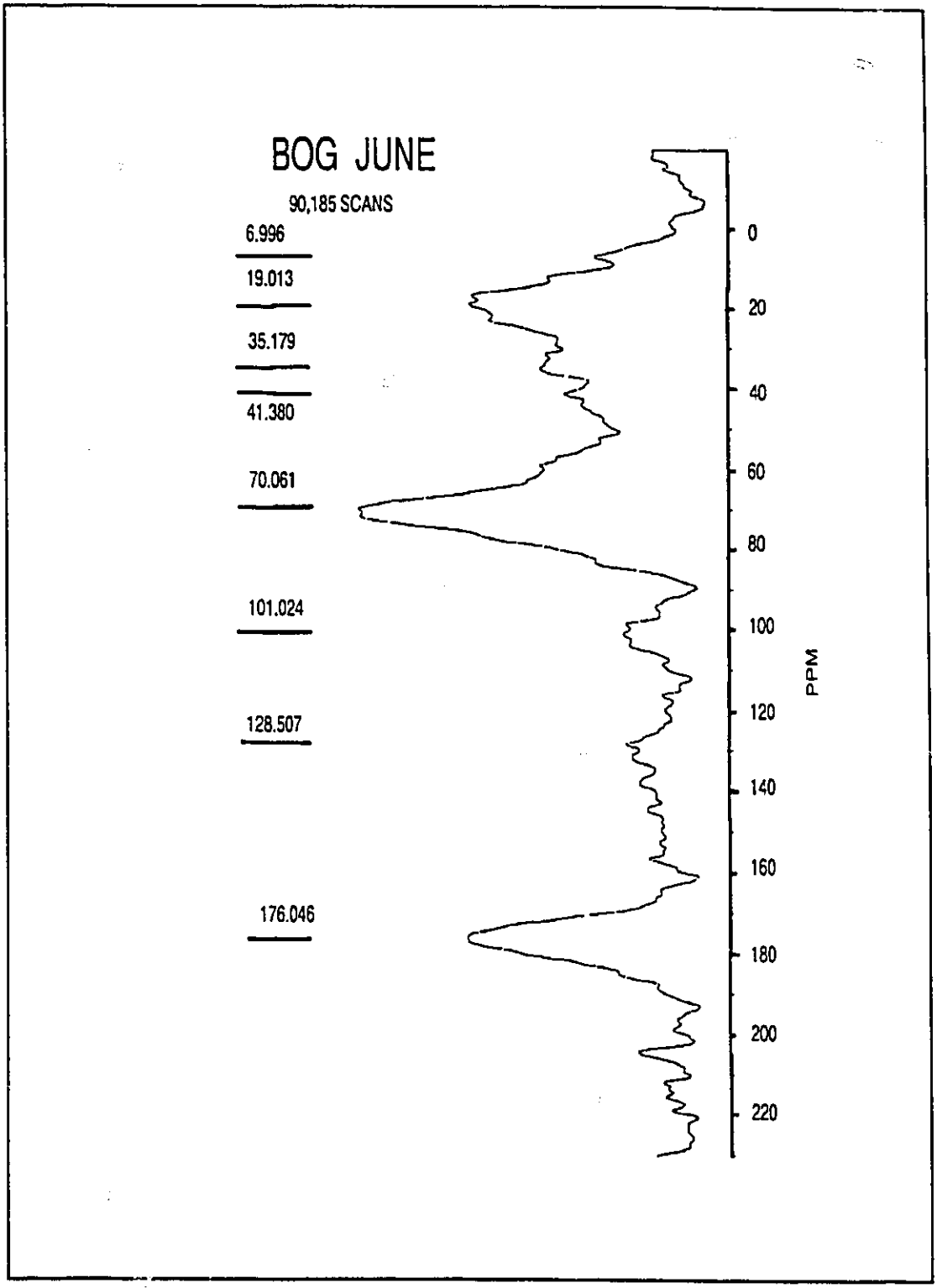
20
40
60
80
100
120
140
160
180
200
220
ppm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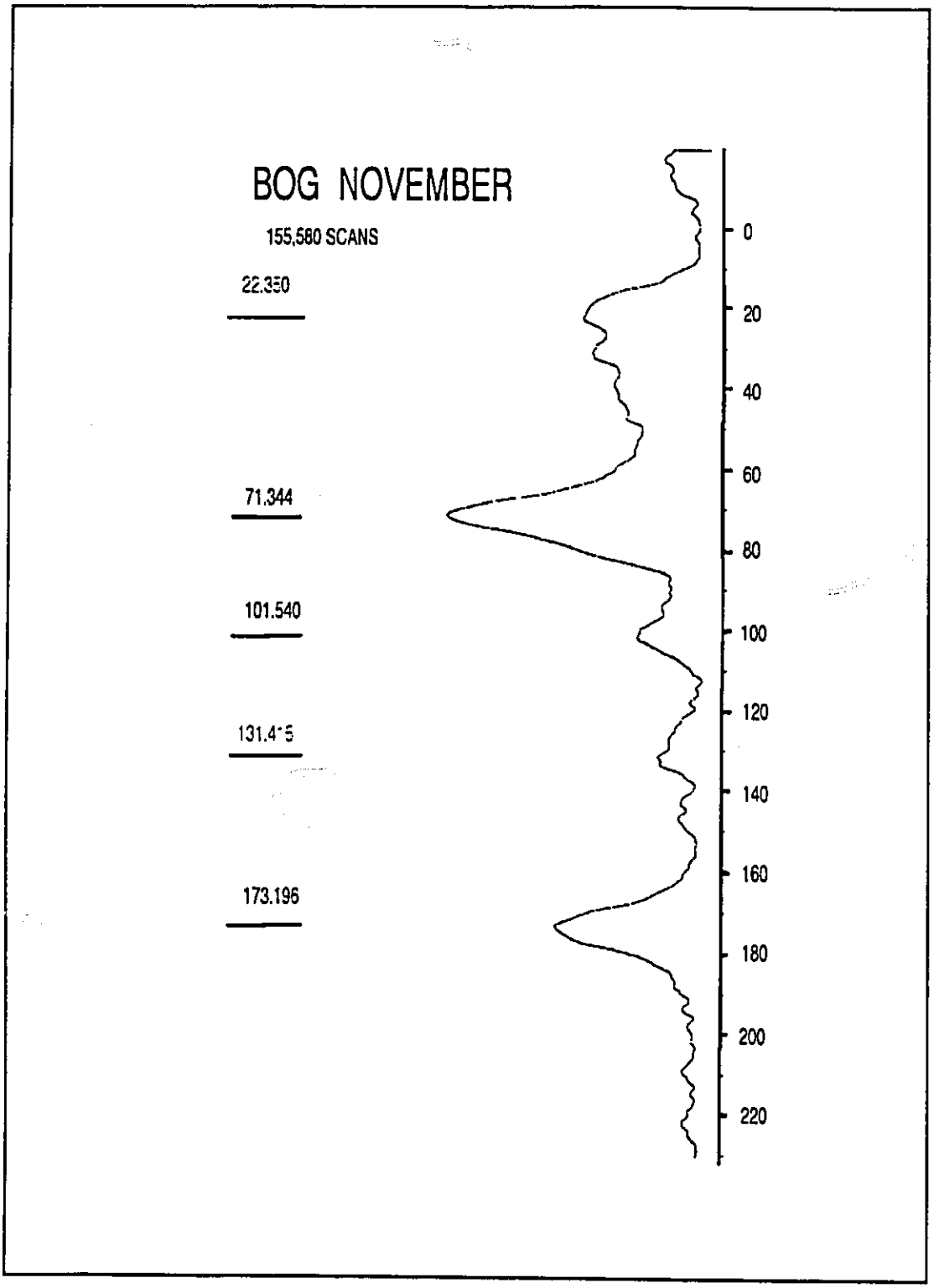
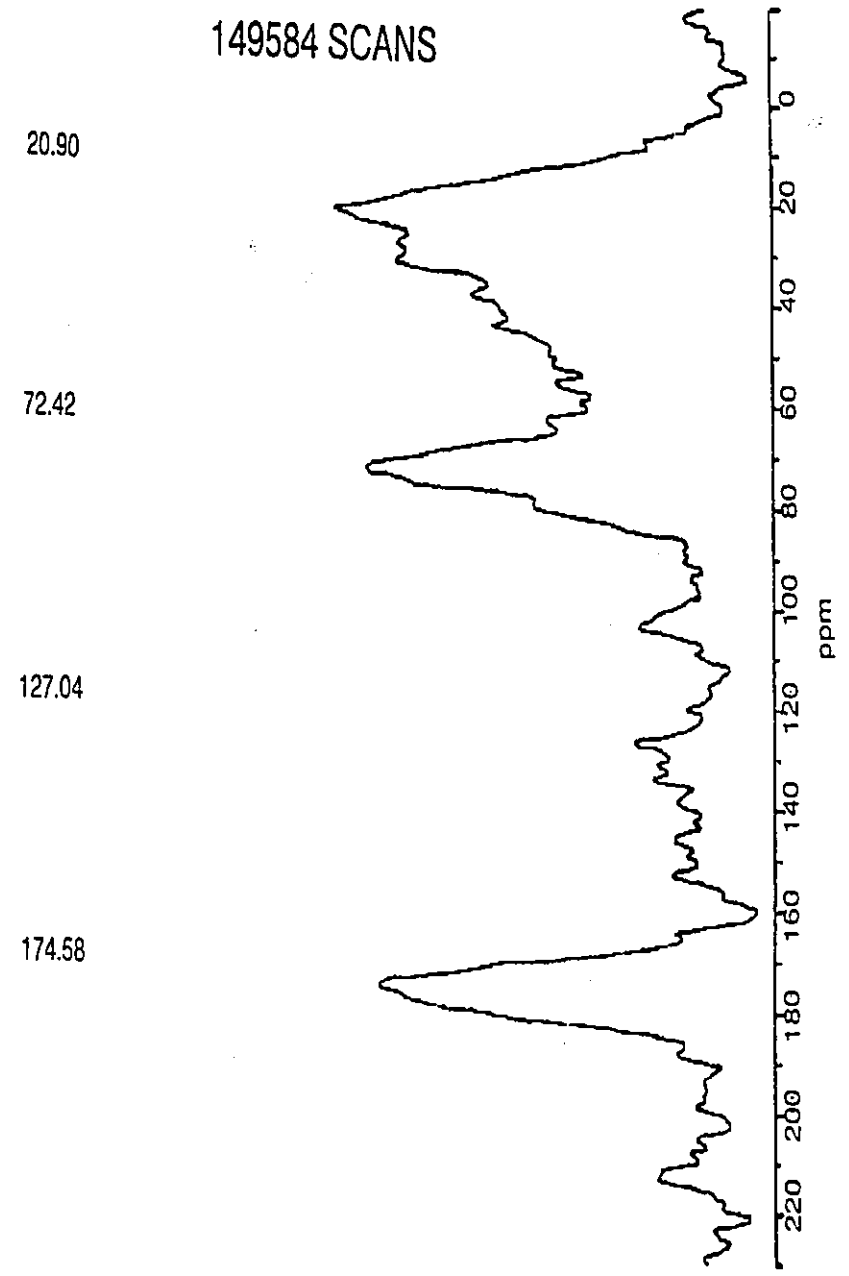
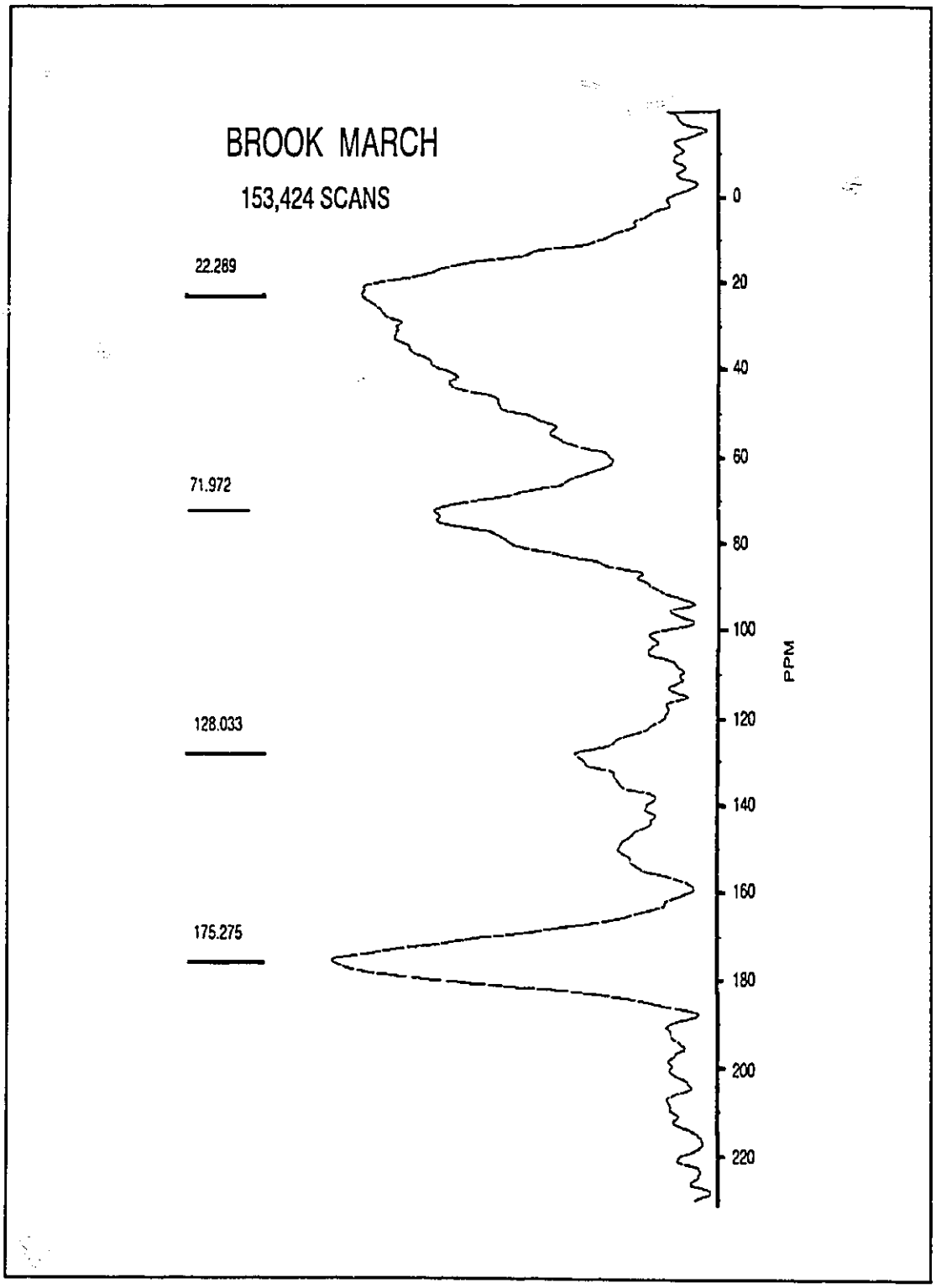
41.380
70.061
101.024
128.507
176.046
0
20
40
60
80
100
120
140
160
180
200
220
PPM


BOG NOVEMBER
155,580 SCANS
22.350
71.344
101.540
131.415
173.196
0
20
40
60
80
100
120
140
160
180
200
220

BROOK JANUARY




BROOK MARCH
153,424 SCANS
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128.033
175.275
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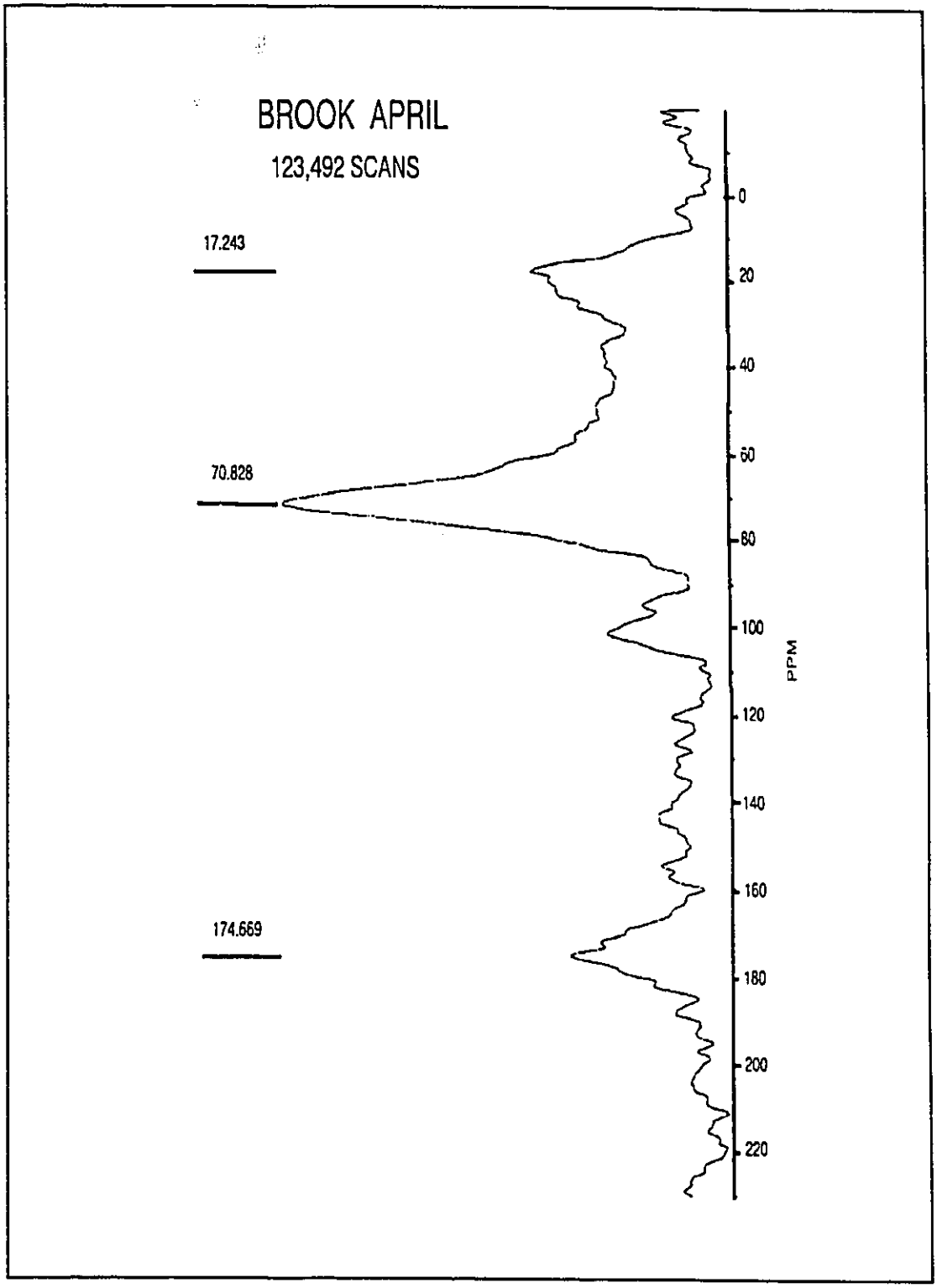
20
40
60
80
100
120
140
160
180
200
220
PPM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123,492 SCANS
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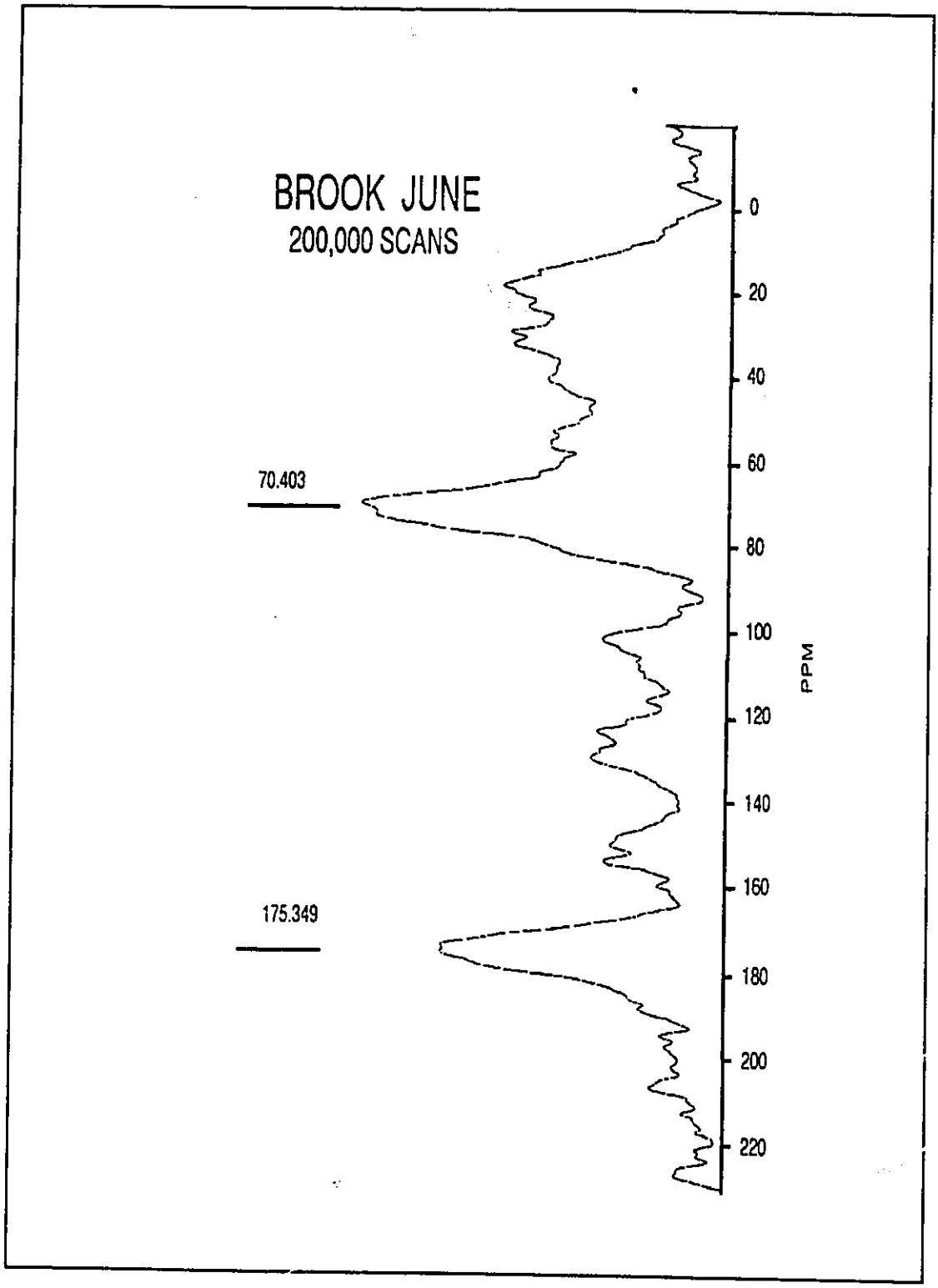
100
120
140
160
180
200
220
PPM


BROOK JUNE
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60
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100
120
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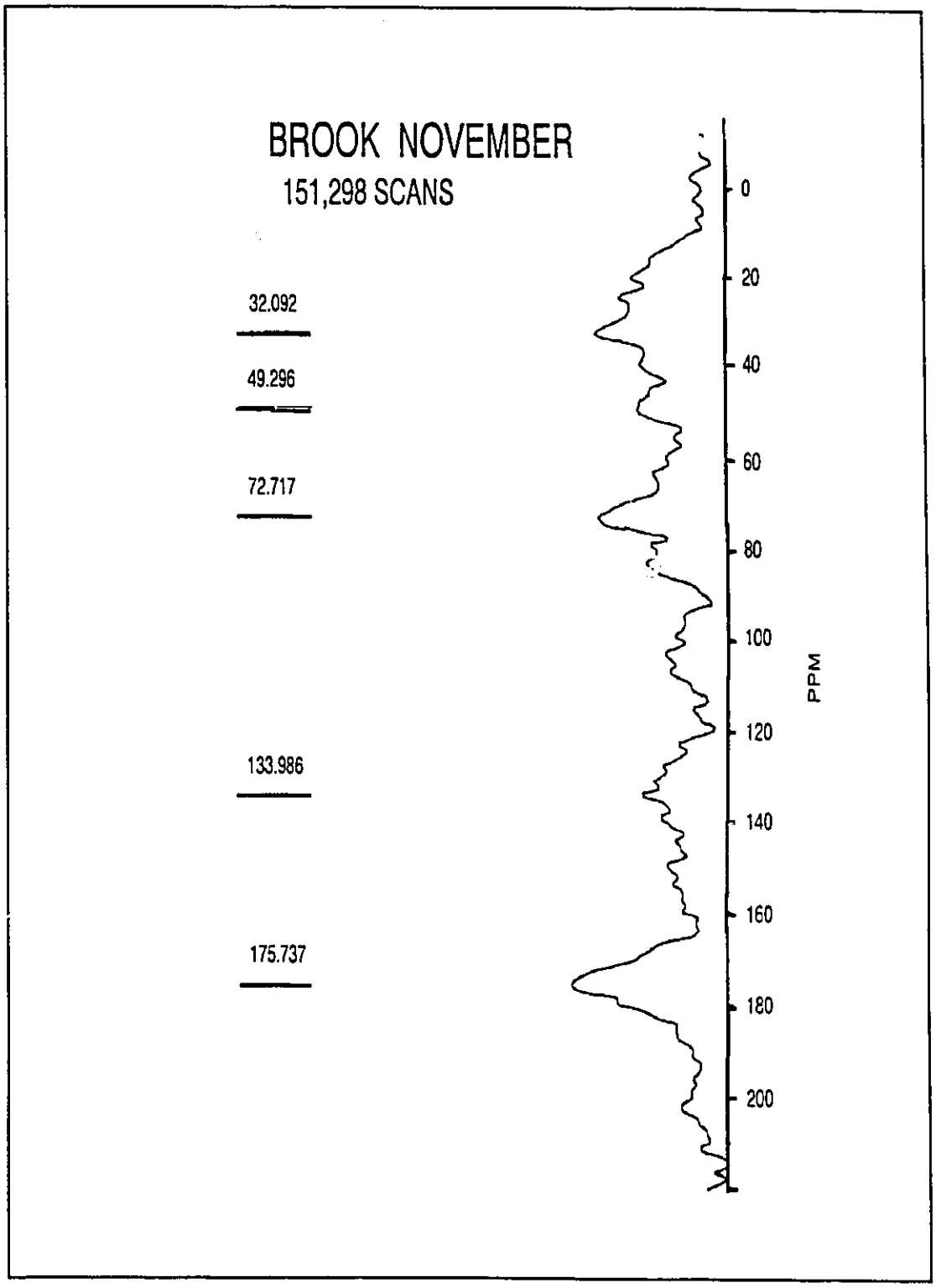
160
180
200
220
PPM


BROOK NOVEMBER
151,298 SCANS
32.092
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20
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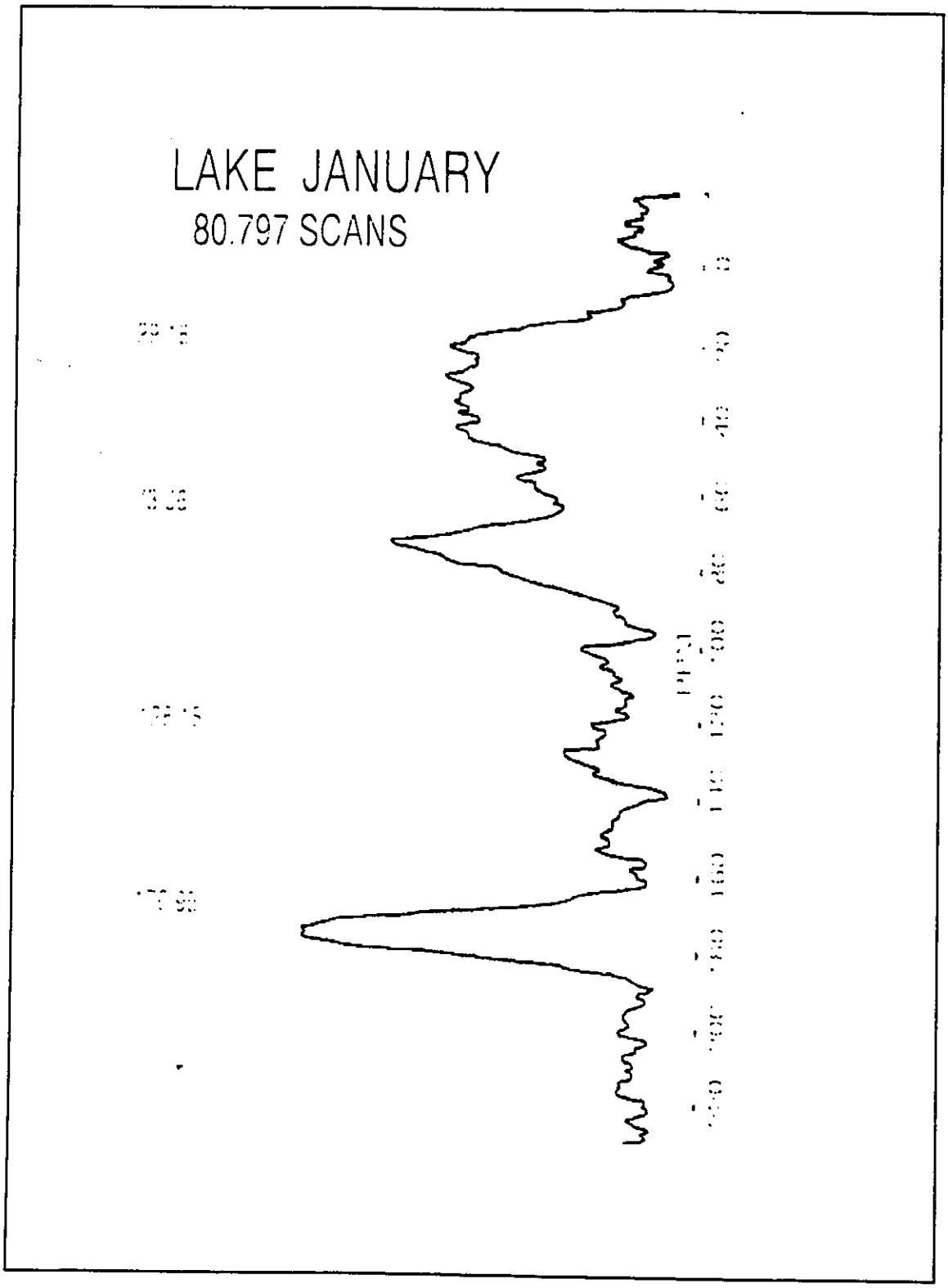
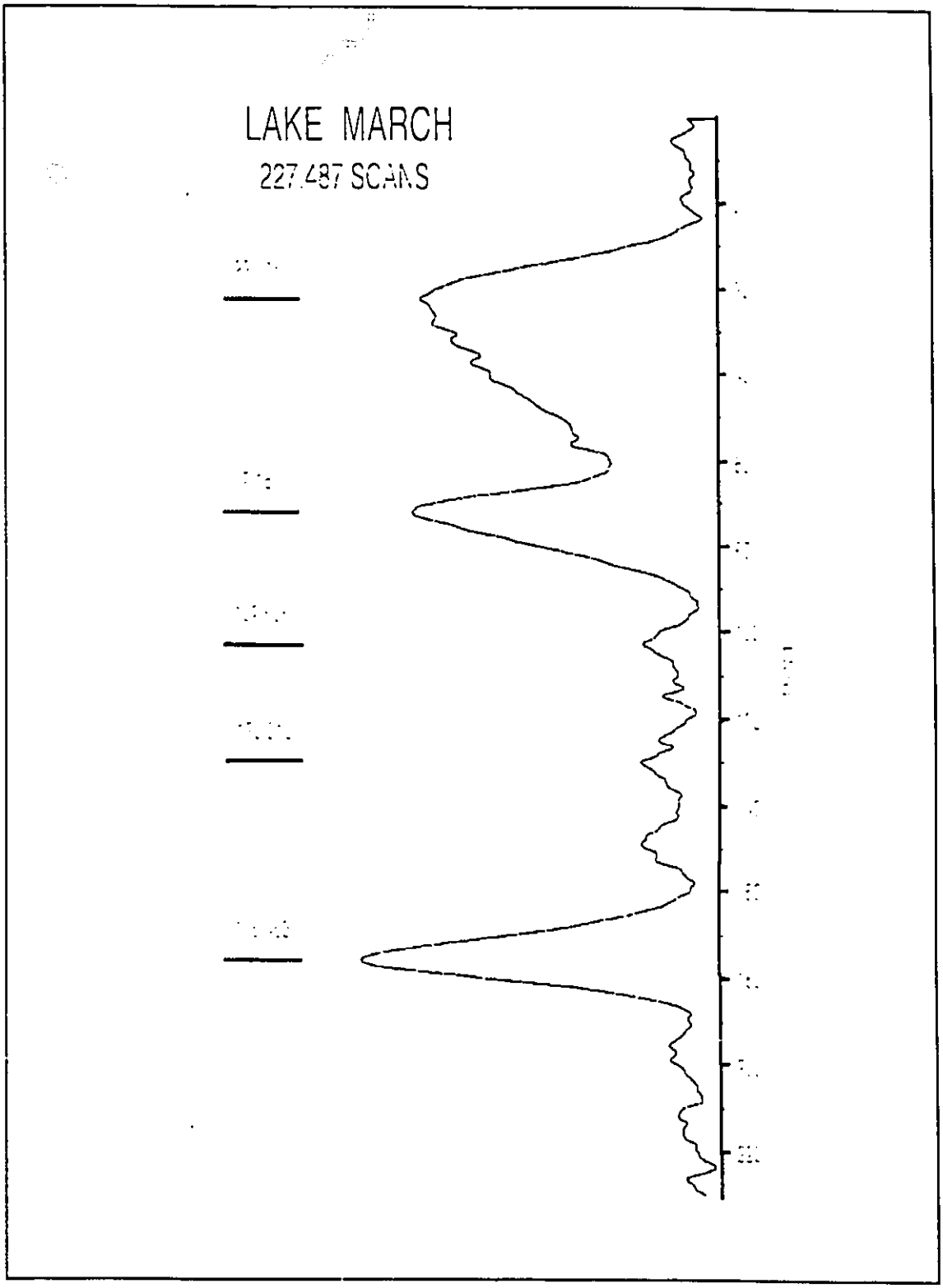
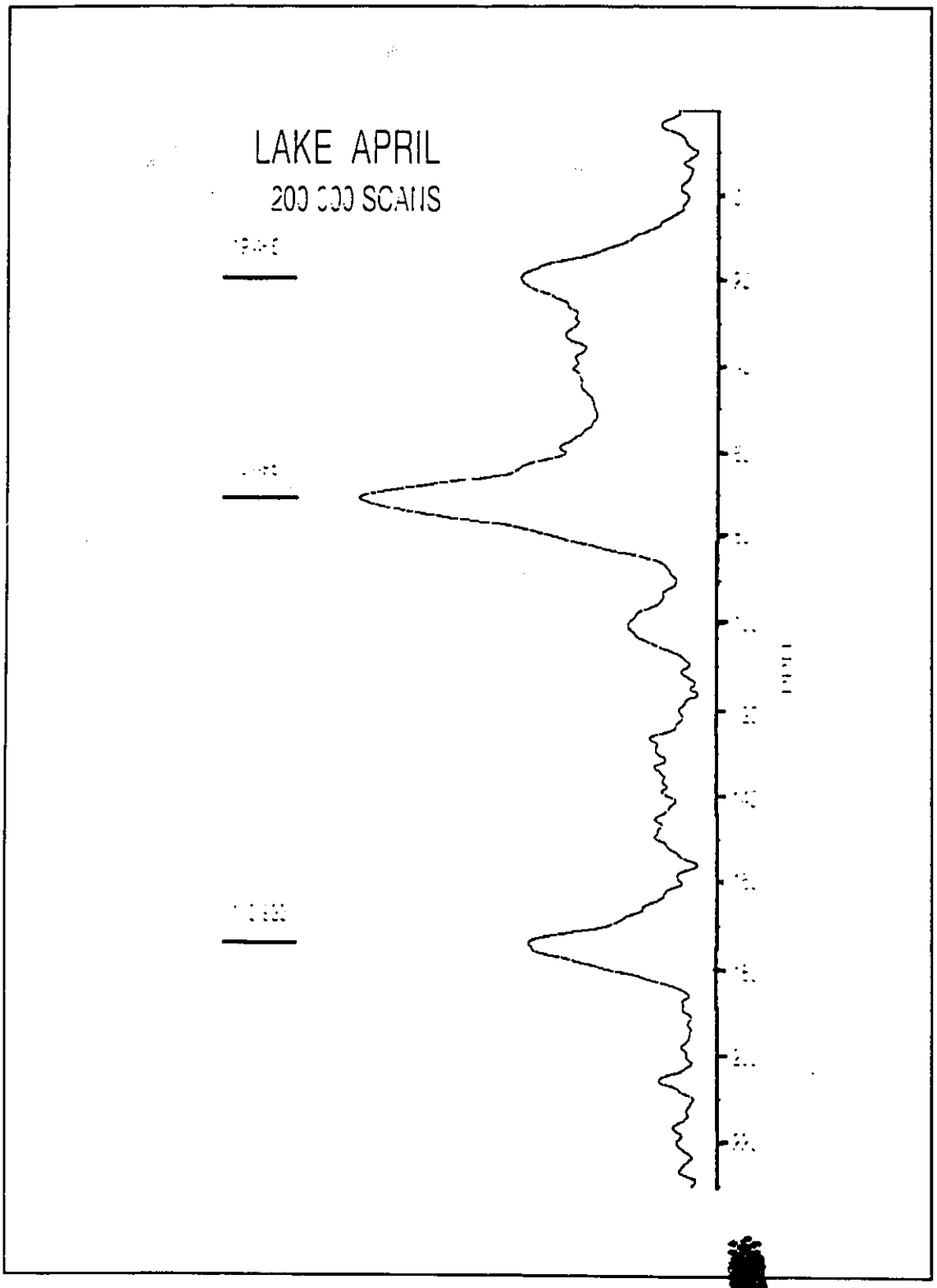
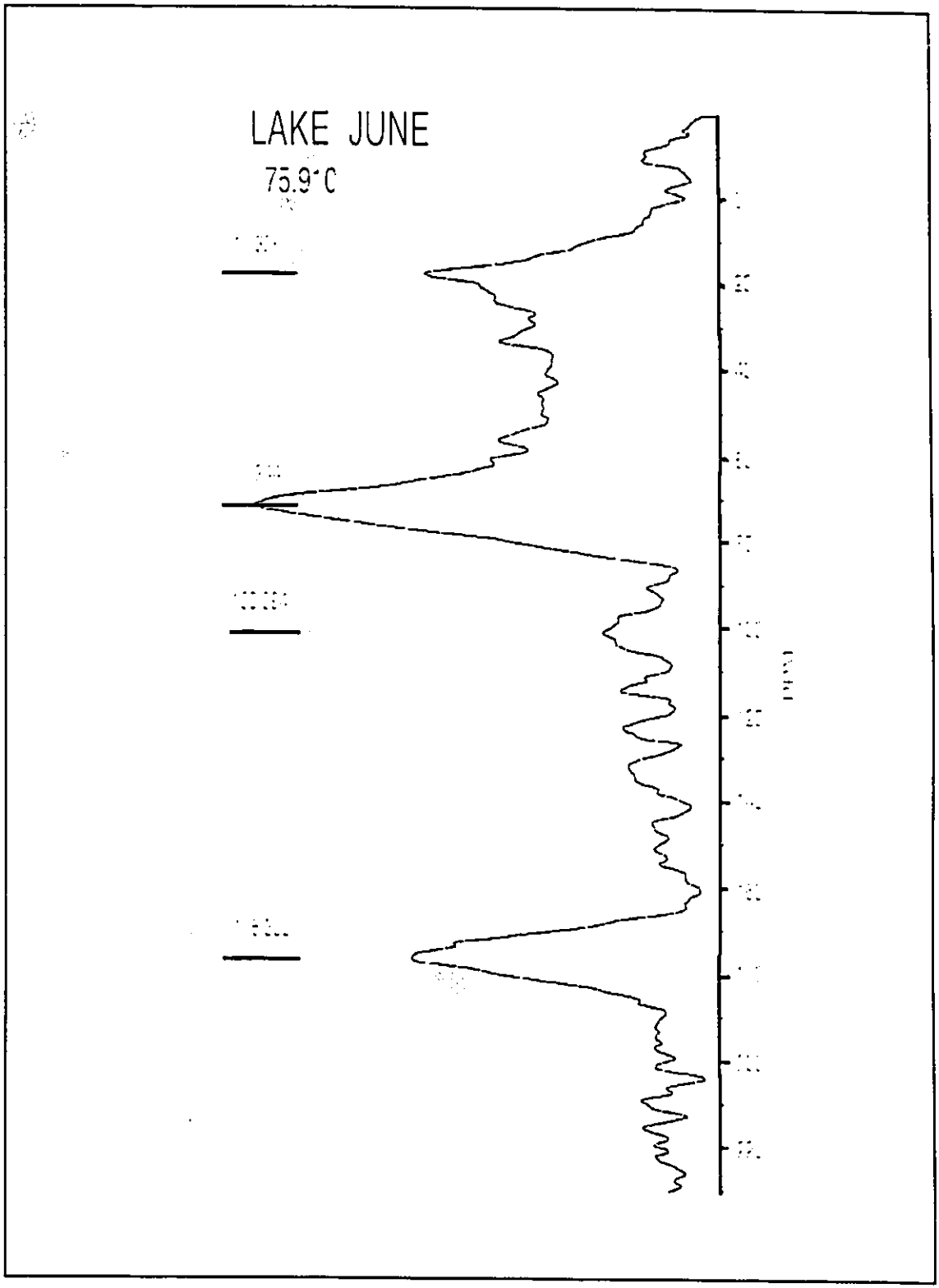
60
80
100
120
140
160
180
200
PPM


LAKE JANUARY
80.797 SCANS


LAKE MARCH
227.487 SCANS


LAKE APRIL
200 000 SCANS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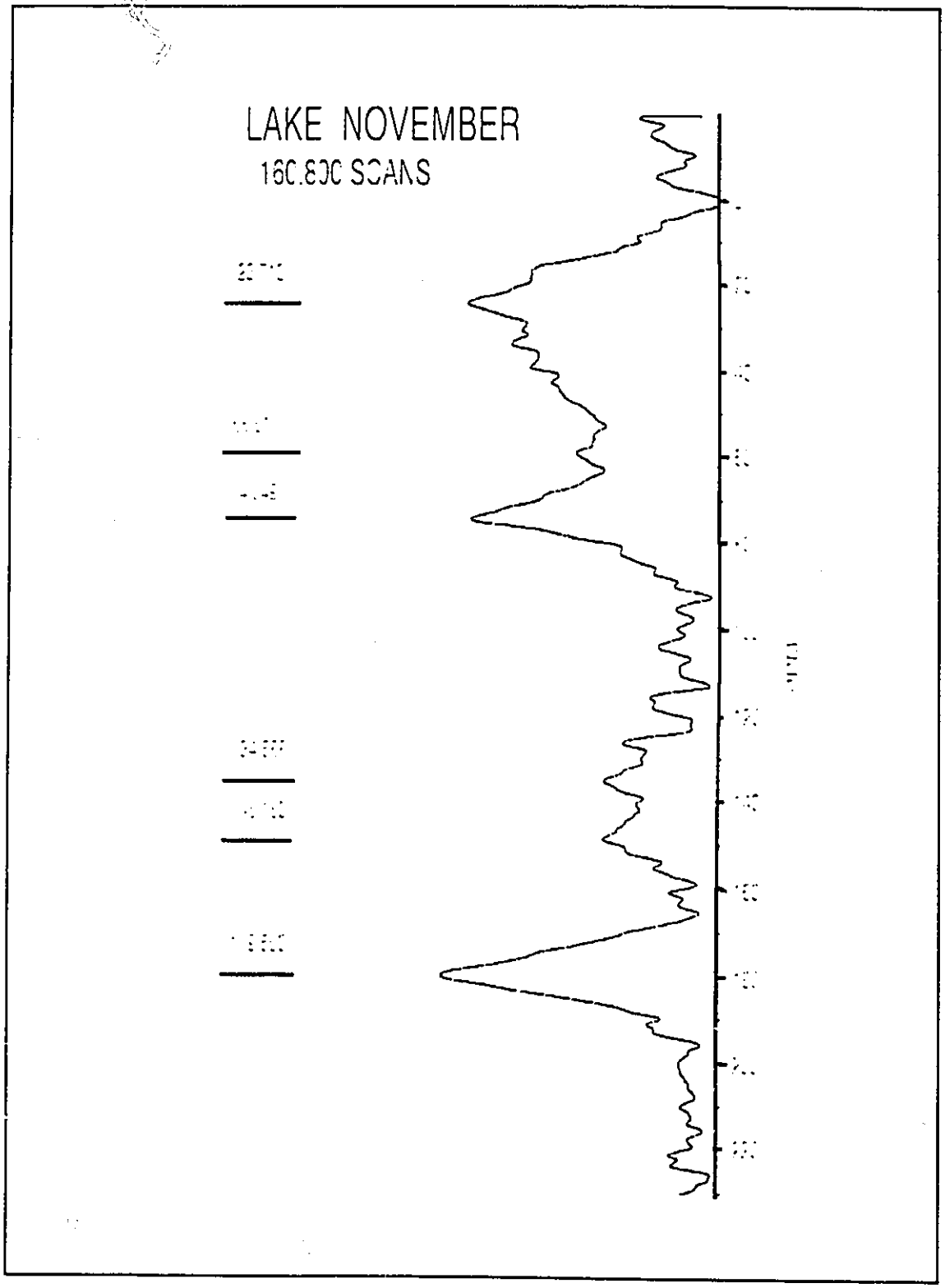
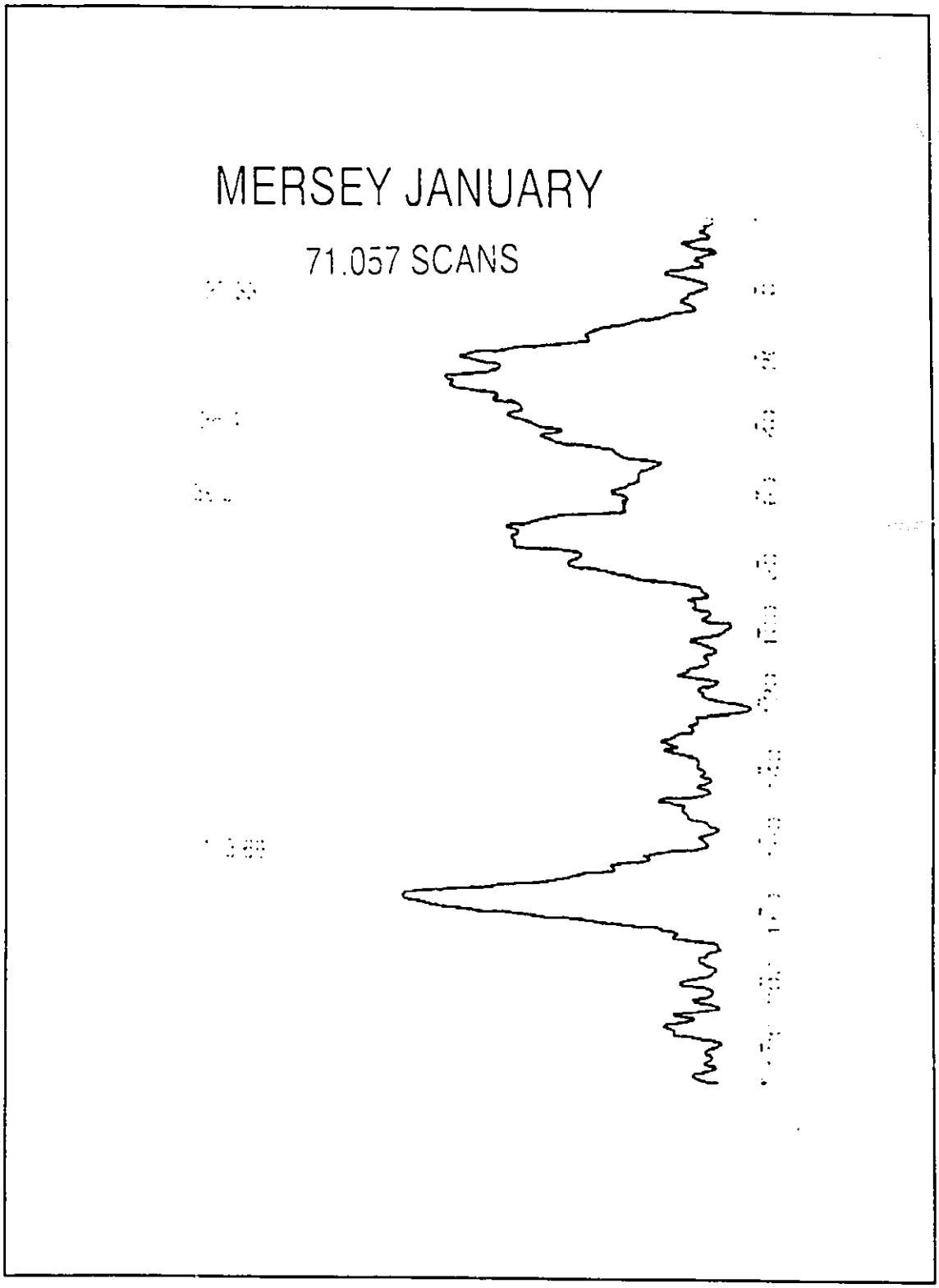
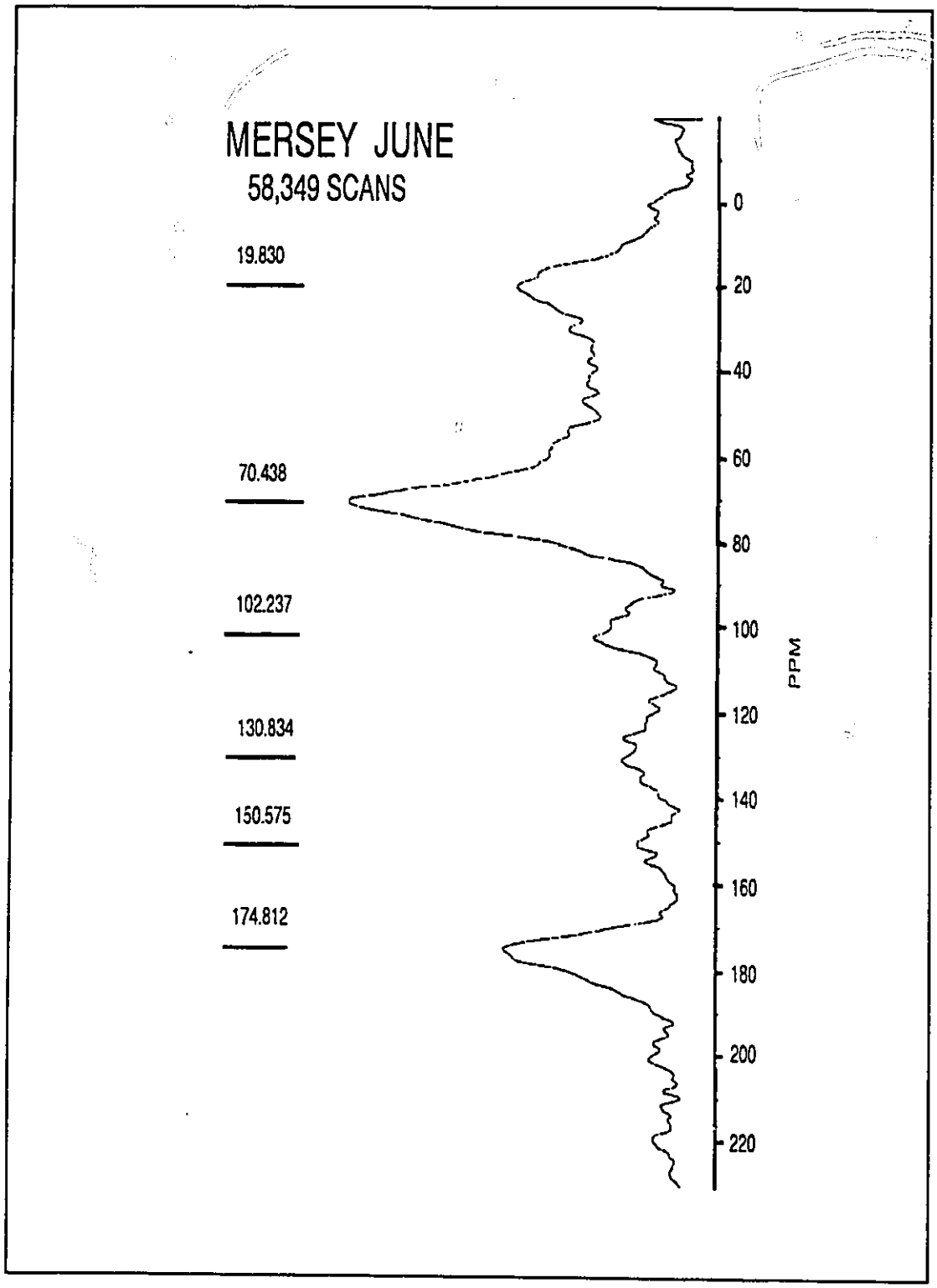
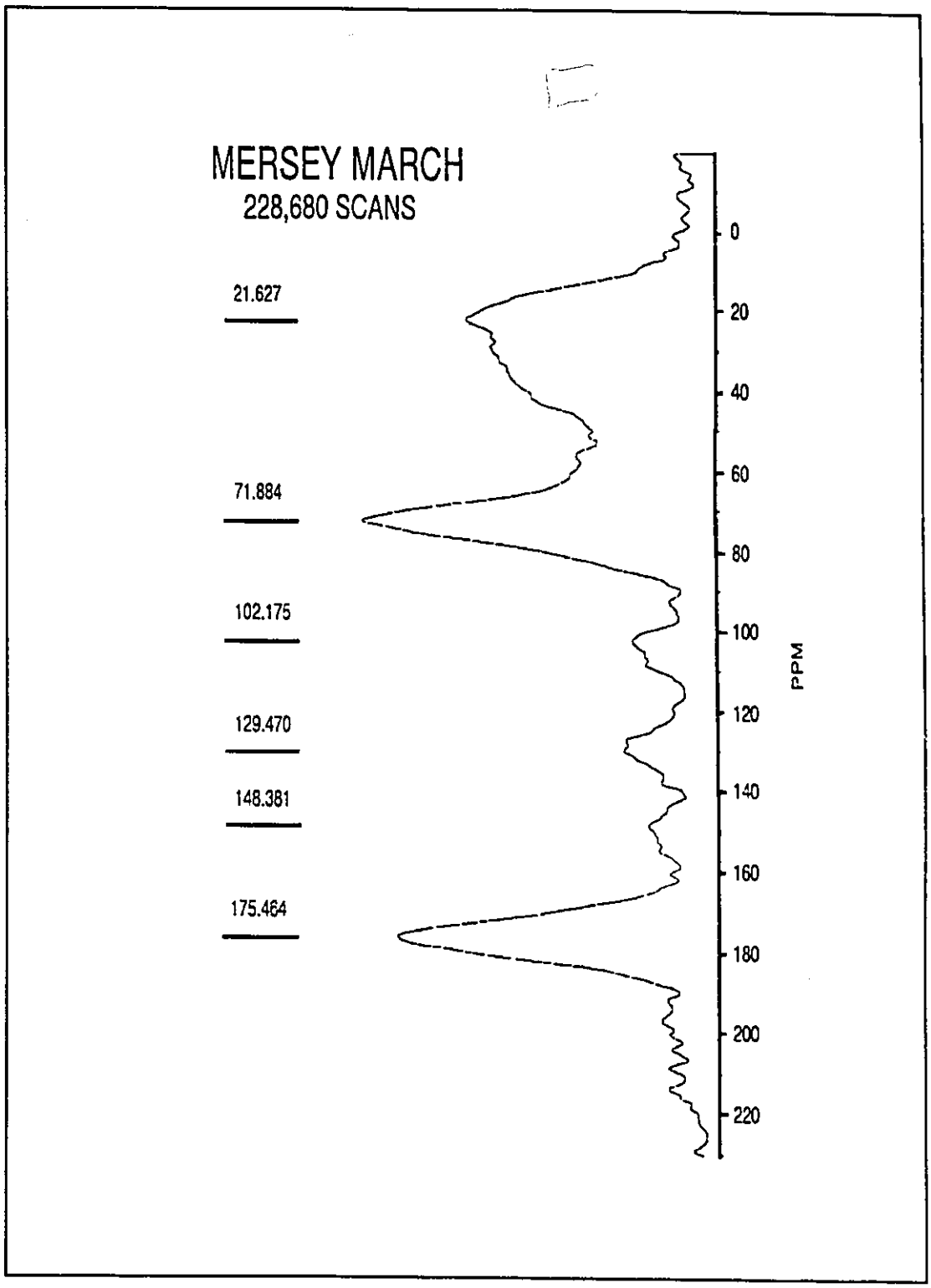
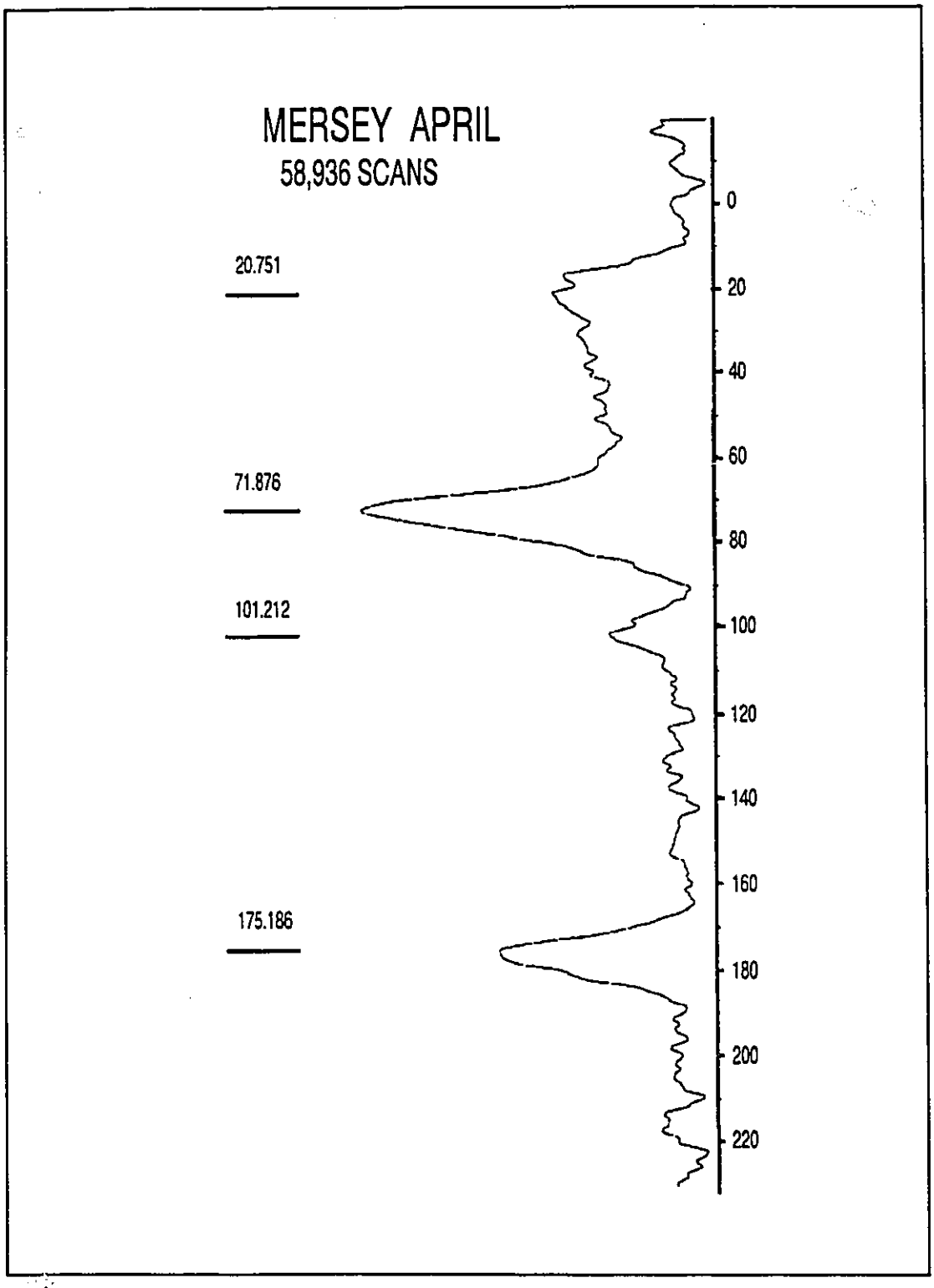
75.9°C

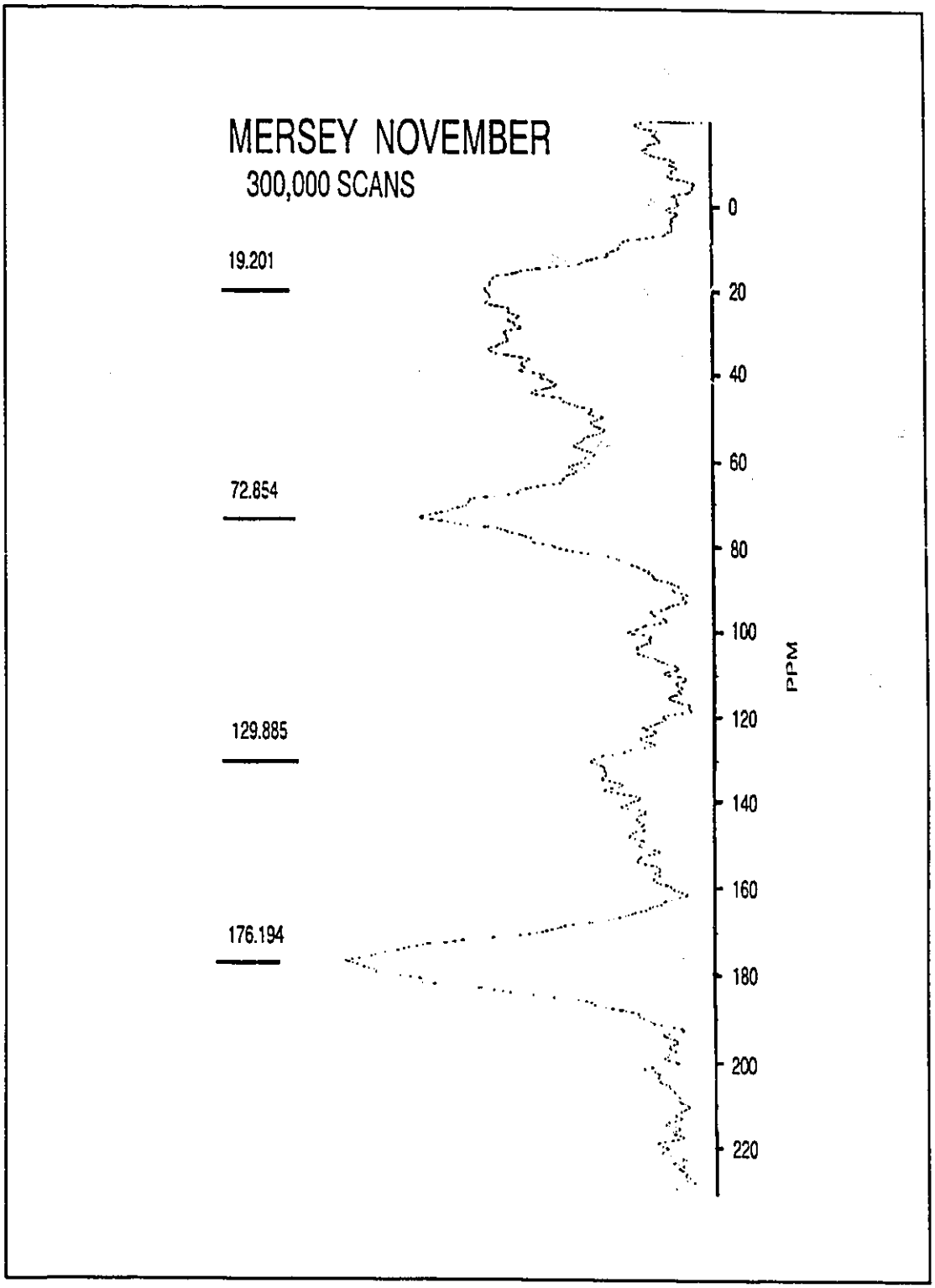

LAKE NOVEMBER


MERSEY JANUARY
71.057 SCANS


MERSEY JUNE
58,349 SCANS
19.830
70.438
102.237
130.834
150.575
174.812
0
20
40
60
80
100
120
140
160
180
200
220
PPM


MERSEY MARCH
228,680 SCANS
21.627
71.884
102.175
129.470
148.381
175.464
0
20
40
60
80
100
120
140
160
180
200
220
PPM


MERSEY APRIL
58,936 SCANS
20.751
71.876
101.212
175.186
0
20
40
60
80
100
120
140
160
180
200
220


MERSEY NOVEMBER
300,000 SCANS
19.201
72.854
129.885
176.194
0
20
40
60
80
100
120
140
160
180
200
220
PPM