Stable Isotopes of Sulphur and Oxygen in Forested Catchments: Insight from New Techniques into Sulphur Cycling and Dissolved Organic Matter Alteration

by

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Author's Declaration For Electronic Submission Of A Thesis

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

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Abstract

Dissolved organic matter (DOM) is present in all forested catchments and can be important in binding metals, absorbing UV, and the transport of nutrients (C, N, S, P). DOM is extremely heterogeneous in time and space, making it difficult to characterize. New techniques have been developed to determine $\delta^{34}S$ and $\delta^{18}O$ in DOM. These techniques have been applied to samples from Harp and Plastic Lake catchments (45º23'N, 79º 08'W, 45º11'N, 78º 50'W) in order to obtain information about sources and sinks of DOM within forested catchments on the Canadian Shield. In conjunction with sulphate and DOC concentrations, this new data provides valuable insight into sulphur cycling and DOM alteration within these catchments. Data generated for $\delta^{34}S$-DOM and $\delta^{18}O$-DOM appears to be the first data reported in the literature for DOM.

The inorganic ($\delta^{34}S$-SO_4^{2-}) and organic S ($\delta^{34}S$-DOM) differs by environment in both catchments. The range of $\delta^{34}S$-SO_4^{2-} is between 3.3‰ and 10.3‰, and the range of $\delta^{34}S$-DOM is from 3.4‰ to 8.7‰. Sulphate in the Harp Lake catchment in most samples is subject to some sort of cycling within the watershed, since $\delta^{34}S$-SO_4^{2-} differs from precipitation. In the Harp Lake catchment, upland $\delta^{34}S$-SO_4^{2-} is influenced by historical precipitation. The $\delta^{34}S$-DOM is derived from leaching and microbial activity of DOM from organic horizons in the soil. The $\delta^{34}S$-SO_4^{2-} and $\delta^{34}S$-DOM of wetland streams is extremely variable, controlled by hydrology. The $\delta^{34}S$-SO_4^{2-} provides information on oxidation-reduction dynamics in the wetland, and $\delta^{34}S$-DOM provides information about sources of DOS in the wetland. The $\delta^{34}S$-SO_4^{2-} and $\delta^{34}S$-DOM are possibly related in Harp Lake. Mineralization of DOS as evidenced by $\delta^{34}S$-DOM and DOS concentrations could be a small input of SO_4^{2-} into Harp Lake.

It is possible $\delta^{18}O$-DOM could be an indicator of DOM alteration. The range of $\delta^{18}O$-DOM is between 8.2‰ and 14.4‰. The $\delta^{18}O$-DOM in the Harp Lake catchment is highly correlated with relative molecular weight, which has been shown to decrease with increasing

alteration. Wetland streams show the largest range in δ^{18}O-DOM, while uplands, groundwater, and Harp Lake are the least varied. The highest δ^{18}O-DOM values are from sources of DOM such as leaf leachates (representative of forest floor litter) and wetlands. The most depleted samples are from groundwater and Harp Lake which typically contain highly altered DOM.

The δ^{34}S-DOM and δ^{18}O-DOM can provide valuable information on sources of DOM and DOM alteration within the catchment. The δ^{18}O-DOM could also allow the separation of autochthonous and allochthonous DOM in lakes.

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Chapter 1: Introduction

1.1 Dissolved Organic Matter in Forested Catchments

Dissolved organic matter (DOM) is a complex heterogeneous mixture of numerous natural organic compounds that result from the decomposition of plants and animals. It can play an important role in forested catchments affecting both aquatic chemistry and biology. Potentially toxic metals have been known to complex with DOM in natural aquatic systems, influencing both the speciation and mobility of metals and, in turn, affect the metals' fate, transport, and toxicity to aquatic life (Hollis et al., 1996, Wu & Tanoue, 2001). DOM can also affect the acid-base chemistry of acid freshwater systems, contributing up to 20% of the total acid buffering capacity (Schiff et al., 1990). The presence of DOM can protect freshwater organisms from exposure to harmful UV radiation by absorbing ultraviolet rays (Schindler & Curtis, 1997). It can also increase mineral weathering rates (Drever, 1997) by increasing the minerals' solubility (Schiff et al., 1990, Schindler & Curtis, 1997). Therefore, it is necessary to understand the composition and character of DOM and how these change DOM as it moves through different flowpaths within forested catchments.

1.2 DOM: Its Definition and Characterization

In most scientific literature, DOM is defined as organic material that passes through a 0.45 micron filter, however, this is a somewhat arbitrary definition. Organic materials can occur in a range of sizes, and 0.45 μm does not represent a fundamental break in this range, except for the fact that it excludes almost all bacteria (Drever, 1997). Other studies have used 0.2 μm as the threshold for DOM, and still other scientific studies do not filter samples at all, considering their samples to be less than 10% particulates (i.e. Ontario Ministry of Environment). Because of inconsistencies in the definition of DOM, caution must be taken in the comparison of DOM from different studies.

Due to the extremely heterogeneous nature of the material, only 3-5% of DOM can be structurally identified (Drever, 1997, USGS, 1994). Numerous attempts have been made to characterize DOM using various methods, including elemental analysis, molecular size/weight determination, UV absorption and isotopes. A widely accepted approach in the literature to DOM characterization is the separation of DOM into six major groups: humic substances (humic/fulvic/humin fractions); hydrophilic acids; carboxylic acids; amino acids; carbohydrates; and hydrocarbons (Bourbonnière & Meyers, 1978, Thurman, 1985). This method of separation was developed by George Aiken and the USGS using XAD resins (Aiken et al., 1985), and is still widely used today. However, the one major shortcoming of this method is that it is biased towards DOM with a higher affinity for the XAD resin. Generally, separation by XAD resin retains only 45-50% of dissolved organic matter.

Other methods of analysis such as isotopic or elemental analysis can either be used in conjunction with resin separation or used on total DOM (Drever, 1997). These methods provide a bulk picture of the DOM, labeling it with a single isotopic value or a single elemental percentage on the total DOM. In terms of omission of a certain portion of DOM, these methods are less discriminatory than resin separation, and can provide additional details on DOM composition or transformation.

1.3 Factors affecting composition and character of DOM

There are many factors that can affect both the composition and character of DOM within the catchment. Origin and source, transport, and subsequent physical, geochemical, and biological processes can transform the nature of DOM. As DOM progresses along various hydrological flowpaths, it can undergo transformations in its character.

The origin and source of DOM is an important determinant of DOM produced within a catchment. One example is the composition and character of DOM from terrestrial or allochthonous sources, differs significantly from autochthonous or aquatic sources (Thurman, 1985). Allochthonous DOM is influenced by original terrestrial vegetation, and differs from uplands to wetlands (Schiff et al., 1990). For example, forest type can play a large role in the

type of DOM exported from a catchment. Therefore, within the catchment, the terrestrial DOM deriving from that vegetation typically differs from uplands to wetlands. The character of autochthonous DOM is determined by the type of aquatic organisms and aquatic vegetation in the lake. However, because lakes receive input from terrestrial sources, it is a mixture of autochthonous/allochthonous components, thereby making it difficult to determine the origin of DOM within that lake. Dillon & Molot (1997) using a mass balance approach found that lakes located on the Canadian Shield are primarily dominated by the allochthonous DOM. Dillon & Molot (1997) found this allochthonous input originated in the wetlands, and that DOC is relatively "young" carbon that has been fixed in recent times (e.g. majority within the last 50 years). This occurs despite a large proportion of organic carbon in the wetland being much older. Thus, the origin and source of DOM greatly influences the character and composition of the DOM as it moves through various flowpaths in the catchment.

As DOM is transported along hydrological pathways in the catchment, it can be subject to transformations through physical, geochemical, and biological processes. These processes include UV degradation, sorption, microbial degradation, and DOM sedimentation within the lake (Dillon & Molot, 1997). DOM in surface waters absorbs ultraviolet and visible light, which both break down the molecules and provides free radicals that may influence other aquatic chemistry (Drever, 1997). Furthermore, microbial breakdown of the labile portion of DOM can completely change the character of the DOM (Thurman, 1985). These processes (and others processes along hydrologic pathways in the catchment), affect both the structure and composition of DOM after its original formation.

As seen above, there are many factors that can affect the structure and composition of DOM in catchments. Therefore, DOM will differ throughout the catchment, both spatially and temporally. It is because of these differences that there is a need for efficient, quick, and effective methods to characterize DOM.

1.4 Characterization of DOM by Isotopes

DOM consists of many different elements, but the five main elements are: carbon; oxygen; hydrogen; nitrogen; and sulphur, in varying amounts. Isotopes of these elements have proven useful in fingerprinting the origin and fate of DOM within a catchment. Considerable research has been done using the isotopes ratios of $^{13}C/^{12}C$ and $^{14}C/^{12}C$, and $^{15}N/^{14}N$ to characterize DOM, providing useful insights into the age, origin, and soil reworking of the DOM (Schiff et al., 1990)). Considerably less research has been performed on S and O isotopes in DOM, although some work has been accomplished in acidification (for sulphur) and paleoclimatic (oxygen) studies (Alewell & Gehre, 1999, Alewell & Novak, 2001, Anderson et al., 2002, Edwards & McAndrews, 1989, Sauer et al., 2001,Wolfe & Edwards, 1997, Zhang et al., 1998).

1.5 Organic Sulphur and its Importance

Organic S is an important constituent of organic matter in forested catchments. Organic S constitutes between 0.1-3.5% of soil humic substances, and 0.5-1.43% of aquatic substances (Drever, 1997, Xia et al., 1998). In forested catchments, about 80-99% of total sulphur in soils is organic sulphur (Mitchell et al., 1998), and about >90% of sulphur in wetlands is organic sulphur (Alewell & Novak, 2001, Brown, 1985, Chapman & Davidson, 2001). Houle et al (1995) showed dissolved organic sulphur (DOS) accounts for 8-22% of total S in Pre-Cambrian Shield lakes in Québec. Nriagu & Soon (1985) found the majority of sulphur (>80%) in sediments in unpolluted lakes on the Canadian Shield is in the form of organic S. Urban et al. (1999) showed that addition of sulphur to organic matter occurs during diagenesis in lake sediments. Despite this abundance within the forested catchment, few studies have been done on the movement of organic S between different pools within the catchment.

Inorganic S cycling

Unlike organic sulphur, many studies have been conducted on inorganic sulphur cycling within the forested catchment. Most of this work was focused in Eastern Canada, Northeastern United States, and Europe, where acid rain deposition within the last 50 years has become harmful to aquatic biology (Gorham, 1998). After regulations to cut back sulphur emissions had been put in place in the early 1990's, studies have attempted to detect recovery of lakes in these regions (Dillon et al., 1997). These studies have focused on the fate and transport of sulphate within the watersheds in order to understand recovery from acidification.

Studies of the fate and transport of sulphate suggest that organic S is important in S cycling within the watershed. Alewell & Gehre (1999) performed a long-term analysis of stream sulphate at the Hubbard Brook Experimental Forest in New Hampshire using isotopes as tracers of sources of sulphate. They determined that sulphate is not conservative, but is subject to many transformations between inorganic and organic forms. They propose that a large proportion of stream sulphate comes from the organic S pool in the catchment.

Other studies investigating the effect of wetlands on sulphate fate and transport have concluded that a large store of sulphur exists within wetlands. Wetlands could possibly act as a source or sink for sulphate, depending upon redox conditions (Evans et al., 1997). Brown (1986) concluded that humic S compounds are a major product of dissimilatory sulphate reduction, with most organic S being formed in the top 7.5cm of the wetland. Mandernack et al. (2000) showed dissimilatory sulphate reduction occurring in wetlands reducing sulphate to organic sulphur. Chapman & Davidson (2001) and Alewell & Novak (2001) determined the fate of the majority of sulphate reduced in peat is storage in the takes the form of organic sulphur.

Importance of Organic S to Metal Binding

In addition to being important to sulphur budgets within catchments, organic sulphur is also important in metal binding. Many studies have shown DOM binds with metals, and that there are strong and weak binding sites in DOM. Recent studies have focused upon sulphur functional groups as being the strong binding sites for metals (O'Driscoll & Evans, 2000). Xia et al (1999) provided mechanistic proof of the ability of reduced sulphur species (such as thiols and disulfides) to strongly bind with Hg (II). This work was conducted using XAS (X-ray Absorption Spectroscopy) studies, which is used to obtain information on the local chemical environments of elements in a variety of geochemical materials (Xia et al., 1999). This study was the first study to demonstrate conclusively the importance of organic sulphur and reduced organic sulphur groups in the binding of metals.

Reduced sulphur functional groups in organic matter can range from 10% of total sulphur in a mineral soil to 50% in an aquatic fulvic acid (Xia et al, 1998). The percentage of reduced sulphur functional groups varies with organic matter; hence the metal binding capacity of the different types of organic matter will also vary. Furthermore, it has been shown the amount of reduced S in organic matter can be influenced by its environmental origin (Xia et al, 1998). From this, and the findings which reduced organic sulphur is related to metal binding, it can be concluded that DOM formed in different environments will display different average metal binding constants.

These studies illustrate the importance of organic sulphur and its environmental origin in metal binding. It can be concluded that the transport of organic sulphur in the watershed can be potentially significant for the fate and transport of metals in the catchment. This illustrates the need for more research to be carried out with respect to organic sulphur.

1.6 Organic Oxygen and Importance

Unlike sulphur, oxygen is extremely abundant, and on average, can constitute about 40-45% of the natural organic molecule in DOM (Thurman, 1985). Since oxygen is so abundant in DOM, much more is known about the functional groups containing oxygen such as carboxyls and phenols, and their role in combining with nitrogen and sulphur in different functional groups (Drever, 1997). Much work has been done to determine controls of $\delta^{18}O$ isotopic composition in carbohydrates (Cernusak et al., 2002, Dillon & Molot, 1997, Epstein et al., 1977, Farquhar & Lloyd, 1993, Sternberg, 1989, Sternberg et al., 1986). In particular, $\delta^{18}O$ fixed in cellulose has been the focus of paleoclimatological studies, using both lake sediment cores and tree ring analyses to interpret past temperature and climate (Edwards et al., 1989, Wolfe & Edwards, 1997, Abbott et al., 2000, Sauer et al, 2001, Anderson et al., 2002).

The isotopic ratio of water is determines the $\delta^{18}O$ composition in cellulose, with an enrichment factor of +27‰ (+26-28‰; Epstein et al., 1977, Sternberg et al., 1986, Sternberg, 1989, Farqhuar & Lloyd, 1993, Sauer et al., 2001). This enrichment of +27‰ is consistent across all plant types (terrestrial or aquatic), regardless of photosynthetic mode (Epstein et al., 1977, Sternberg et al., 1986, Sternberg, 1989). Experiments have shown that the oxygen derived from CO_2 equilibrates fully with water prior to being fixed as cellulose, even though the oxygen in carbohydrates is incorporated from both H_2O and CO_2 (Epstein et al, 1977, Sternberg, 1989, Sauer et al, 2001). Sternberg et al. (1986) shows that this enrichment does not occur during uptake of soil water in the plant, but rather it most likely occurs at the carbonyl hydration step where oxygen is fixed. This consistent enrichment among all plants allows paleoclimatologists to make inferences about the $\delta^{18}O$ composition of the water that the cellulose was formed in, allowing them to infer past climates.

Oxygen isotopes in cellulose of terrestrial vascular plants can possibly undergo further fractionation from local groundwater due to evapotranspiration of leaf water (Sternberg, 1989, Farquhar & Lloyd, 1993, Sauer et al, 2001, Anderson, 2002). When leaf water

evaporates, it will become enriched in $\delta^{18}O$, and since the oxygen isotopic signature of cellulose is derived from water, it will display the fractionated isotopic signature of the evapotranspired leaf water (Sternberg, 1989). Therefore, for terrestrial plants, the site of cellulose synthesis (leaf vs. stem) can be important in $\delta^{18}O$ studies (Sauer et al, 2001).

Given that the $\delta^{18}O$ composition of terrestrial plants is dependent upon evapotranspiration, it would be mainly controlled by climate. Anderson et al. (2002) state the $\delta^{18}O$ isotopic composition of tree ring cellulose is linked to climatic variables such as temperature, relative humidity, and amount of precipitation. In fact, when studying the $\delta^{18}O$ composition in tree rings, there are four important factors that are considered to control the $\delta^{18}O$ isotopic composition: 1) the isotopic composition of the water utilized in cellulose production; 2) the biologic fractionation between cellulose and water; 3) evaporative enrichment of leaf-water due to stomatal transpiration; and 4) isotopic exchange of oxygen atoms during the transfer of sucrose produced in the leaves to sites of cellulose production (Anderson et al., 2002 and references therein).

Aravena & Warner (1992) found that $\delta^{18}O$ signatures of Sphagnum moss growing on hummocks are enriched by 2‰ over the submerged Sphagnum species. They attributed this enrichment to microclimate differences in evapotranspiration. Similarly, Sauer et al. (2001) found terrestrial moss to be generally more enriched and more variable than submerged mosses, and attributed this to the effects of evapotranspiration. It is apparent from these studies that the $\delta^{18}O$ signature will be different in terrestrial plant species than aquatic species. The $\delta^{18}O$ composition of terrestrial plants near Dorset, Ontario, would be enriched by a factor of 3-5‰, giving a total enrichment of 30-32‰ (Dr. Tom Edwards, pers. comm.)

It is important to note that DOM consists of a large suite of organic molecules, and cannot be expected to have the same $\delta^{18}O$-DOM as cellulose. However, the $\delta^{18}O$ should be similar to cellulose (Cernusak, 2002). This difference in $\delta^{18}O$ between allochthonous or autochthonous cellulose could mean that $\delta^{18}O$-DOM could be extremely useful in the determination of the origin of DOM. Other studies have attempted to differentiate between

allochthonous/autochthonous DOM using various methods, with varying degrees of success. Using $\delta^{18}O$ could prove to be very valuable in DOM studies, as allochthonous and autochthonous DOM influence the DOM in the lake very differently.

1.7 Research Objectives

The characteristics of DOM vary within the forested catchment, due to differences in origin and transformations occurring within the catchment, both spatially and temporally. Little information is available on the cycling of organic S within the watershed, as well as the ability of different DOM to bind metals. Also, there is no simple way to differentiate allochthonous vs. autochthonous DOM. More research needs to be performed in the areas of organic sulphur and oxygen to understand sulphur cycling and allochthonous/autochthonous origin of DOM.

The main objective of this research is to characterize DOM in terms of organic oxygen and sulphur. The specific objectives of this research are to develop a new technique to enable the characterization of DOM in terms of $\delta^{34}S$, C/S ratios, and $\delta^{18}O$.

1.8 Thesis Organization

This thesis is composed of a general introduction to organic S and O in DOM (Chapter 1), site description (Chapter 2), and is followed by three chapters of results and discussion. The first of these three chapters (Chapter 3) presents details of the methods and analytical protocols developed to characterize DOM in terms of $\delta^{34}S$, C/S ratios, and $\delta^{18}O$. Chapter 4 presents results of $\delta^{34}S$-SO_4^{2-} and $\delta^{34}S$-DOM. Chapter 5 is a presentation of $\delta^{18}O$-DOM results. The final chapter consists of a summary of chapters 4 and 5, conclusions, and recommendations.

Chapter 2: Site Description

2.1 Harp and Plastic Lake Watersheds

Harp Lake (45º23'N, 79º 08'W) and Plastic Lake (45º11'N, 78º 50'W) catchments are located approximately 200 km north of Toronto, Ontario, Canada (Fig. 1). Both sites have been intensively investigated as part of the Ontario Ministry of Environment's acidic precipitation research program. These catchments are situated near the southern limit of the Precambrian Shield in south-central Ontario and have similar physiography, geology and some hydrological and geochemical characteristics (Hinton et al., 1994).

2.2 Climate

Annual precipitation in the area is 900-1100mm with 240-300 mm falling as snow between December and April. The mean January and July air temperatures are –10ºC and 17.7º C, respectively. Annual runoff is similar in both catchments, varying between years from 400 to 600mm.

2.3 Geology, Hydrology, Vegetation of Catchments

The geology, hydrogeochemistry, and hydrology of the catchments are outlined in detail in Jeffries & Snyder (1983).

Geological Setting of Catchments

Both catchments are underlain by impermeable Precambrian metamorphic silicate bedrock covered with thin basal till. The underlying bedrock in the Harp Lake catchment consists primarily of biotite and horneblende gneiss with amphibolite (69%) and schist (28%) in the remaining portion of the basin. The overburden consists of glacial till deposits,

varying in thickness from 0 to 15 meters (Jeffries & Snyder 1983). Soils are poorly developed podzols formed upon the generally thin, sandy basal tills (Schiff et al., 1997).

The underlying bedrock in the Plastic Lake catchment is a hummocky granitic gneiss and amphibolite (Lazerte, 1993). Overburden in the Plastic catchment is classified as thin till-rock ridges (less than 1m depth) with a small area (10%) of sandy till 1-1.5m in depth. The upland forest soils consist of sandy, shallow (~0.5m) podzols, while the conifer swamps are peaty, organic mucks and gleysols (mean depth 2-3m, 7m max. depth) (Lazerte, 1993).

Hydrology of Catchments

The terrestrial catchment area of the Harp Lake catchment has been divided into six major subcatchments (Fig. 2). Each stream is gauged with a weir at a convenient location proximal to the lake edge. Harp 4–21 (a sub basin within the Harp 4 basin) has been the site of intensive investigations on the role of groundwaters in streamflow generation (Hinton et al. 1994). Harp 4-21 is atypical in that it has deeper tills and no wetland areas (Hinton et al., 1994). Wetlands are present in most subcatchments (Fig. 2), with the main wetland types being beaver ponds and conifer swamps. Harp Lake is a dimictic oligotrophic lake with an area of 71.4 ha, with a mean depth of 13.2m. It is a soft water lake with an average alkalinity of approximately 60μeq/L.

The study area at Plastic Lake catchment is the PC-1 catchment (Fig. 3). This catchment comprises upland streams feeding into a low-lying conifer swamp, which then outlets as PC-1, into Plastic Lake. Each stream is gauged with a weir, located proximal to the bottom of the subcatchment, and PC-1 is gauged proximal to the lake.

The input to the Plastic swamp is the upland stream PC1-08, which drains an upland catchment fed primarily by groundwater. PC1-08 is the only input stream gauged into the Plastic swamp.

Biogeochemical Setting of Catchments

The vegetation in the Harp catchment is a mixed deciduous-conifer forest of primarily sugar maple (*Acer* spp.) and birch (*Betula* spp.) on the dry uplands and a coniferous forest (white cedar (*Thuja occidentalis*) hemlock and balsam fir) in low-lying wetland areas.

The Plastic uplands are forested primarily with stands of white pine (*Pinus strobus*) hemlock (*Tsuga canadensis*) and balsam fir (*Abeis balsamea*). The dominant vegetation in the lowland conifer swamps is white pine (*Pinus strobus* L.) and black spruce (*Picea mariana*) with sphagnum spp. as the dominant ground cover (Lazerte, 1993).

Plastic conifer swamp (2.2 ha) occupies a central bedrock depression and represents about 10% of the sub-catchment basin area of 21.1 ha (Fig. 2). The swamp is forested primarily with white cedar and black spruce with some birch and maple. There is an understorey of *Alnus* spp., *Ilex vericillata,* and a well-developed layer of *Sphagnum.* A hummock-hollow micro-topography has developed throughout the swamp. Peaty humic mesisols up to 6m depth (average 2-3m) overlie regions of gyttja and deposits of silt, clay, sand and gravel up to 1m depth in the bedrock basin (Eimers, 2002).

2.4 Location of Sample Sites

At the Harp Lake catchment, samples were collected at the weir outlet of the Harp 4, Harp 4-21, Harp 5, and Harp 6 subcatchments (Fig. 2). Groundwater samples were collected from Wells 57, 59, 60, and 61 in Harp 4-21. These samples were combined to obtain a representative sample of shallow groundwater. A deep groundwater sample was collected from Well 55, located near the Lake. The Harp Lake sample was obtained from the epilimnion.

At the Plastic Lake catchment, samples were collected from the weir of PC-108 – one of the inflows to the wetland, and at the weir of PC-1 – the outflow from the wetland into the lake (Figure 2). Bulk precipitation samples were collected from collection buckets (screened to prevent large debris from falling into the sample), located approximately 200m from the

edge of the lake and 400m north of PC-1. Throughfall samples were collected using eavestroughing-type channels that accumulated water in buckets. LFH water samples were collected from different zero-tension lysimeters located within the uplands (Fig. 3) and combined together to provide a large enough volume for reverse osmosis concentration.

Figure 1. Location of Harp and Plastic Lake watersheds.

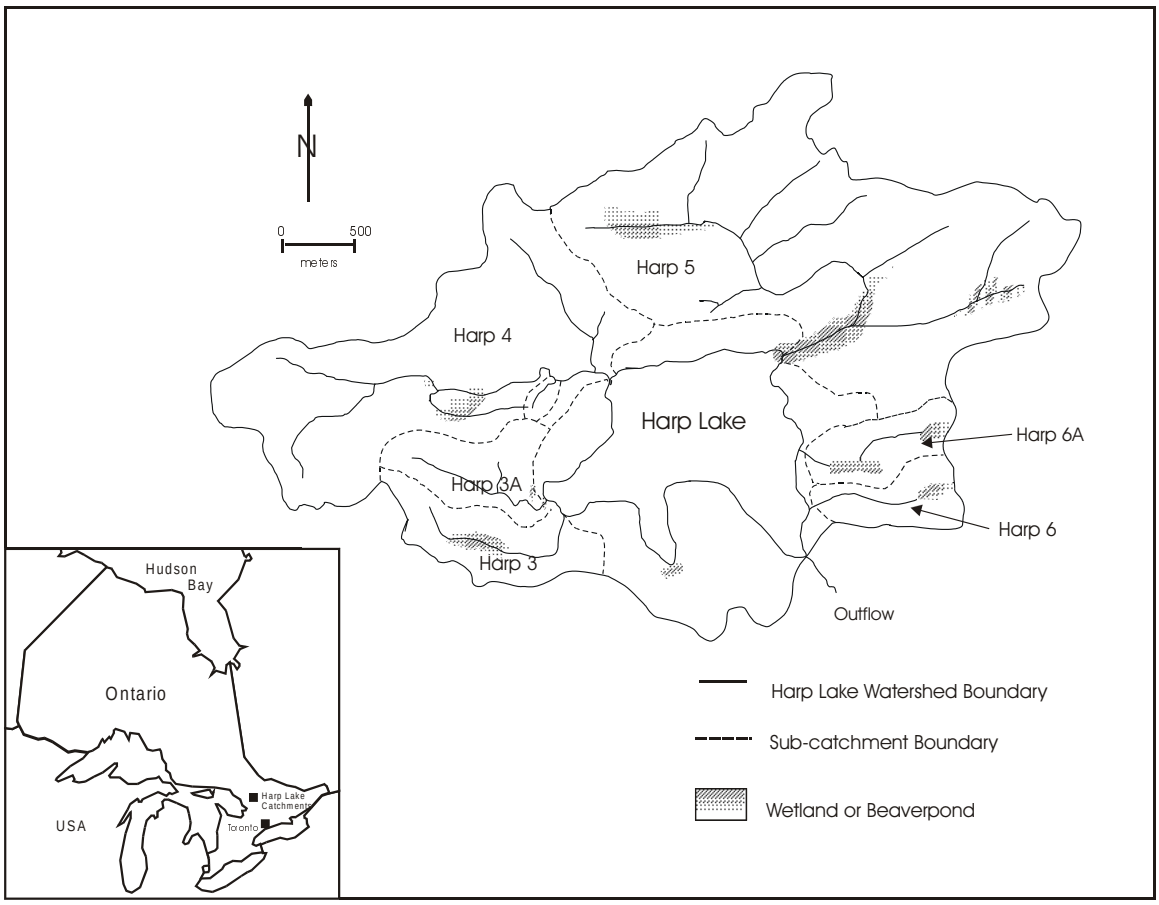

Figure 2. Detailed map of Harp Lake Catchment.

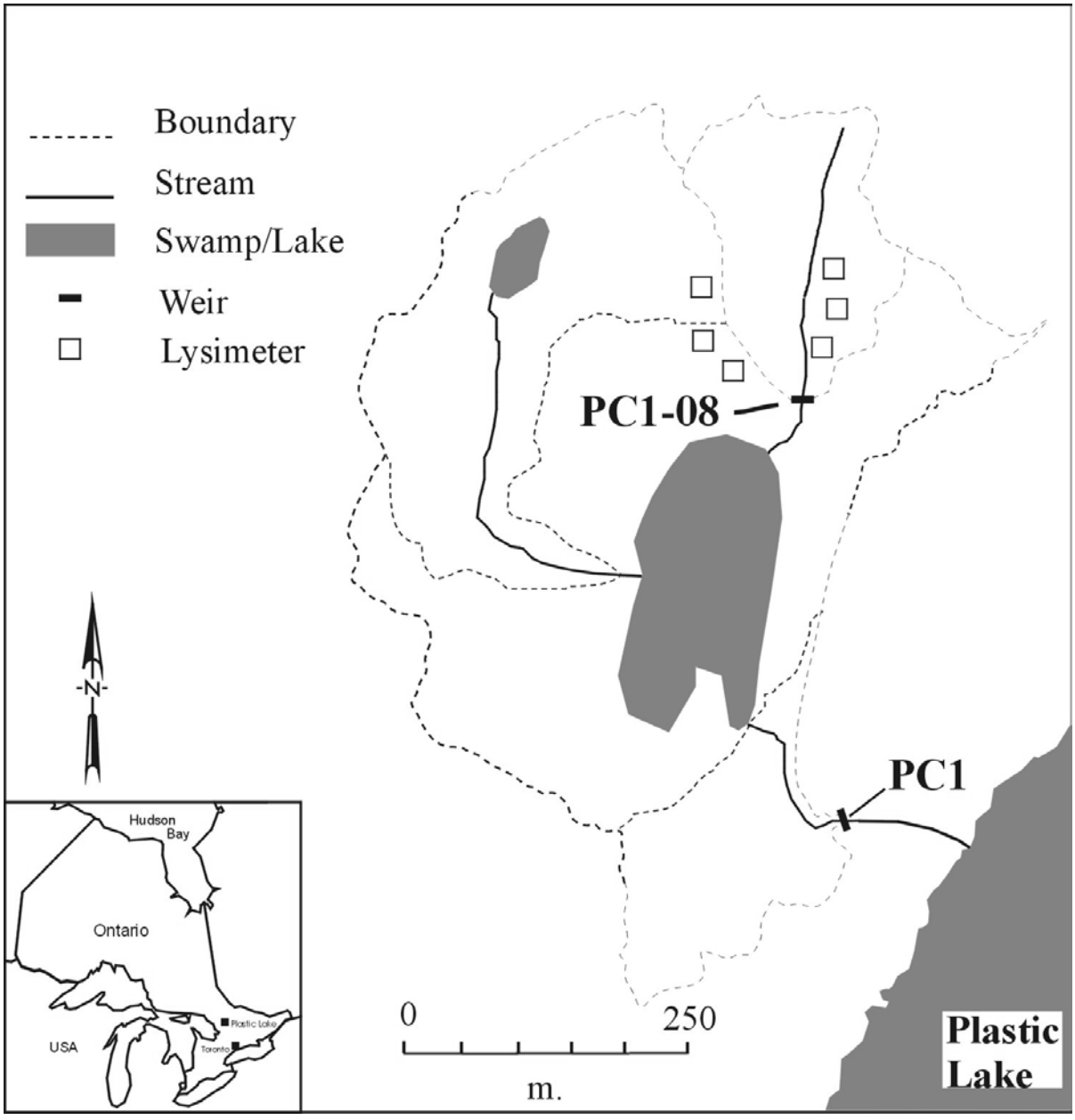

Figure 3. Detailed map of Plastic Swamp investigated at Plastic Lake Catchment.

Chapter 3: Isotopic Characterization of Sulphur and Oxygen in Dissolved Organic Matter

3.1 Introduction

Organic sulphur constitutes the largest pool (80-99%) of total sulphur in temperate forest soils (Mitchell et al., 1998) and wetlands (>90%; Alewell & Novak, 2001). Despite its abundance, the role of organic sulphur in sulphur cycling is not well understood because of the diversity of organic sulphur compounds (Krouse et al., 1992). Sulphur has a wide range of oxidation states (-2 to +6), and thus has a tendency to form a variety of compounds with a multitude of elements, most commonly carbon, oxygen and itself. This, in addition to methodological problems in isolating organic sulphur (Krause et al., 1992) and the fact that it has a low abundance in organic matter (0.1-3.5%; Xia et al., 1999), creates difficulties in understanding both organic sulphur chemistry and cycling in natural ecosystems.

Little work has been done in the field of dissolved organic sulphur (DOS), since it is difficult to separate dissolved organic matter from the inorganic sulphate (which interferes with the sulphur signal). Houle et al (1995) determined DOS as the difference between total S and inorganic sulphate. This differential procedure implies the remainder of the sulphur in the sample to be DOS, but does not obtain a direct measurement of DOS itself. To date, there are relatively few papers in the literature on the determination of DOS in waters, and none which determine directly the presence of natural DOS in waters. Schnitzler & Sontheimer (1982) established a method of determining the dissolved organic sulphur in water, but their study was focused more on pollutants. In their method, organic compounds were adsorbed to sulphurous active carbon at a pH of 3, with any adsorbed sulphate was removed by washing. Then, organic compounds were analysed for organic sulphur and labeled as DOS. One problem with this method is the carbon adsorption surface contains sulphur, approximately 2mg sulphur per gram of carbon. This sulphur could potentially contaminate the organic sample, particularly if organic sulphur levels were low (as in natural

waters). Also, there is the potential for isotopic exchange between the two organic sulphur species. Therefore, the signal obtained from this analysis could potentially be incorrect, both in DOS concentrations and $\delta^{34}S\text{-}S_{org}$. Secondly, it appears not all of the organic matter will be adsorbed to the active carbon, resulting in the loss of organic matter. Therefore, as of this time of publication, there is no effective method of determination of DOS in the literature.

Organic Oxygen

Listed in order of abundance, dissolved organic matter consists of: carbon, oxygen, hydrogen, nitrogen and sulphur. Organic oxygen can constitute between 23 to 45% by weight of the DOM molecule (Thurman, 1985). Oxygen is important in many functional groups in DOM (Drever, 1997), and oxygen accounting has been used to obtain information about these functional groups (Thurman, 1985). Other than oxygen accounting, few studies have been performed on organic oxygen in DOM, despite its abundance and importance in functional groups in DOM.

The $\delta^{18}O$-DOM could possibly be a potential tool for differentiating terrestrial (allochthonous) DOM from aquatic (autochthonous) DOM. This is due to the difference in $\delta^{18}O$ of the water used in photosynthesis (Sternberg, 1989). Evapotranspiration effects in the leaf cause the $\delta^{18}O$ signature in terrestrial plants to be enriched and more varied than aquatic plants (Sauer et al., 2001). Aravena & Warner (1992) found the $\delta^{18}O$ signatures in submerged Sphagnum cellulose were on average 2‰ more depleted than Sphagnum located on hummocks in wetlands, and attributed this difference to microclimatic differences in evapotranspiration. Sauer et al. (2001) determined ^{18}O differences between subaerial and submerged moss cellulose, and concluded ^{18}O in terrestrial organic matter would have a different ^{18}O signature than aquatic organic matter. Therefore, using these findings, terrestrial DOM could potentially be differentiated from aquatic DOM.

This chapter will focus on techniques to isolate total DOS and dissolved organic oxygen. The research centres on total DOM, and does not separate the DOM into different fractions. Thus, analyses of total DOM enables a more complete picture of the characteristics of total DOM while allowing comparisons between samples of DOM from different environments. Techniques have been developed to determine the $^{34}S/^{32}S$, $^{18}O/^{16}O$, and the C/S ratios for application to sulphur cycling, environmental origin and metal binding. The $\delta^{34}S_{org}$ signal should provide insight into environmental origin, and furthermore the C/S ratio should give some hints about the nature of metal binding. Concurrent analysis of ^{34}S is conducted to allow comparison of $\delta^{34}S_{org}$ with $\delta^{34}SO_4^{2-}$. The $\delta^{18}O$-DOM should yield insight into the autochthonous or allochthonous nature of the DOM. Also, this new data may provide new understanding into sources and processes affecting dissolved organic matter along different flowpaths.

3.2 Methods

Analysis protocols of DOC, SO_4^{2-}, NO_3^-, $\delta^{34}S$, $\delta^{18}O$

DOC concentrations were determined using a Rosemount Analytical Dohrmann (DC190) high temperature total carbon analyzer at the Environmental Geochemistry Laboratory, Department of Earth Sciences, University of Waterloo. This apparatus had a detection limit of 0.5mg/L DOC.

Sulphate (SO_4^{2-}) and nitrate (NO_3^-) concentrations were determined by ion chromatography at the Analytical Chemistry Services Lab, Chemical Engineering, University of Waterloo. A Dionex 500 with a Dionex IonPac 4-mm AS11column with an eluent of 10 mM NaOH delivered isocratically at 1 ml/min was used to perform analysis. The apparatus had a detection limit of 0.03 mg/L for non-diluted samples, and 0.3 mg/L for samples diluted by a dilution factor of 10.

Both inorganic and organic samples were run for $\delta^{34}S$ using an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental

Analyzer (CHNS-O EA 1108) in the Environmental Isotope Laboratory (EIL), University of Waterloo. The detection limit of this apparatus was 0.3‰ for clean BaSO4, and 0.3-0.6‰ for organic S. The range in error for organic S is dependent on the amount of S within the organic sample. As the %S decreases in a sample, the error increases. Although there are no international organic sulphur standards, a representation of sample reproducibility can be gained through sample repeats.

Organic samples were run for $\delta^{18}O$ using a Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA 1108) with a high T combustion. This apparatus has a detection limit of ±0.2‰ for $\delta^{18}O$ of cellulose.

Samples were run for Nitrogen and Carbon analysis on an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carla Erba Elemental Analyzer (CHNS-O EA1108).

All mass spectrometers were located in the Environmental Isotope Laboratory (EIL), University of Waterloo.

The following sections describe the methods used on all samples in this thesis to isolate organic S and O, as well as protocols used to test the isolation procedure. In overview, the sample procedure to remove sulphate involves concentration of samples by reverse osmosis, and isolation of organics by barium sulphate precipitation techniques. The resulting isolated organic matter can be analysed for $\delta^{34}S$ and $\delta^{18}O$ as well as C/S ratios.

Large Volume Sample Collection and Field Filtration

A large volume sample was collected to allow technique development and analysis of other DOM parameters. The volume of sample required was dependent upon the DOC concentration at the time of sampling. Historical data were used to determine the volume to be collected and the sample size ranged from 50 to 200L. Subsamples of each sample were

submitted to the Ministry of Environment Dorset Research Center in Dorset, Ontario, for chemical analysis.

Large volume samples were field filtered with Nitex mesh (150-200 μm) and transported to the University of Waterloo where they were kept at 4°C until subsequent processing.

In the laboratory, large volume samples (50-200L) were filtered using a Balston inline glass fiber filter (7μm) stainless steel aluminum 20 μm prefilter followed by a Geotech 147mm inline filter containing a 0.7μm glass fibre filter (Whatman GF/F, 0.7μm nominal size). The 0.7μm GF/F filters were precombusted (550°C, 6hrs). Samples were stored in amber glass bottles with no exposure to light and kept at 4-5°C.

The rationale behind filtering each sample to a nominal 0.7μm using glass fibre filters (GF/F) is: 1) they are glass filters which are binder free, allowing them to be burnt to remove organic matter and, 2) the glass filters allow isotopic analysis of the filter, without introducing any organic material from the filter itself. The nominal size of 0.7μm is the minimum pore size available in glass fibre filters, and is the current choice of filters. In this study, DOM is defined as that passing through the 0.7μm filter.

After filtering, 20ml subsamples were taken for analysis of DOC, nitrate, and sulphate at the University of Waterloo.

Concentration of Dissolved Organic Matter by Reverse Osmosis

A reverse osmosis (RO) system with a 300 Dalton membrane was used to concentrate the DOM (Fig. 3). Volumes of the original samples were recorded before RO. With a commercial reverse osmosis system, the organic matter and other solutes would be rejected to waste. However, in this application, to concentrate a sample, the rejected material is returned to the pot (source water) and becomes the “retentate”. This retentate consists of the concentrated solutes, including all organic and inorganic species < 0.7 μm, and is kept for

analysis. For more detailed information on the reverse osmosis procedure, see Serkitz and Perdue (1990), Clair et al (1991), and Sun et al (1995).

Samples were concentrated from the original volume (50-200L) to approximately 4-5.5L of retentate. During concentration, the permeate DOC concentration was monitored and collected to be used for displacing the water in the membrane and cleaning the system. Approximately 1L of the concentrated sample remains in the pot and approximately 4L is retained within the membrane. The retentate in the pot was drained (see drain-Figure 4) and the retentate in the membrane was displaced with permeate water and collected in a separate bottle (henceforth called "flushed retentate"). DOM in the retentate is typically more concentrated than the flushed retentate, because the flushed retentate is diluted as it is flushed with permeate water. During the RO process, 20ml samples were taken from the permeate water to ensure retention of solute within the membrane. DOC concentrations from both the pot and flushed retentates to determine the amount of sample to be used in subsequent isolation procedures.

Previous work has demonstrated that approximately 99% of organic matter is rejected by the RO membrane utilized in this study (Richard Elgood, unpublished data). Although a carbon mass balance was not performed for each sample, it is assumed that the rejection by the membrane was consistently better than 98%. Analysis of DOC from the 20ml permeate samples collected during the RO process shows little to no loss of DOC for each sample. However, minimal loss of C in the permeate does not mean that 99% of carbon is recovered, as some organic material may be lost, possibly due to operator error (e.g. spillages, membrane not totally flushed).

Isolation Procedures

In order to conduct analyses of $\delta^{34}S$ and $\delta^{18}O$ in DOM, the original sample must be purged of sulphate (SO_4^{2-}) and nitrate (NO_3^-). Sulphur from sulphate and oxygen from both sulphate and nitrate could interfere with the desired isotopic signals of $\delta^{34}S$ and $\delta^{18}O$ in DOM. These anions can be present in appreciable concentrations. Table 1 is an example of

the interference that differing amounts of sulphate can have on $\delta^{34}S$ and $\delta^{18}O$ signatures. The assumptions in these calculations are: 1) the organic matter sample contains 1%S and 40%, 2) the $\delta^{34}S$ of sulphate is 5‰, and 3) the $\delta^{18}O$ of sulphate is 12‰ (average values from Van Stempvoort et al, 1991, 1992).

Table 1. Table showing example of interference from sulphate (SO_4^{2-}).

Theoretical $\delta^{34}S$ of Organic Sample	Theoretical $\delta^{18}O$ of Organic Sample	DOC (mg/L)	S_{org} (mg)	O_{org} (mg)	SO_4^{2-} (mg/L)	S_{inorg} (mg)	O_{inorg} (mg)	Resulting $\delta^{34}S$	Resulting $\delta^{18}O$
8	20	200	4	160	100	33.33	66.67	5.32	17.65
8	15	200	4	160	20	6.67	13.33	6.13	14.77
3	20	200	4	160	100	33.33	66.67	4.79	17.65
3	15	200	4	160	20	6.67	13.33	4.25	14.77

As can be seen in Table 1, the resulting of $\delta^{34}S$ and $\delta^{18}O$ of the total sample can be substantially different from the $\delta^{34}S$ and $\delta^{18}O$ value of the DOM. Therefore, as shown by the theoretical calculations in the above table, the inorganic isotopic signatures can considerably alter the resulting isotopic signature and sulphate must be removed.

Removal of Sulphate (SO_4^{2-})

Several different methods (including ultrafiltration, dialysis, and barium sulphate precipitation) were tried in an effort to remove sulphate (SO_4^{2-}) and nitrate (NO_3^-) from the organic S in the surface and groundwater samples.

The removal of SO_4^{2-} by ultrafiltration was attempted in previous experimentation using a Pall-Gelman Centramate system with a 1000D tangential flow membrane. This method proved to be ineffective in removing a sufficient amount of the sulphate and is further limited by the loss of a significant quantity of low molecular weight organics (Richard Elgood, unpublished data, 2000).

Removal of SO_4^{2-} using dialysis membranes was attempted. Feuerstein et al (1997) demonstrated that dialysis of nitrate (NO_3^-) can be achieved using a 100 Dalton membrane over periods of up to 2 days. Similar dialysis experiments were conducted for sulphate.

Experiments used 100 and 500D Spectra-por cellulose ester membranes. Some membranes were washed with distilled water (DI), and some with Extran soap and then immersed in large volumes of DI. Experiments were carried out for up to 10 days (240hrs). A maximum of 50% of sulphate mass was removed using the membranes washed with Extran (Fig. 5), which is not sufficient removal for isotopic analysis. Therefore, the use dialysis membranes proved to be unsuccessful in removing sulphate

Removal of sulphate by dialysis was attempted using a Spectra-por 50mm Macrodialyzer with dialysis membranes of 100 and 500D. This technique was also unsuccessful in removing adequate sulphate amounts. Therefore, experiments on the use of dialysis membranes as a method to remove sulphate were discontinued.

Protocol for the Isolation of Dissolved Organic Sulphur and Oxygen

Precipitation of barium sulphate proved to be an effective method for the removal of sulphate, and is the method used in the remainder of this thesis (Fig 6.). This method involves the reaction of free Ba^{2+} cations with SO_4^{2-} anions to form the insoluble salt barium sulphate ($BaSO_4$):

$$Ba^{2+} + SO_4^{2-} \Rightarrow BaSO_4 \qquad \text{Eq. 3.1}$$

The addition of free Ba^{2+} cations was accomplished by the reaction of barium carbonate ($BaCO_3$) with hydrochloric acid (HCl):

$$BaCO_3 + HCl \Rightarrow Ba^{2+} + H^+ + CO_3^{2-} + Cl^- \qquad \text{Eq. 3.2}$$

At each step in this procedure DOC concentrations are recorded for mass balance purposes. This monitoring allows calculation of carbon removed at each step in the process and will give an indication of the loss of organic matter at each step.

Precipitation of Organic Matter

The addition of $BaCl_2$ or $BaCO_3$ to samples with high organic carbon content results in the precipitation of organic matter, as well as adsorption of organic matter to the barium sulphate precipitate. In an attempt to minimize the loss of organic matter with the barium sulphate precipitation procedure, a method was developed that removes solid organic matter prior to the addition of the barium salts.

1. The concentrated solution collected from the RO machine is further concentrated by freeze drying. Concentrated samples which had insufficient mass of DOM for S analysis were evapo-concentrated in order to obtain sufficient mass.

2. DI (between 20-40 ml) is added to the freeze-dried sample, and the sample is transferred into a 50ml centrifuge tube. After freeze drying and subsequent hydration, some organic material does not re-hydrate, leaving a layer of organic precipitate at the bottom of the centrifuge tube (Fig. 7).

3. Hydrochloric acid (3ml) is added to the sample, thereby acidifying the sample to a pH of less than 2. This will effectively precipitate out the humic acids, the fraction of DOM that is insoluble at pH lower than 2 (Bourbonnière & Meyers, 1978, Thurman, 1985). This, in combination with precipitate from freeze drying/ hydration, can remove up to 30% of carbon from solution as precipitate (Fig. 8).

4. The sample is left overnight in a refrigerated environment to allow organic precipitate to settle. After organic precipitation, the sample is placed in a centrifuge and spun at 8000 rpm to ensure suspended material is concentrated in the tip of the centrifuge tube. The organic precipitate is subsequently removed by pipette, and placed in 2ml centrifuge tubes. To further recover the supernatant solution, the 2ml tubes are spun at 4000 rpm. The resulting supernatant solutions from both 50ml and 2ml centrifuge tubes are combined and placed in a beaker for $BaSO_4$ precipitation. The remaining organic precipitates in the 2ml centrifuge tubes are retained for later combination with the sulphate/nitrate free solution for freeze drying (Fig. 6).

Precipitation of Barium Sulphate

The high affinity of Ba^{2+} for exchange sites within the concentrated organic matter creates problems when trying to precipitate $BaSO_4$. Free barium is known to bind strongly with organic matter and has been used in soil science to determine cation exchange capacity (CEC) (Ellis & Mellor, 1995, Foth, 1984, Hendershot & Duquette, 1986). Thus, when free Ba^{2+} is added to a concentrated organic solution, loss of free Ba^{2+} to exchange sites within the organic matter will occur. The result of this loss of free barium is a decreased availability of Ba^{2+} to react with SO_4^{2-} for the formation of barium sulphate.

This problem was resolved by the addition of concentrated acid (10% HCl) to the concentrated solution. The addition of HCl appeared to saturate the exchange sites within the organic matter with hydrogen (H^+) ions, effectively freeing Ba^{2+} ions into solution. This method is used in determining the cation exchange capacity by "proton complexation". The addition of protons by proton titration removes all other bound cations from the organic matter, giving an estimatation of cation exchange (Sposito et al., 1982). Preliminary tests with and without acidification showed a difference of 45-50% sulphate removal between both acidified and non-acidified samples.

For the supply of free barium ions, $BaCO_3$ was chosen over $BaCl_2$ because $BaCO_3$ removed a lesser amount of organic matter when added to the concentrated solution (Tables 5 and 6). Experiments were performed using both $BaCl_2$ and $BaCO_3$ salts as the source of barium in order to determine which particular salt results in the lowest percentage of organic matter removal. The loss of organic matter was much larger for precipitation by $BaCl_2$ (18-35.1%) than by $BaCO_3$ precipitation (4.8-16.9%).

Table 2. Summary of Carbon Lost by $BaCl_2$ precipitation.

Sample	Original DOC (mg/L)	Final DOC (mg/L)	Carbon Retained (%)	Carbon Lost (%)
Harp 4 Oct 23/00	106.0	73	68.9	31.1
Harp 4 Oct 23/00 (after 1 week)	106.0	69	65.1	34.9
Harp 5 Oct 23/00	160.6	104.2	64.9	35.1
Harp 5 Oct 23/00	160.6	107.7	67.1	32.9
Harp 4-21 Oct 23/00	22.5	12.7	56.6	43.4
PC1 2000	106.6	87.4	82.0	18.0
Harp 5 Oct 23/00 (retentate- pot)	81.9	62.6	76.4	23.6

Table 3. Summary of Carbon Lost by $BaCO_3$ precipitation.

Sample	Original DOC (mg/L)	Final DOC (mg/L)	Carbon Retained (%)	Carbon Lost (%)
Harp 4 Oct 23/00	106.0	94	88.7	11.3
Harp 4 Oct 23/00 (after 1 week)	106.0	93	87.7	12.3
HP 6 Oct 01 – BaCO3	131.9	125.5	95.2	4.8
HP 5 Oct 01 – BaCO3	235.0	207.0	88.1	11.9
HP 4 Oct 01 – BaCO3	115.4	95.9	83.1	16.9
HP Lk Oct 01 – BaCO3	86.2	76.3	88.5	11.5

In addition, the $BaCO_3$ + HCl procedure is more effective in removing SO_4^{2-} from the solution (Table 4). The removal of SO_4^{2-} is only approximately 60% with $BaCl_2$, but with $BaCO_3$ + HCl it is near 100 %.

Table 4. Removal of SO_4^{2-} by $BaCl_2$ and $BaCO_3$.

Sample	Salt used	Original SO_4^{2-}	Final SO_4^{2-}	SO_4^{2-} Removed (%)
Harp 4 Oct 23/00	$BaCl_2$	130.7	49.11	62.4
Harp 4 Oct 23/00 (after 1 week)	$BaCl_2$	130.7	51.58	60.5
Harp 4 Oct 23/00	$BaCO_3$	130.7	0.79	99.4
Harp 4 Oct 23/00 (after 1 week)	$BaCO_3$	130.7	0.74	99.4

Barium must be added in excess of stoichiometric requirements for $BaSO_4$ precipitation (Eq. 3.2), due to binding of Ba^{2+} by organic matter. It was determined from tests that 6x the required stoichiometric amount of $BaCO_3$ is the most effective in removing SO_4^{2-} from the solution from all samples.

After the addition of $BaCO_3$ and HCl, the solution is stirred for ten minutes on a stir plate to ensure complete reaction. The beaker is then covered and placed in a refrigerator to allow the $BaSO_4$ precipitate to settle overnight.

Evaluation of Sulphate Removal

After allowing adequate time for the $BaSO_4$ precipitate to settle, the remaining solution is analysed for SO_4^{2-} in order to ensure complete removal of SO_4^{2-}. DOC concentrations are taken, to determine the percentage carbon lost during the precipitation of the $BaSO_4$.

Assuming 1% sulphur content (Xia et al., 1999) within the organic matter, the ratios of inorganic sulphur (determined from SO_4^{2-} concentration) and organic sulphur (determined from 1%S of organic matter) are compared as a percentage. If the inorganic sulphur is <10% of the theoretical organic sulphur, then the procedure moves to the next stage. If it is >10%, additional BaCO3 is added in order to precipitate more SO_4^{2-} from the solution (Fig 6).

When the inorganic sulphate is less than 10% of organic S, the $BaSO_4$ must be separated from the remaining solution. This was done by decantation with a pipette.

Washing Procedures

The $BaSO_4$ precipitate contains some portion of organic matter adsorbed to it. In an effort to recover all of the organic matter, washing procedures were developed in an attempt to recover some of the adsorbed organic matter. Methods employed included washing the $BaSO_4$ precipitate with concentrated HCl and NaOH, as well as DI. Results from carbon mass balance analyses showed that HCl and NaOH removed a larger proportion of carbon (6%, 3%, respectively; Fig. 9). However, the addition of extra salt to the sample proved to be

too great for a signal of $\delta^{34}S\text{-}S_{org}$ to be analysed by the elemental analyser. Therefore, it was decided that one wash of the precipitate with DI would be the most effective in recovering carbon from the precipitate without having to add any extra salt. To wash the barium sulphate, 150 ml of DI were added to the precipitate. The mixture was stirred for 10 minutes, and left to settle overnight in the refrigerator.

The precipitate and DI solution are separated by pipette as above, and this DI solution is added to the concentrated original supernatant solution (Fig. 6). This washing procedure can recover between 1 and 10% of the original carbon (Appendix X).

Removal of Nitrate

In order for the $\delta^{18}O$ signal of the product to accurately reflect the $\delta^{18}O$ signal in DOM, nitrate must also be removed (provided there is sufficient nitrate to interfere with the signal). Nitrate contains 3 oxygen atoms, and, given appreciable amounts of nitrate, its isotopic signal could interfere with the organic $\delta^{18}O$ signature. Dialysis, as outlined by Feuerstein et al. (1997), was used for removing nitrate from the sample. Dialysis of nitrate was achieved using membranes with a molecular weight cutoff of 100 Daltons.

Because of the abundance of O in organic matter, and the fact that most samples contain little to no nitrate, most samples do not require dialysis. If the sample contained less than 15% inorganic oxygen in relation to organic oxygen, then it was decided not to use dialysis.

The dialysis process itself involves the placement of a portion of the sample in a dialysis membrane (Spectra-por 100D, 3.1ml/cm) inside a large volume (40L) of DI. Over a period of 24-48 hrs, nitrate and other salts (with a molecular size smaller than 100D) diffuse across the membrane. After dialysis, the concentrated sample in the dialysis membrane is analysed for nitrate, and if it is removed, the sample is placed in a beaker for freeze drying.

Preparation of Inorganic and Organic samples

After the $BaSO_4$ precipitate is washed with DI and separated, it is washed with acid (HCl) to eliminate any $BaCO_3$ from the precipitate. The precipitate is subsequently dried in the oven, ground, and analysed for $\delta^{34}S$.

Organic samples are placed in beakers, acidified (pH <2) and stirred to remove carbonate species. They are frozen overnight and placed in the freeze dryer. After the sample has been dried, it is carefully homogenized and transferred to a small glass vial for storage. The dried material is then ready to be run for $\delta^{34}S$-DOM and $\delta^{18}O$-DOM.

Method Verification: Recovery and Isotopic Integrity of $\delta^{34}S$-S_{org} and $\delta^{18}O$-DOM

Two standards were used to verify the isolation procedure: Florida Pahokee Peat reference sample obtained from the International Humic Substances Society (IHSS) and a leaf leachate which is derived from leaching of leaves in the Harp 6A catchment. Before the isolation procedure, both samples were analysed for SO_4^{2-} and NO_3^- to test for any appreciable inorganic S and O. The Florida peat sample (40mg) was dissolved using 1ml NaOH (pH=13). The above isolation procedures were tested using the IHSS standard and leaf leachate with added sulphate salts. For the Florida peat, 80mg was dissolved in 200ml of DI (198ml DI, 2ml NaOH). Sulphate (SO_4^{2-}), in the form of potassium sulphate (K_2SO_4) was added to the solution (40mg). Processing of the sample followed the same procedure as described above. For the leaf leachate, 72.6mg of K_2SO_4 ($\delta^{34}S$ = -0.7‰, $\delta^{18}O$ = 17.2‰) was added to 200mL of solution (DOC = 413mg/L). Processing of the sample followed the same procedure described as above. Duplicates of the same sample were also subject to the same isolation procedures to determine sample reproducibility. The samples were: PC1 June 7/2001 and PC1-08 June 22/2001. These are samples from two different environments, and the DOM found in each is very different. PC1 is an wetland stream, and DOM from this site is high in molecular weight and high in C/S. PC1-08 is an upland stream, and DOM consists of low molecular weight and low C/S.

3.3 Results and Discussion

Concentrations of DOC for the dissolved IHSS standard and leaf leachate standard are 205mg/L and 413mg/L, respectively. Sulphate concentrations were 0.5mg/L for the dissolved IHSS standard and 0.5mg/L for the leaf leachate. Concentrations of nitrate in both samples were below detection limits.

The sulphur from sulphate in the dissolved solution has the potential to interfere with the $\delta^{34}S$ and $\delta^{18}O$ analyses. To determine whether this is a factor in this study, the inorganic sulphur was compared to the organic sulphur content in the organic matter. The IHSS standard has a 0.71% sulphur content, corresponding to 0.57mg of organic S. The mass of inorganic S from sulphate (0.033mg) is 5.9% of the mass of organic S, which is considered an acceptable result.

Assuming a 1% sulphur content by weight for the leaf leachates (Xia et al., 1999), the mass of organic sulphur from 200mL of solution would be 0.83mg. The mass of inorganic S from sulphate (0.033mg) in this sample is 4.0% of organic S, and is also considered acceptable.

DOC concentrations of large volume samples used for developing the isolation procedures range between 4.3 and 14.3mg/L for original samples and 84.9 to 150.7mg/L for retentates (Table 5). Sulphate concentrations for these samples ranged from 3.2 to 6.2mg/L for original samples and 37.8 to 139.2mg/L for retentates, respectively.

DOC recoveries from concentration by RO ranged from 98 to 99% for all samples used in technique development.

Table 5. Concentrations of DOC and sulphate for large volume samples used for procedure development.

Sample	Date	DOC (orig) (mg/L)	DOC (conc) (mg/L)	SO_4^{2-} (orig) (mg/L)	SO_4^{2-} (conc) (mg/L)	DOC Recovery by RO (%)
PC1	June 7, 2001	13.4	150.7	3.2	37.8	99.0
PC1-08	June 22, 2001	4.3	106.6	6.2	139.2	99.0

Evaluation of Removal of Sulphate and Carbon loss on $BaSO_4$ Precipitate

After its removal, sulphate concentrations ranged from 0.41 to 1.88mg/L, with sulphate removal percentages ranging between 95.1 and 99.6% (Table 6). The mass of carbon lost during the isolation procedure was estimated as a percentage of the mass of carbon in the original solution, which ranged between 0.4 to 4.0%.

Table 6. Sulphate concentrations, percentage removal of sulphate, Inorganic/organic S ratios, and DOC loss to $BaSO_4$ precipitate in organic standards and duplicates

Sample	Date	SO_4^{2-} (mg/L)	SO_4^{2-} Removed (%)	Ratio of Inorganic S to Organic S	DOC adsorbed to Precipitate (%)
IHSS Std.	-	0.79	99.6	6.6	0.4
Leaf Leach	-	0.86	99.5	7.3	2.0
PC1	June 7, 2001	1.88	95.1	9.2	4.0
PC 1	June 7, 2001	1.68	97.1	7.8	3.0
PC-108	June 22, 2001	0.41	99.7	6.2	2.7
PC1-08	June 22, 2001	1.21	99.4	8.3	1.9

These results indicate the effectiveness of the procedure in removing inorganic sulphate while minimizing loss of organic matter. Despite only 95 to 99% sulphate removal, the organic matter was concentrated enough that the isotopic signature would be considered negligible. The inorganic S in these samples is below 10% of an assumed 1% organic S content, which is negligible in influencing the isotopic signatures of the dissolved organic S.

$\delta^{34}S\text{-}S_{org}$ and $\delta^{18}O$-DOM of Standards and Duplicate Samples

When comparing samples before and after isolation procedures consideration must be given to the precision of analysis of the mass spectrometer and reproducibility of the samples during isolation procedures. In order to obtain a statistically accurate precision of analysis and reproducibility, calculations included the $\delta^{34}S$-DOM and $\delta^{18}O$-DOM for all samples in this research. Precision of analysis for $\delta^{34}S$-DOM and $\delta^{18}O$-DOM for samples processed by

isolation procedures is 0.9‰ and 1.2‰, respectively, determined from samples repeats. Reproducibility of $\delta^{34}S\text{-}S_{org}$ and $\delta^{18}O$-DOM was found to be 1.1‰ and 1.2‰, respectively.

It should be noted that the $\delta^{18}O$ signature of the IHSS peat standard changes when it is dissolved in NaOH. Isotopic exchange appears to occur when the NaOH is added. Before dissolving in NaOH, $\delta^{18}O = 13.43$, after dissolving, $\delta^{18}O = 12.1$. No exchange seems to occur when $\delta^{34}S\text{-}S_{org}$ of the peat was analysed (Table 7)

Table 7. $\delta^{34}S\text{-}S_{org}$ and $\delta^{18}O$-DOM of organic standards before and after isolation procedures

		IHSS Std.	Leaf Leach
$d^{34}S\text{-}S_{org}$ (‰)	Before NaOH	13.4	-
	After NaOH, Before isolation	8.6	9.0
	After Isolation	6.4	10.9
$d^{18}O$-DOM (‰)	Before NaOH	13.8	-
	Before Isolation	12.1	23.6
	After Isolation	10.1	23.0

The results of $\delta^{34}S\text{-}S_{org}$ and $\delta^{18}O$-DOM from the organic standards suggest that isolation procedures are successful in reflecting the actual $\delta^{34}S$ and $\delta^{18}O$ of the dissolved organic matter (Table 7).

Duplicates of actual samples also proved to be within the error of reproducibility (Table 8).

Table 8. $\delta^{34}S\text{-}S_{org}$ and $\delta^{18}O$-DOM of samples run through the same isolation procedures

		PC1 Jun 7	PC1-08 Jun 22
□$^{34}S\text{-}S_{org}$ (‰)	Sample 1	5.6	7.1
	Sample 2	6.8	5.83
□^{18}O-DOM (‰)	Sample 1	14.3	10.1
	Sample 2	12.6	10.0

The potassium sulphate added to the organic standards has a $\delta^{34}S$ of -0.7‰, which is substantially different from the $\delta^{34}S$-S_{org} of the organic standards. The $\delta^{18}O$ of the potassium sulphate is 17.2‰, which is also different from the $\delta^{18}O$ of the organic standards. This means that a sufficient amount of depleted sulphate is removed by the isolation procedures to preserve the $\delta^{34}S$ and $\delta^{18}O$ of the organic material.

3.4 Conclusions

The $\delta^{34}S$-S_{org} and $\delta^{18}O$-DOM results from the standards and duplicates show that removal of sulphate by $BaSO_4$ precipitation is possible without altering $\delta^{34}S$ and $\delta^{18}O$ isotopes of organic matter.

This procedure allows determination of $\delta^{34}S$-S_{org} and $\delta^{18}O$-DOM in natural DOM samples with only a small loss of original organic matter (up to 4%). These results show that there appears to be no bias or error introduced by the isolation procedures. Despite this finding, however, it is recommended that this procedure be tested with other organic standards (Such as other IHSS standards) in order to verify rigour of this isolation procedure.

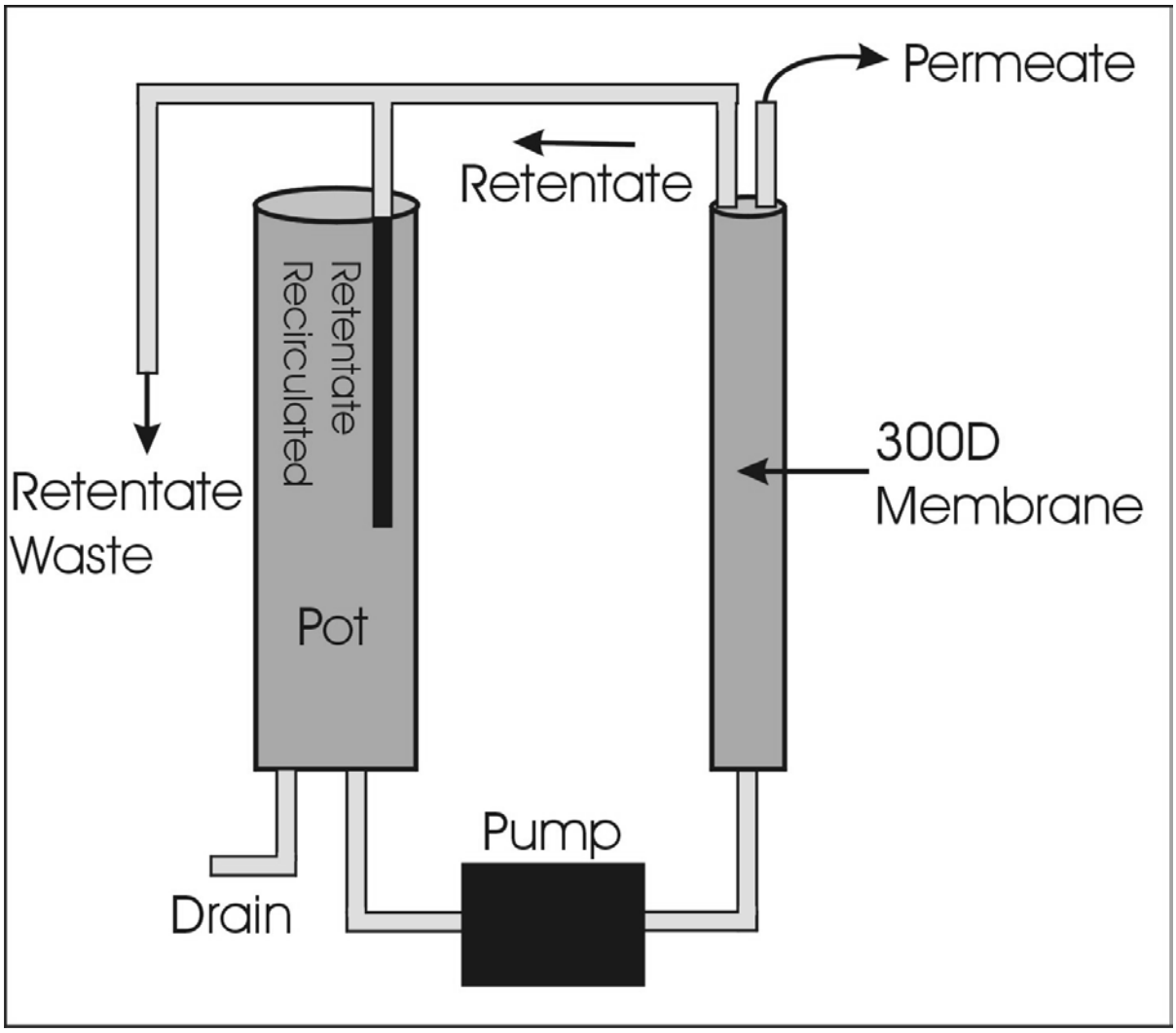

Figure 4. Detailed schematic diagram of reverse osmosis apparatus.

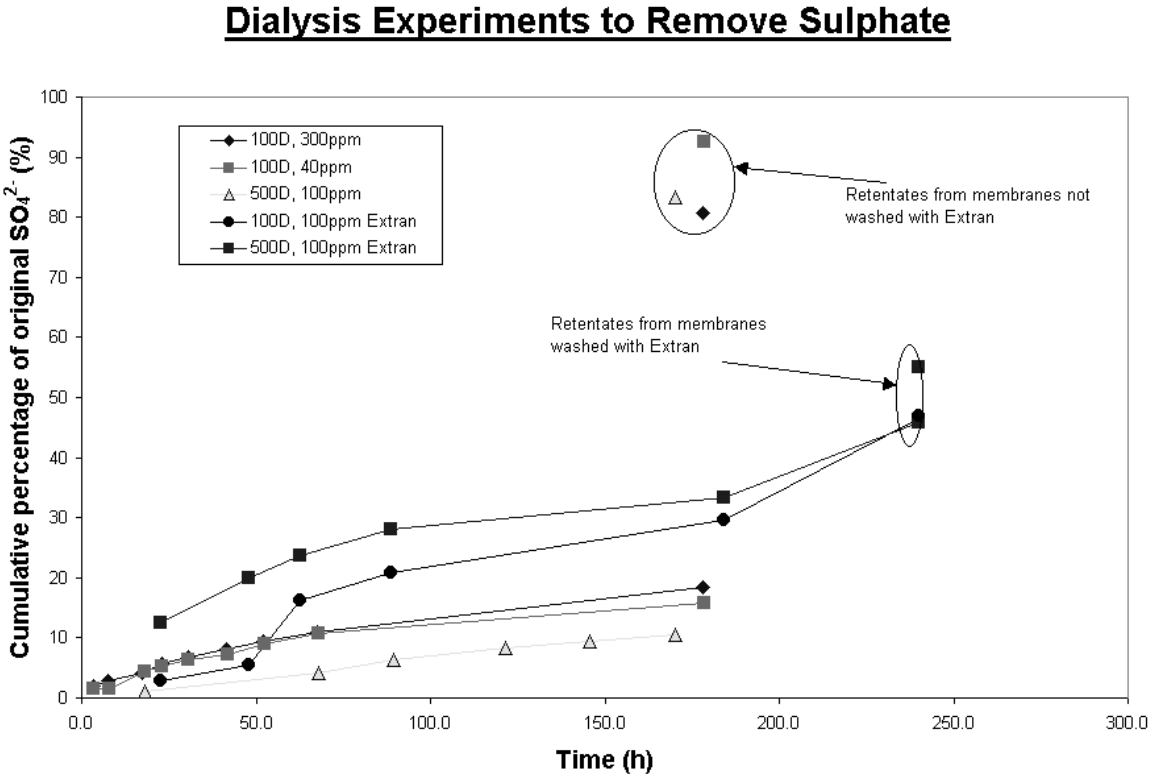

Figure 5. Results from attempted sulphate removal using dialysis experiments. Greater removal of sulphate was achieved with membranes washed by Extran (data in Appendix A).

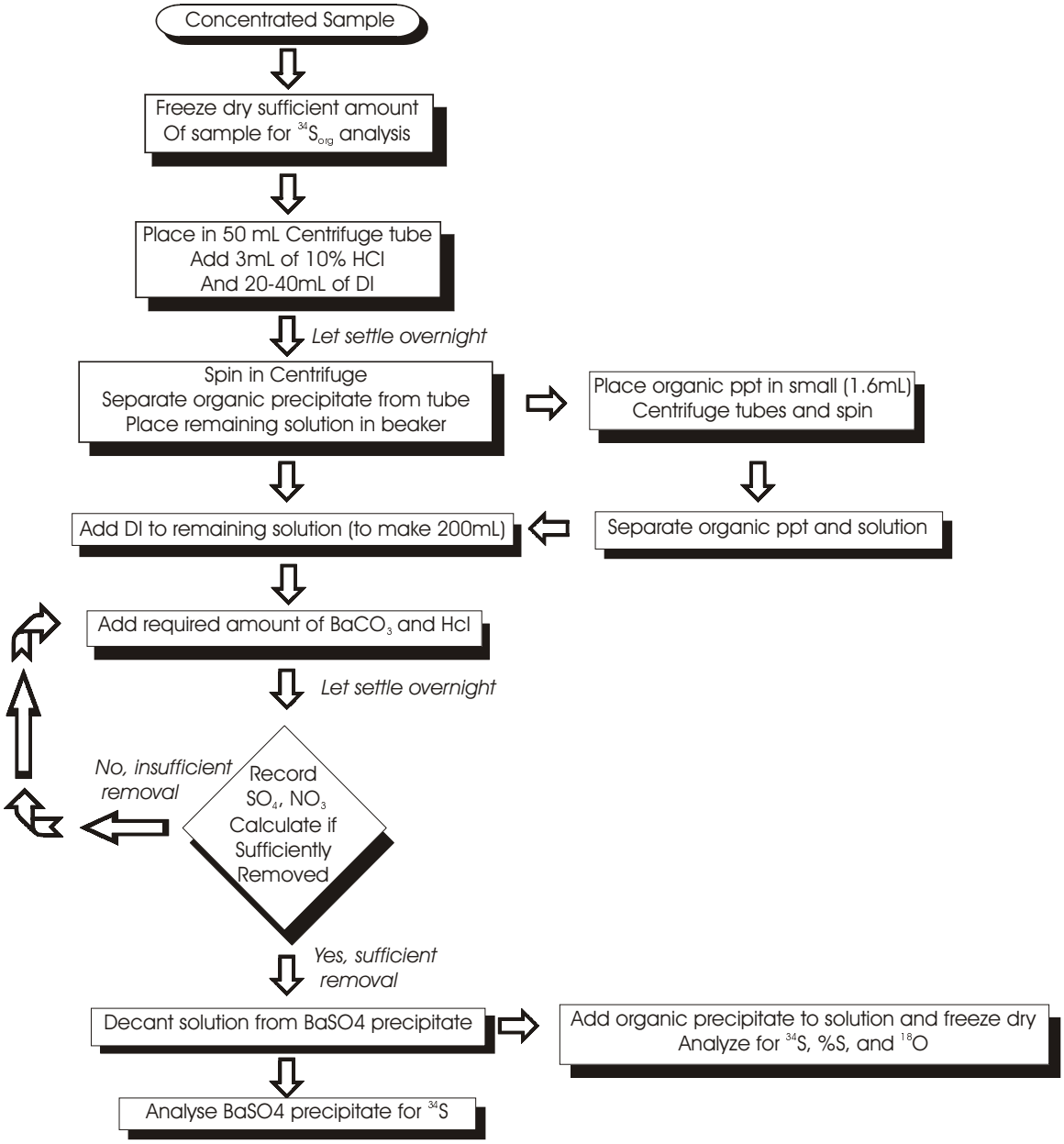

Figure 6. Flowchart of isolation procedures.

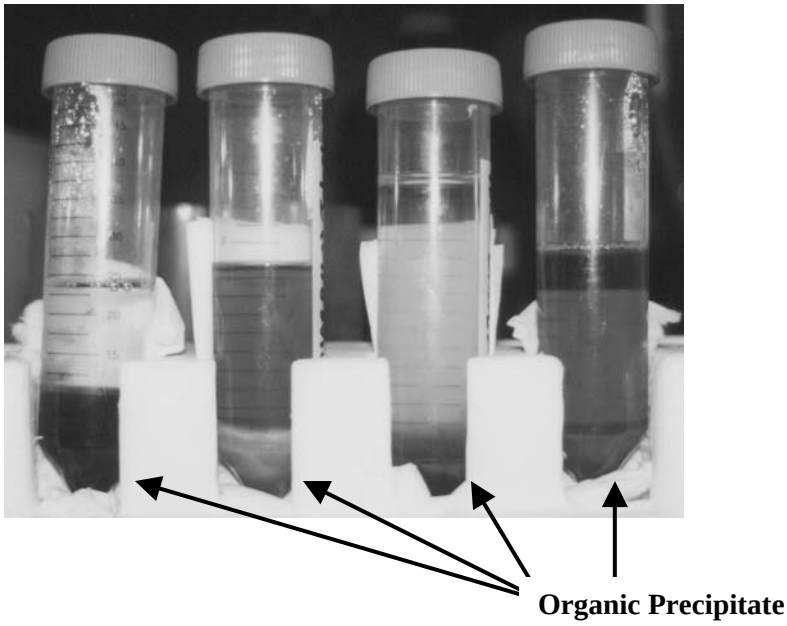

Figure 7. Organic Precipitation after re-hydration.

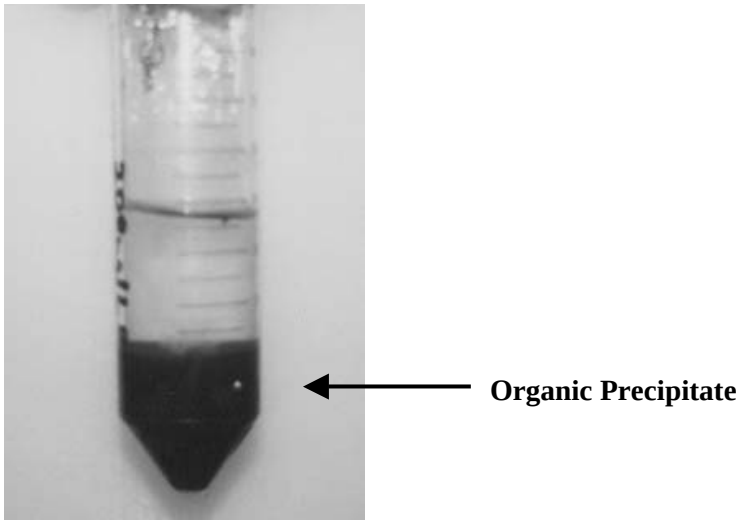

Figure 8. Organic Precipitation after HCl addition.

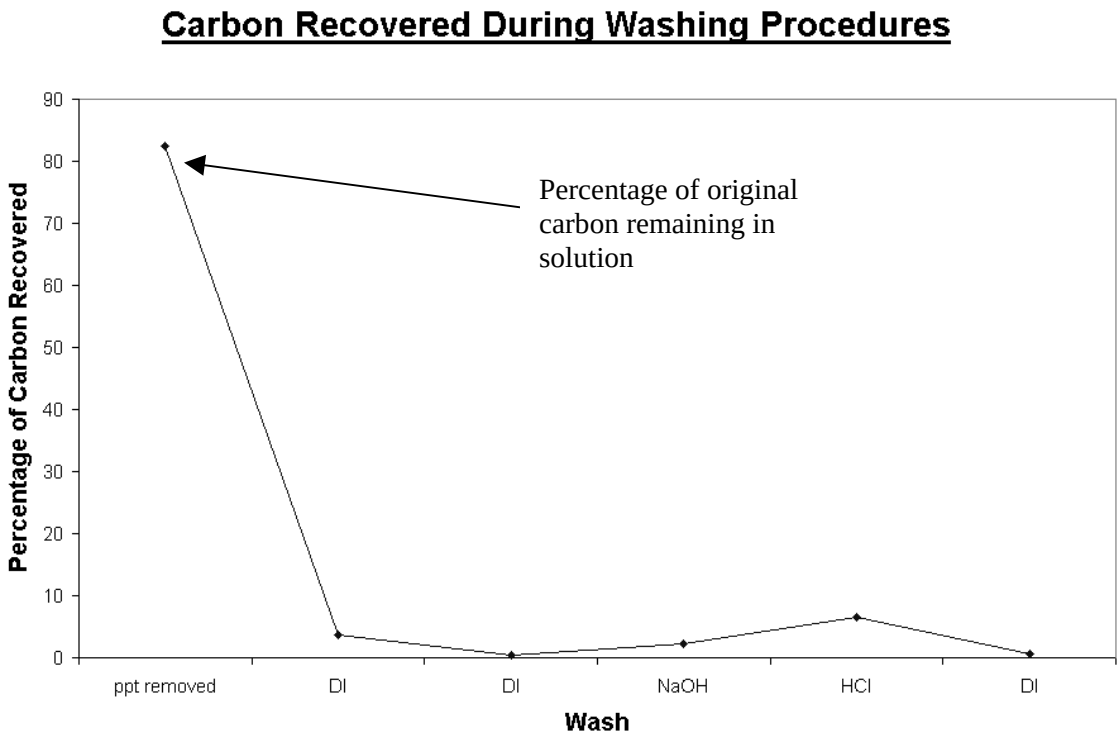

Figure 9. Percentage of carbon recovered during washing procedures for sample Harp 4Oct/2000. The first point on the graph refers to the amount of original carbon that is still reaming in the solution. The remainder of points refer to the washing of the $BaSO_4$ precipitate (data in Appendix B).

Chapter 4: Sulphate and Dissolved Organic Sulphur in Forested Catchments: New Insight from $\delta^{34}S$

4.1 Introduction

The largest input of sulphur into forested catchments on the Canadian Shield is in precipitation. Acid precipitation has deposited high levels of anthropogenic inorganic S into catchments over the last 50 years, resulting in acidification of aquatic ecosystems and losses of base cations from forest soils (Dillon et al., 1987, Gorham, 1998). Many studies have investigated the fate of sulphate within forested catchments in areas of high acid rain deposition (Hesslein et al., 1988, Evans et al., 1997, Alewell & Gehre, 1999, Chapman, 2001). One central conclusion from these studies was that wetlands play a large role in the storage and cycling of sulphate within the catchment.

In particular, the hydrology of wetlands plays a large role in the storage and release of sulphate from the wetland to downstream streams (Devito & Hill, 1997). A considerable portion of this release of sulphate from wetlands can be attributable to seasonal effects, due to the drawdown of the water table and resulting low flow conditions during the summer (Hesslein et al., 1988, Devito & Hill, 1997). This low flow regime causes a concomitant increase in the residence time of the water (and therefore sulphate) within the wetland, resulting in an enriched residual $\delta^{34}S\text{-}SO_4^{2-}$ from isotopic fractionation by dissimilatory sulphate reduction (DSR) within the wetland (Hesslein et al., 1988, Mitchell, 1998, Devito & Hill, 1997). In addition, it is probable that higher temperatures in the summer contribute to increased sulphate reduction in the wetland.

The long-term product of DSR in freshwater wetlands is organic sulphur in peat, which is correspondingly depleted in $\delta^{34}S\text{-}SO_4^{2-}$ (Brown, 1986, Evans et al., 1997, Mandernack et al., 2000, Chapman & Davidson, 2001, Alewell & Novak, 2001, Eimers, 2002). Furthermore, as hydrologic conditions change to higher flow in the fall (decreased

evapotranspiration and increased precipitation results in higher groundwater tables), the enriched sulphate is typically flushed out of the system (Hesslein et al., 1988, Mitchell et al., 1998, Mandernack et al, 2000). This phenomenon is also accompanied by a "pulse" of increased sulphate concentrations which have been well documented (Devito & Hill, 1997, Devito et al., 1999).

Organic Sulphur

The dominant fraction of sulphur in soils in forested catchments is organic sulphur (Houle et al., 1995, Mitchell et al., 1998). This trend also exists in wetland peats, where organic sulphur consists of greater than 90% of total sulphur (Alewell et al., 1999). Alewell & Novak (2001) confirmed that S in organic matter in wetlands acts as a long-term sink of sulphate (SO_4^{2-}) in forested catchments. Organic S also dominates in lake surficial sediments in unpolluted lakes, taking the long-term form of carbon-bonded sulphur (Nriagu & Soon, 1985). Despite the prevalence of organic S in these pools, little attention has been focused on the movement of dissolved organic S in the transport of S in forested catchments.

The movement of dissolved organic S (DOS) could be an important pathway for S transport between different environments in forested catchments. Houle et al (1995) showed DOS accounts for 8-22% of total S concentrations in Pre-Cambrian Shield lakes in Québec. Recently, Houle et al. (2001) suggested organic sulphur export from forest floors could contribute to the long-term S export from the catchment. Houle (Pers. comm., 2002) suggests that as much as 30% of total S output from forested watersheds could be in the form of DOS.

These studies highlight the importance of organic S in the transport and storage of total S in forested catchments. However, more research is required with respect to both the movement between different sulphur pools and the storage of sulphur within these pools in the watershed.

Dissolved Organic Sulphur and Environmental Origin

The amount of organic S can vary in dissolved organic matter. The S content in DOM ranges from 0.1-3.5% in soil humic substances, and from 0.5-1.43% in aquatic substances (Xia et al, 1998). These ranges are a result of both the environmental origin of the organic matter and the processes which add or remove organic sulphur.

The most common methods of forming organic S are assimilatory sulphate reduction and dissimilatory sulphate reduction (Luther & Church, 1992, Edwards, 1998). Each of these processes can be dominant in various environments within the catchment and each, in turn, can create different types of organic S compounds.

Organic S in soil and aquatic systems is usually divided into two main types of compounds: S directly bonded to C and sulphate esters (Thurman, 1985, Luther and Church, 1992, Edwards, 1998). Most of the literature usually categorizes organic S into these two fractions, but Krouse et al. (1992) state there are problems with the analytical determination of these fractions. Edwards (1998) states that in soils, sulphate esters result from two sources: microbial biomass material and microbially formed materials. C bonded S, however, is derived solely from plant material (Edwards, 1998). Recent studies have speculated that C bonded S also forms in freshwater environments from reduced inorganic S (Wieder & Lang, 1988, Mandernack et al., 2000, Chapman & Davidson, 2001).

The distinction between the mechanisms of formation of these types of organic sulphur has been problematic. For instance, Chapman & Davidson (2001) state that the mechanism of ester sulphate formation is not fully understood. They demonstrated that most of the sulphate in the wetland is stored over the long term as C bonded S, formed during anaerobic incubation. Also, they showed sulphate to be immobilized by the microbial biomass by assimilatory reduction, becoming part of the organic S pool when the biomass turns over. Urban et al (1999) found sulphur added to organic matter in lake sediments during diagenesis is in the form of organic sulphides and thiols (C bonded S). Moreover, Mandernack et al. (2000) established that organic sulphur formation in wetlands is a result of dissimilatory

sulphate reduction, stored as C bonded S. They could not specify, however, whether this organic S is formed either by direct sulphate reduction or by indirect assimilation of reduced inorganic S. These studies establish that the mechanism of formation of organic S is difficult to determine, and organic S dynamics within wetlands are poorly understood.

Many studies have shown that long-term S storage in wetlands is in the C bonded form (Luther & Church, 1992, Chapman & Davidson, 2001). Sulphate esters have been found to be less resistant to degradation than C bonded S (Edwards, 1998). It is speculated that sulphate esters could even be the source of sulphate in streams draining wetlands during oxidizing conditions (Mandernack, 2000). In addition, since organic S has a variety of oxidation states (Luther & Church, 1992), its oxidation state will be dependent upon the environment in which it was formed. Xia et al (1998) determined that organic sulphur formed in different environments comprises different oxidation states. They concluded that organic matter derived from more reducing environments –such as wetlands –contained more organic sulphur and reduced sulphur functional groups than the organic matter derived from more oxidizing environments. It follows that the amount of organic sulphur in organic matter can vary by environment, and is dependent upon the method of assimilation of sulphate into the organic matter. Brown (1986) concluded that humic S compounds are a major product of dissimilatory sulphate reduction (DSR), with most organic S being formed in the top 7.5cm of the wetland. The method of assimilation into organic matter is most likely dependent upon the amount of reduction occurring at each respective site, since organic matter originating from wetlands tends to have both larger amounts of organic sulphur and reduced sulphur compounds. Studies of stable S isotopes in DOM within the wetland could possibly lead to obtaining additional information on environmental origin and oxidation states of organic sulphur.

Organic S and Metal Binding

It is widely accepted that DOM has a high affinity for binding metals. Organic sulphur functional groups are thought to be the principal strong binding sites in DOM molecules

(O'Driscoll & Evans, 2000). Xia et al (1999) provided mechanistic proof of the ability of reduced organic sulphur species to bind strongly with Hg (II). Reduced sulphur functional groups such as thiols and disulphides in organic matter are also shown to be the principal binding sites for Hg (II), and the abundance of these groups in organic matter is dependent upon its environmental origin (Xia et al, 1998).

In this chapter, both inorganic sulphate and dissolved organic sulphur (DOS) in the Harp and Plastic Lake watersheds will be characterized using isolation procedures presented in Chapter 3. Both seasonal and environmental differences in $\delta^{34}S$ signatures and C/S ratios will be examined.

4.2 Methods

Samples were collected from Harp and Plastic Lake watersheds, located approximately 200km north of Toronto, Ontario, Canada. Sampling schedules and locations were different for each catchment. Samples were collected at Harp Lake to get a range in samples as a function of season and environment; samples were collected at Plastic Lake to enable a more intensive insight into the seasonal dynamics of Plastic swamp. Plastic swamp has a high retention of S and a high export of S following droughts.

To characterize any seasonal differences, samples were collected from the Harp Lake catchment during the months of April, July, and October. Samples in April were collected during snowmelt, a period of high groundwater tables and high stream discharge. Samples in July were collected at a time of low groundwater tables and low streamflow. October sampling occurred just after leaf fall when groundwater tables rise and stream discharges are increased in comparison to summer.

Dry leaves were collected from the Harp 6A catchment after leaf fall in October 2002, in an attempt to determine the influences of leaf litter.

More intensive sampling was conducted at the Plastic Lake catchment to focus on temporal changes in the wetland and its input. Samples were collected every 2-3 weeks from the months of April to July, and on a monthly basis from September to December in 2001. Between the sampling dates of July 16 and September 25, flow was insufficient for collecting large volumes, or sometimes even non-existent. Samples of precipitation were collected from precipitation buckets, located in a clearing approximately 200m from the edge of the lake and 400m north of PC-1. The precipitation sample consisted of a combined sample from the months of July-September. A mixed throughfall sample was collected from the months of October-November in throughfall collectors; the collectors consisted of eavestroughing-type channels that accumulated water in buckets, located 20m from the clearing containing the precipitation buckets.

Sample Collection

Sample volumes collected at each site were variable, depending upon DOC concentrations estimated from historical data. Volumes ranged between 50 to 200L. This was to ensure sufficient mass of DOM after the reverse osmosis (RO) process. Subsamples were submitted to the Ministry of Environment Dorset Research Center in Dorset, Ontario for chemical analysis. Large volume samples were field filtered with a Nitex mesh (200μm).

Laboratory Methods

Samples were filtered using a Balston stainless steel aluminum 20 μm pre-filter followed by a Geotech 147mm inline filter containing a 0.7 μm precombusted glass fiber filter (Whatman GF/F, 0.7μm nominal size). These samples were subsequently concentrated by RO, using a membrane cutoff of 300 Daltons. Recovery of DOC suggests an efficiency of 99%. The RO process concentrated solutes by a factor of 8 to 20, and samples were reduced to 4 to 5L.

Concentrated samples were then subjected to isolation procedures to remove sulphate from the solution. These procedures consisted of a combination of the addition of HCl and

$BaCO_3$ to the solution, effectively precipitating out SO_4^{2-} as $BaSO_4$. Steps were taken to ensure maximum recovery of organic matter. Details of procedures for sample isolation and SO_4^{2-} removal are found in Chapter 3.

A portion of the leaves collected from the Harp 6 catchment was progressively leached with deionized water (DI). Leaves were leached once with DI, drained, and then left for 2 days at 4°C. Subsequently, more DI was added. The second leach was then drained, and after a similar rest period, a third volume of DI was added. Then, the final leachate was drained, and samples of leachate are subsequently known as leaf leaches 1, 2, and 3.

No organic standards exist for sulphur, so samples were compared with inorganic IAEA standards at the Environmental Isotope Laboratory in the University of Waterloo. Precision of the mass spectrometer for $\delta^{34}S\text{-}S_{org}$ is calculated to be ± 0.9‰, while reproducibility between samples is estimated to be ± 1.1‰. Precision of the mass spectrometer for $\delta^{34}S\text{-}SO_4^{2-}$ is 0.6‰.

C/S Ratios

Ratios of C/S can be used as an indication of the amount of sulphur contained in the organic molecule. C/S ratios are determined using %C and %S, which are acquired from the elemental analyzer (EA) coupled to the mass spectrometer. Since there are large amounts of salts added during the isolation procedure, the %S given from the EA is the portion of sulphur as a total of the organic sample and salts added to the solution. Therefore, using %S as a measure of the amount of sulphur in an organic molecule is not accurate. In order to accurately determine the amount of S in the organic molecule, C/S ratios must be calculated. The molar C/S ratio is determined by taking the quotient of %C and %S and multiplying through by molecular weights:

$$C:S = \frac{\%C}{\%S} \times \frac{32.066}{12.011} \qquad \text{Eq. 4.1}$$

4.3 Results

Harp Lake Catchment

Harp 4, a stream with contributions from both uplands and wetlands, was relatively constant in sulphate concentrations, $\delta^{34}S\text{-}SO_4^{2-}$, and $\delta^{34}S\text{-}S_{org}$ (Table 6). In contrast, streams in catchments with higher DOM from wetlands (Harp 5 and 6) varied in sulphate concentrations and $\delta^{34}S\text{-}SO_4^{2-}$. These streams exhibited a wide range of sulphate concentrations, with a maximum in the fall and minimum in the summer; Inorganic $\delta^{34}S$ in Harp 5 and 6 in the fall is different to the spring and summer, having significantly high SO_4^{2-} concentrations and enriched $\delta^{34}S\text{-}SO_4^{2-}$. The $\delta^{34}S\text{-}S_{org}$ in Harp 5 also differs in summer and fall.

Harp 4-21, the upland catchment, and shallow groundwater (SGW), which feeds Harp 4-21, exhibit relatively constant sulphate concentrations, and $\delta^{34}S\text{-}SO_4^{2-}$. Only one $\delta^{34}S\text{-}S_{org}$ was obtained for Harp 4-21 and none were generated for SGW in this catchment, because of time constraints in analysis.

Samples obtained from Harp Lake were also constant for all three seasons and not similar to other samples in the catchment. The $\delta^{34}S\text{-}S_{org}$ in DOS in Harp Lake is similar to the streams supplying the lake.

Deep groundwater, collected from Well 55, had a sulphate concentration of 13.03 mg/L and a $\delta^{34}S\text{-}SO_4^{2-}$ of 8.3‰. The $\delta^{34}S\text{-}S_{org}$ was not determined for this sample, due to analysis constraints.

Leaves from Harp 6A had a $\delta^{34}S\text{-}S_{org}$ of 6.9‰, while leaf leachate 2 showed a $\delta^{34}S\text{-}S_{org}$ of 7.3‰. These values, however, had lower than normal peak areas, so caution must be used when considering them in scientific analyses.

Table 9. Sulphate concentrations, $^{34}S\text{-}SO_4^{2-}$, $^{34}S_{org}$, DOC concentrations and C/S ratios for samples from the Harp Lake catchment.

	April 22, 2001					July 16, 2001					October 8, 2001				
Sample	**$[SO_4^{2-}]$ (mg/L)**	**$^{34}S\text{-}SO_4^{2-}$ (‰)**	**$^{34}S_{org}$ (‰)**	**DOC (mg/L)**	**C/S Ratio**	**$[SO_4^{2-}]$ (mg/L)**	**$^{34}S\text{-}SO_4^{2-}$ (‰)**	**$^{34}S_{org}$ (‰)**	**DOC (mg/L)**	**C/S Ratio**	**$[SO_4^{2-}]$ (mg/L)**	**$^{34}S\text{-}SO_4^{2-}$ (‰)**	**$^{34}S_{org}$ (‰)**	**DOC (mg/L)**	**C/S Ratio**
HP 4	5.55	6.9	5.1	5.7	48.4	5.43	5.2	4.9	6.8	45.0	5.53	5.9	4.6	8.5	89.3
HP 5	5.78	6.1	4.7	8.3	83.6	0.87	6.2	3.4	25.9	96.7	7.24	10.3	4.9	24.3	137.0
HP 6	6.29	6.4	5.6	5.3	77.6	0.96	5.5	4.9	14.5	75.5	12.72	7.6	5.7	9.1	53.2
HP 4-21	7.30	3.9	-	2.0	-	6.57	4.7	-	2.4	-	8.37	3.3	8.7	3.7	40.2
HP Lake	5.93	5.1	5.8	3.7	47.8	4.23	4.9	6.4	4.2	70.4	5.94	5.3	6.0	4.4	84.8
SGW	6.37	3.3	-	0.4	-	6.63	3.3	-	1.2	-	7.68	4.1	-	1.0	-

Plastic Lake Catchment

Throughfall at Plastic Lake catchment had a slightly higher sulphate concentration than precipitation, 3.35 mg/L compared to 2.84 mg/L. Inorganic$\delta^{34}S$ for throughfall was within precision of the precipitation ($\delta^{34}S\text{-}SO_4^{2-}$ of 3.7‰ compared to 4.2‰). Organic $\delta^{34}S$ of throughfall, however, was significantly different than precipitation, having a $\delta^{34}S\text{-}S_{org}$ of 4.7‰ compared to 6.3‰.

Sulphate concentrations, $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{34}S\text{-}S_{org}$ in the upland PC1-08 stream are relatively constant. The output from the wetland (PC1), however, is not constant, appearing to have seasonal dynamics. Concentrations of sulphate in PC1 are relatively low in the spring (2.20-4.78 mg/L), and decrease into the summer (0.99 mg/L). Flow between July 16 and September 25, 2001 was insufficient to obtain enough volume for the RO process. Sufficient flow began on September 25 and an elevated sulphate concentration was observed during this sampling period (14.09 mg/L). In the months of October, November, and December, sulphate concentrations decrease and remain relatively steady through to April 4, 2002 (6.24-6.9mg/L).

Similar to sulphate concentrations, the inorganic $\delta^{34}S$ of the PC1 samples also had $\delta^{34}S\text{-}SO_4^{2-}$ which showed a large range (4.7‰-10.1‰). All samples had $\delta^{34}S\text{-}SO_4^{2-}$ above those of PC1-08 (4.5‰-5.6‰). The sample with the lowest $\delta^{34}S\text{-}SO_4^{2-}$ (4.7‰) occurred on the September 25 sampling date after a long period of no flow conditions. The sample with the highest $\delta^{34}S\text{-}SO_4^{2-}$ (10.1‰) occurred on October 8.

Dissolved Organic $\delta^{34}S$ at PC1 showed a range of 4.9‰ to 8.7‰, but did not vary as substantially as inorganic $\delta^{34}S$ (4.7‰-10.1‰).

Table 10. Sulphate concentrations, $\delta^{34}S\text{-}SO_4^{2-}$, $\delta^{34}S\text{-}S_{org}$, DOC concentrations, and C/S ratios for PC1 and PC1-08 in the Plastic Lake catchment.

	PC1					PC1-08				
Date	$[SO_4^{2-}]$ (mg/L)	$^{34}S\text{-}SO_4^{2-}$ (‰)	$^{34}S_{org}$ (‰)	DOC (mg/L)	C/S Ratio	$[SO_4^{2-}]$ (mg/L)	$^{34}S\text{-}SO_4^{2-}$ (‰)	$^{34}S_{org}$ (‰)	DOC (mg/L)	C/S Ratio
April 22, 2001	4.878	7.6	6.9	10.6	143.1	NF	NF	NF	NF	NF
May 12, 2001	2.197	9.5	8.7	18.4	138.6	NF	NF	NF	NF	NF
June 7, 2001	3.18	9.2	6.8	13.4	180.9	7.12	5.6	6.4	2.5	NA
June 22, 2001	2.99	5.9	5.7	12.5	167.0	6.15	4.5	7.1	4.3	56.4
July 16, 2001	0.99	8.9	5.6	23.8	95.5	NF	NF	NF	NF	NF
September 25, 2001	14.09	4.7	5.3	16.7	96.0	6.72	NA	NA	3.2	NA
October 8, 2001	7.21	10.1	8.1	15.2	204.4	NF	NF	NF	NF	NF
November 2, 2001	6.51	6.9	6.7	10.5	131.7	6.73	4.7	6.7	2.8	NA
December 6, 2001	6.36	6.3	4.9	9.1	61.1	6.6	4.5	6.3	2.0	NA
April 4, 2002	6.24	6.5	5.7	6.6	90.9	6.44	4.5	NA	2.2	NA

*NF = no-flow conditions at the weir, NA = not analysed

4.4 Discussion

Inorganic Sulphur in the Harp Lake Catchment by Environment

Results from sulphate concentrations and $\delta^{34}SO_4^{2-}$ confirm the seasonal and environmental trends observed in other studies of forested catchments containing wetlands (Mitchell et al., 1998). These trends, controlled both by hydrology and biogeochemical processes, are consistent with most other wetland-containing catchments on the Canadian Shield (Hesslein et al., 1988, Devito & Hill 1999, Mandernack, 2000). Eimers (2002) found that $\delta^{34}SO_4^{2-}$ in the Plastic Lake catchment could be consistently predicted from discharge, but there is no apparent relationship between $\delta^{34}SO_4^{2-}$ and SO_4^{2-} concentrations.

Few samples fall within the range of known precipitation in the area (1.3-2.8 mg/L, 5.2 ±0.6‰; Eimers, 2002), or the range where samples could be concentrated by evapo-concentration (Fig. 10). All samples from Harp Lake, one sample from Harp 4-21, one from Harp 4, and the Harp 4 beaver pond, are within the range of evapo-concentrated

precipitation. Samples that lie outside of this range are presumed to have undergone some sort of cycling within the watershed.

All of the samples falling above the precipitation range (higher $\delta^{34}S$) were taken from streams containing wetlands (Harp 4, 5, 6), with the exception of the deep groundwater sample (Fig. 10). In the wetland streams, these altered signatures are likely attributable to DSR within the wetlands. As mentioned previously, DSR serves to enrich the reactant sulphate in $\delta^{34}S$, shifting the samples to higher $\delta^{34}S$.

Samples that are below the precipitation range all originate from the Harp 4-21 catchment (Harp 4-21, Shallow groundwater). Since Harp 4-21 is fed solely by groundwater, these samples could have some historical influence due to residence time of groundwater; precipitation in 1986 had a $\delta^{34}S\text{-}SO_4^{2-}$ ranging from +3 to 5‰ (Van Stempvoort et al., 1991, 1992) compared to 5.2 ±0.6‰ in current precipitation (Eimers, 2002). These samples also have higher SO_4^{2-} concentrations (Fig. 10), which indicate an evapo-concentration effect.

Additionally, samples taken from the wetland streams show a large range of seasonal variability, particularly those taken from Harp 5 and 6 (Fig. 10). Differences in hydrologic conditions in each season affect residence times in the wetland, in turn affecting sulphate reduction in the wetland. These seasonal and environmental effects in the Harp Lake catchment reflect similar trends observed in studies carried out in Southeastern Canada/ Northeastern U.S. (Hesslein et al., 1988, Mitchell et al., 1998, Devito et al., 1999).

Harp Lake

Harp Lake is the only sampling station in which all three seasonal samples fall within the range of known precipitation. This means there is some process occurring which serves to buffer seasonal differences in stream inputs from each catchment. Eimers (2002) found responses to seasonal changes of Harp and Plastic lakes to be more gradual and less dramatic than the streams in each respective catchment. In order for the Harp Lake samples to plot within the precipitation range (Fig. 10), the input of the streams that plot above the

precipitation box would have to be balanced by input which is depleted in $\delta^{34}S\text{-}SO_4^{2-}$ (input that would plot below the precipitation box).

Each input to Harp Lake contributes differently in volume, and also varies in its contribution of mass of solutes to the lake, such as sulphate. Precipitation has the highest input by volume into Harp Lake, and plots below the precipitation box (depleted in $\delta^{34}S\text{-}SO_4^{2-}$; Fig. 10). Input of $\delta^{34}S\text{-}SO_4^{2-}$ from precipitation would serve to place the samples from Harp Lake in the precipitation box, balancing input from streams with large wetland areas, such as Harp 5 and 6. It should be noted, however, that wetland catchments show a net S export (Evans et al., 1997) and therefore inputs by volume will not properly reflect inputs of S to the lake.

Another possible reason why the Harp Lake samples plot within the box could be due to the long residence time in the lake. This could buffer the seasonal effects observed in the streams which input the lake. It is also possible that processes within the lake could change the sulphate concentrations and $\delta^{34}S\text{-}SO_4^{2-}$.

Harp 4-21 and Shallow Groundwater (SGW)

Both Harp 4-21 and SGW undergo little seasonal change. Harp 4-21 has a slightly higher sulphate concentration in the fall (8.37mg/L), which is not significantly different from spring, but significantly different from summer sulphate concentrations. Harp 4-21 is fed by shallow groundwater and has a similar $\delta^{34}S\text{-}SO_4^{2-}$ signature to the shallow groundwater samples. These samples show little seasonal effect because there is probably no DSR occurring within the shallow groundwater.

The majority (5 out of 6) of the samples taken from Harp 4-21 and from the shallow groundwater are both higher in sulphate concentrations and depleted in $\delta^{34}S\text{-}SO_4^{2-}$ when compared to precipitation. The Harp 4-21 sub-catchment does not contain any wetland area. Groundwater feeding Harp 4-21 has a residence time of 3-4 years. One explanation for the relatively depleted $\delta^{34}S\text{-}SO_4^{2-}$ values when compared to precipitation could be that the

groundwater consists of historical water with had lower $\delta^{34}S\text{-}SO_4^{2-}$ (than current precipitation). Data from Van Stempvoort (1991,1992) show that historical $\delta^{34}S\text{-}SO_4^{2-}$ of precipitation could be as low as 3.0‰. Also, there could be a small contribution of SO_4^{2-} from the deeper till in the sub-catchment. The elevated sulphate concentrations seen in the Harp 4-21 subcatchment (6.57-8.37mg/L) could be a result of due to further concentration from evapotranspiration by trees in the subcatchment.

Another explanation could be that sulphate is released from organic matter in the upper litter layers of the subcatchment. Eimers (2002) also observed a net export of sulphate from the upland catchment, PC1-08. The mineralization of organic substrate in the upland catchment could lead to sulphate which is relatively depleted in $\delta^{34}S\text{-}SO_4^{2-}$ when compared to precipitation. Alewell & Novak (2001) and references therein found 32S to be preferentially mineralized in organic matter. From this information, it is plausible that mineralization of organic matter could be the cause of the slightly depleted $\delta^{34}S\text{-}SO_4^{2-}$ values and elevated sulphate concentrations seen in the shallow ground water and Harp 4-21 samples.

Deep Groundwater

The deep groundwater sample has a high sulphate concentration (13.03 mg/L) and a relatively high $\delta^{34}S\text{-}SO_4^{2-}$ (8.3‰).

This sample has been found to be contaminated with road salt, which could account for the high concentrations of sulphate and $\delta^{34}S\text{-}SO_4^{2-}$. The presence of high chloride in this well (109.5mg/L) is extremely high for the Harp watershed. Although road salt primarily consists of chloride salts, small amounts of sulphate salts such as gypsum could have been present in the same formation from which the salt was mined. Sulphate salts (such as gypsum) typically have very high $\delta^{34}SO_4^{2-}$ (Clark & Fritz, 1997), but they are most likely present in low abundances –so mixing with natural waters could account for the $\delta^{34}SO_4^{2-}$ of 8.3‰.

Another explanation could be that the water in the deep groundwater is a result of historical deposition from 1960-70, a time where SOx deposition was at a maximum (Robertson et al., 1989). In this case, the enriched $\delta^{34}S\text{-}SO_4^{2-}$ could be due to reduction of sulphate in the deep groundwater.

Harp 5 and Harp 6

Seasonal effects observed in Harp 5 and 6 are most likely due to drawdown of water levels within the wetland during the summer and subsequent flushing during the fall. In the spring, the residence time of the water in the wetland is low enough and its volume of water flushing through the wetland is sufficiently high, that sulphate reduction is relatively ineffective in changing either isotopes or concentrations (Fig. 11).

In the summer season, however, evapotranspiration lowers the groundwater tables, and water levels diminish within the wetland. The lowering of water levels results in an increased residence time in the wetland and net discharge occasionally ceases in some streams exiting the wetland. An increased proportion of sulphate is reduced by DSR in the wetland during these times of little to no flow from the wetland. A kinetic isotopic fractionation occurs from reduction by DSR, causing residual sulphate to be enriched and the concentrations of sulphate to be decreased (Clark & Fritz, 1997). Therefore, samples from summer would be expected to have low sulphate concentrations, and have an enriched $\delta^{34}S\text{-}SO_4^{2-}$. Samples from Harp 5 and 6 in the summer season show the expected decrease in sulphate concentrations (0.86, 0.96 mg/L, respectively), but have $\delta^{34}S\text{-}SO_4^{2-}$ fairly similar to that of precipitation (6.2, 5.5‰, respectively). Thus the small amount of SO_4^{2-} leaving these catchments in summer has not been affected by DSR.

The $\delta^{34}S\text{-}SO_4^{2-}$ of Harp 5 and 6 collected during the summer are very similar to $\delta^{34}S\text{-}SO_4^{2-}$ of samples collected in the spring, though the sulphate concentration has decreased by a factor of approximately 7. One possible explanation for this result could be a significant groundwater input. Harp 6 can have a significant groundwater input in the lower part of the catchment (Schiff et al., 2002), and either no flow from the wetland or the mixing of water

from the wetland with no SO_4^{2-} and groundwater input would explain both the $\delta^{34}S\text{-}SO_4^{2-}$ and the low concentrations.

During the fall season, after leaf fall, the groundwater table rises, attributable to diminished evapotranspiration and increased precipitation. Enriched sulphate in the porewaters of the wetland is subsequently flushed from the wetland into streams, resulting in the enriched signal seen in the streams in the October samples (Fig. 10).

Harp 4

Unlike Harp 5 and 6, samples from Harp 4 do not show extremely large differences between seasons (Fig 10). There are slight differences in $\delta^{34}S\text{-}SO_4^{2-}$ (5.2-6.8‰), and concentrations of SO_4^{2-} are relatively constant (4.53-5.55 mg/L). The percentage of wetland in Harp 4 (5%) is much lower than that of Harp 5 or 6 (13%, 10%). Since sulphate dynamics in wetlands are largely controlled by season, a lack of wetland area could the reason for the relatively constant $\delta^{34}S\text{-}SO_4^{2-}$ and sulphate concentrations throughout the year.

Temporal Analysis of Inorganic Sulphur in Plastic Swamp

Plastic swamp shows the same seasonal pattern as seen in other forested catchments containing wetlands (Hesslein et al., 1988, Mitchell et al, 1998, Devito et al. 1999). Eimers (2002) observed a highly coherent pattern in SO_4^{2-} concentrations and export in PC1; high SO_4^{2-} export could be predicted by the number of days with no stream flow or stream flow below a certain threshold. Therefore, climate is the controlling factor in SO_4^{2-} export from the PC1 catchment.

Evapo-concentration of sulphate in the PC1-08 subcatchment can be estimated as the difference between the sulphate concentrations in the subcatchment and precipitation (Fig 12). This estimate is a maximum for evapo-concentration, since PC1-08 has been shown to export SO_4^{2-} (Eimers, 2002).

The differences in concentrations and $\delta^{34}S\text{-}SO_4^{2-}$ between input (PC1-08 is representative of uplands feeding PC1-08) and output of the swamp are caused by sulphur oxidation-reduction dynamics caused by different hydrologic flow conditions in the wetland. DSR in the wetland causes sulphate concentrations at PC1 to be lower than the PC1-08 input during the spring and summer, but not in the fall season (Fig. 12). From $\delta^{34}S\text{-}SO_4^{2-}$ of sulphate in PC1, it is evident that DSR is occurring. With the exception of the Sept 25 sampling date, PC1 $\delta^{34}S\text{-}SO_4^{2-}$ is consistently higher (at least 1-2‰) than the input of PC1-08, which agrees with data from Eimers (2002).

The relatively low $\delta^{34}S\text{-}SO_4^{2-}$ (4.7‰) recorded on September 25 could be a result of incoming precipitation (5.2 ± 0.6‰), or could be caused by a reoxidation of reduced sulphur in the upper layer of peat. When SO_4^{2-}/Cl^- ratios of the PC catchment are compared to the combined precipitation sample (10.3), it becomes apparent there is a source of SO_4^{2-} other than precipitation on this sampling date (Fig. 14).

This sample was taken after a drought period, and is comparable to historical data documenting similar relatively depleted samples after a drought (Eimers, 2002). When a wetland first starts flowing after a drought, depleted sulphate is remineralized from the upper layers of peat. This sulphate is released to the stream, supplying a relatively depleted signal. Data from Eimers (2002) shows the upper layers of peat to be relatively depleted in $\delta^{34}S\text{-}SO_4^{2-}$ (between –1.5 and +3.2‰), which, upon remineralization, would provide depleted $\delta^{34}S\text{-}SO_4^{2-}$ to the PC1 stream. Then, as the groundwater tables rise and the wetland wets up, residual porewater in the wetland is flushed out, as evidenced by SO_4^{2-}/Cl^- ratios. This porewater contains enriched sulphate and explains the relatively high $\delta^{34}SO_4^{2-}$ (10.1‰) seen on October 8th.

When $\delta^{34}S\text{-}SO_4^{2-}$ is compared to SO_4^{2-}/Cl^- ratios, additional information on sources and sinks within the wetland can be acquired (Fig. 15). Insight can be made into S retention by reduction in the wetland and S release by oxidation from the wetland. Samples plotting above the precipitation range, in the upper left-hand corner (low SO_4^{2-}/Cl^- ratios, high

$\delta^{34}SO_4^{2-}$), are samples in which the sulphate has undergone reduction by DSR and S is retained within the wetland. The sulphate is reduced, increasing the $\delta^{34}SO_4^{2-}$ and decreasing sulphate concentrations (decreasing SO_4^{2-}/Cl^- ratios). Samples that plot below the precipitation range, to the lower left corner (Fig. 15), are samples where the peat has released reduced S by oxidation (mineralization). The mineralized sulphate is depleted in $\delta^{34}SO_4^{2-}$, and the SO_4^{2-}/Cl^- ratio is increased.

The majority of the samples from the wetland (PC1) indicate S retention by the wetland for the greater part of the year. Samples which do not follow this trend are samples from June 7, September 25, and December 6. The PC1 sample from June 7 has a high SO_4^{2-}/Cl^- ratio (22.3), but when compared to the input to the swamp on that date (18.44) it is plausible that the output is just a reflection of the input into the swamp. The sample from September 25 has been discussed above, but the plot is further evidence of oxidation of reduced S providing sulphate.

$\delta^{34}S\text{-}S_{org}$ in the Harp and Plastic Lake Catchments

Organic sulphur content can be increased in organic matter in freshwater environments by the reduction of sulphate (Brown, 1985, 1986, Urban et al. 1999, Mandernack et al., 2000, Alewell & Novak, 2001). These studies have found formation of organic sulphur in wetlands to be a long term process, having a relatively depleted $\delta^{34}S$ signature from isotopic fractionation by DSR. Some studies speculate organic S is assimilated from reduced inorganic S by microbes (Wieder & Lang, 1988, Mandernack et al., 2000, Chapman & Davidson, 2001).

The $\delta^{34}S\text{-}S_{org}$ of dissolved organic sulphur (DOS) has not been reported in the literature, therefore one can only speculate to the expected $\delta^{34}S\text{-}S_{org}$ of DOS. If organic S is added to organic matter in the wetland by assimilation of reduced inorganic S from DSR, then release of organic S in the form of DOS from the wetland should result in $\delta^{34}S\text{-}S_{org}$ which is relatively depleted in $\delta^{34}S$ compared to sulphate. Peat in the upper layers of wetlands is

typically depleted in $\delta^{34}S$ (-4 to +3‰; Novak et al., 1999, Eimers, 2002). It would therefore be expected that DOS derived from this organic S would be depleted by a similar amount when compared to precipitation. In environments where there is little or no DSR occurring, the DOS might not show a depleted signature, and other factors could influence the $\delta^{34}S\text{-}S_{org}$ in these samples, such as vegetation type or amount of mineralization.

Dissolved Organic Sulphur in the Harp and Plastic Lake Catchments

The $\delta^{34}S$-DOM in the Harp and Plastic Lake catchments show a wide range from 3.4 to 8.7‰ (Fig. 16). When comparing $\delta^{34}S$-S-DOM of samples to DOC concentrations, environmental differences become apparent (Fig. 17). The $\delta^{34}S$-S-DOM and DOC concentrations in uplands and lake do not vary greatly, but wetlands are extremely variable.

C/S Ratios in Dissolved Organic Matter

The C/S ratios in DOM from wetland streams are higher (53-204) than either uplands streams (8-56) or lakes (47-84; Fig. 18). When C/S ratios are compared with $\delta^{34}S$-DOM (Fig. 19), no trend appears to exist, but environmental differences can be differentiated.

^{34}S-DOM by environment in the Harp Lake Catchment

Upland streams in Harp and Plastic Lake catchments

Upland streams (PC1-08, Harp 4-21) show a much higher $\delta^{34}S$-DOM (an average 1.2‰ enriched) than wetland streams. They display a similar $\delta^{34}S$-DOM to throughfall, and leaf leachates (Fig. 16).

The source of DOM in upland catchments is typically a combination of both groundwater and upper soil horizons, depending on antecedent moisture and groundwater flowpaths (Hinton, 1998). Houle (2001) showed the dissolved organic sulphur in a coniferous forest in Québec to be derived from litterfall. They found DOS was adsorbed to

the B horizons in the soil, and transported through the soil horizons via percolating soil solution.

As water from interflow or groundwater interacts with the LFH layers in the upper soil, the DOS could be leached from these horizons. This DOS would then be transported into upland streams. This could be reflected in the similarity of δ^{34}S-DOM in the upland streams (PC1-08, Harp 4-21) and the δ^{34}S-DOM of leaves and leaf leachates. Therefore, it appears that the δ^{34}S-DOM is determined by the δ^{34}S of the sources of DOM.

Wetland streams in the Harp and Plastic Lake Catchments

The δ^{34}S-DOM originating in wetlands is much more enriched than expected (Fig. 16); these values are similar to δ^{34}S found in organic S in soils found in the Muskoka area (4.0 – 6.0‰ Van Stempvoort, 1991, 1992). The upper layers of wetland soils are typically depleted in δ^{34}S-S_{org} (Alewell & Novak, 2001, Eimers, 2002); peat in Plastic swamp has a δ^{34}S-S_{org} range of -1.21‰ to +3.41‰ in the first 50cm due to the effects of reduction (Eimers, 2002). Also, since DOM originating from wetlands has been shown to contain reduced sulphur species (Xia et al., 1998), it would be expected that DOS originating in wetlands would show a similar depleted δ^{34}S-S_{org} signature to peat.

Wetland streams in both Harp and Plastic Lake catchments generally show a depleted δ^{34}S-DOM when compared to δ^{34}S-SO_4^{2-} (Fig. 20). Every sample which contains a wetland within its catchment has δ^{34}S-DOM < δ^{34}S-SO_4^{2-}, most likely indicating S added from dissimilatory sulphate reduction (DSR). The exception to this is the PC1 sample collected on September 25, which has been explained already as the reoxidation of peat in the upper layers of the wetland. It should be noted that the δ^{34}S-DOM = δ^{34}S-SO_4^{2-} for the September 25 sample, and could possibly be an indicator of mineralization. However, with the exception of the September PC1 sample, all of the other wetlands plot consistently below the 1:1 line (Fig. 20).

A direct comparison of $\delta^{34}S\text{-}S_{org}$ of DOS and $\delta^{34}S\text{-}SO_4^{2-}$, however, may not be valid. Formation of organic S occurs over the long term (Luther & Church, 1992, Alewell & Novak, 2001, Chapman & Davidson, 2001), while reduction of sulphate by DSR is a short term process. The histories of each respective chemical species are different; the reactant, sulphate, is typically not retained in the catchment whereas the product, organic S, is kept within the wetland. Therefore, Figure 20 can be used to conclude that DSR does occur within the wetland.

Thus, the $\delta^{34}S$-DOM could be a reflection of past processes that occurred within the wetland (ie. DOM that was leached from $\delta^{34}S$ reduced peat in the past). Alewell & Novak (2001) found a similar phenomenon in the peat horizon of the fen Schlöppnerbrunnen. They determined the $\delta^{34}S\text{-}S_{org}$ seen in certain layers of the peat to be due to differing reduction processes (assimilatory vs. dissimilatory) within the wetland, referring to it as a "historic fingerprint".

Throughout the hydrologic year, water levels within the wetland vary and differing hydrologic flowpaths transport DOM from different source areas in the wetland. Differing source zones of DOM within the wetland itself could be the reason for the unexpectedly enriched $\delta^{34}S$-DOM values from wetlands. When hydrologic flowpaths in the wetland are shallower, DOM derived from upper layers of the wetland is released. The organic material in the upper horizons of the wetland is "freshest" and consists of organic material deposited relatively recently. The most enriched $\delta^{34}S$-DOM in PC1 occurs during spring and fall, seasons in which the water levels are usually the most shallow (Fig. 20). DOS derived from the fresh organic material would be enriched, showing a signature similar to the fresh material (similar to leaf leachates and leaves).

When hydrologic flowpaths are deeper, DOM is typically transported from the porewaters of the wetland (Schiff et al., 1997). DSR occurs below the water table; therefore incorporation of sulphate into organic S by DSR must occur at deeper depths. The DOS could therefore contain a portion of organic S reduced by DSR. This organic S would be

depleted in $\delta^{34}S$, so it follows that the $\delta^{34}S$-DOM would be relatively depleted. The $\delta^{34}S$-DOM is lowest in summer, when flowpaths are deeper, which supports this hypothesis.

Another reason for the higher than expected $\delta^{34}S$-DOM could be the proportions of organic sulphur species within the DOM (ester sulphates vs. carbon-bonded S) are different. The isotopic fractionation of $\delta^{34}S$ into each fraction of organic sulphur has been found to differ; ester sulphates are typically less depleted than C bonded S. Mayer et al. (1992), in Mitchell et al. (1998) found fractionations of +3.6‰ for ester sulphates and –1‰ for C-bonded S. Mandernack (2000) found ester sulphates to range from –9.1 to –14.7‰, while C bonded S was more depleted, ranging from –11.9 to –16.8‰.

The C/S ratios in the PC1 wetland stream suggest an influence of reduction (Fig. 22); the $\delta^{34}S$-DOM generally decreases as C/S ratios decrease. As sulphur is added to organic matter by DSR of sulphate, the $\delta^{34}S$ and C/S ratios of organic S in peat would be expected to decrease. Studies have shown C/S ratios to decrease in reducing environments such as wetlands and lake sediments (Nriagu & Soon, 1985, Luther & Church, 1992).

The DOS concentrations (calculated from C/S ratios and DOC concentrations) from wetland streams vary substantially (0.07 to 0.27mg/L), and can constitute between 1.6 and 61.2% of the total S. The largest proportion of total S from DOS is at the beginning of the fall, when wetlands begin to wet up (Harp 5, Harp 6 and PC1 show 61.2, 53.6, and 52.8% of sulphate). This is significant, because it shows that a portion of S export from wetlands can be from DOS. This needs to be confirmed by discharge, however, and as of the time of this publication there were no data on discharge.

Harp Lake

Similar to $\delta^{34}S$-SO_4^{2-}, Harp Lake has a different $\delta^{34}S$-DOM to that of the input of the streams (Fig. 16). The $\delta^{34}S$-DOM is more enriched than either of its largest inputs, Harp 4 and 5. Precipitation, the largest input by volume into Harp Lake, does not provide any appreciable DOS; Houle et al. (2001) state precipitation does not contain significant

quantities of DOS. Therefore, it is assumed there must be processes occurring within the lake itself which serve to deplete the δ^{34}S-DOM.

Dillon & Molot (1997) showed 50% of the DOM in Harp Lake is lost to processes within the lake. DOS concentrations in the lake (0.05 to 0.08mg/L) are much lower than input streams (0.07 to 0.27mg/L) suggesting that DOS is mineralized within the lake. If DOS in streams provide between 8-22% of total S to the lake (Houle et al., 1995), then the proportion of DOS to total S in the lake could be significant. This could mean that the input of sulphate from mineralization of DOS could be significant.

Mineralization of DOS in the lake could serve to enrich the δ^{34}S-DOM in the lake. The addition of depleted $\delta^{34}S\text{-}SO_4^{2-}$ from DOS could possibly explain why the inorganic sulphate in Harp Lake reflects that of precipitation (Fig. 10).

4.5 Summary and Conclusions

Sulphur dynamics in forested catchments are very complex. Information about various processes causing sulphur transformations within the catchment can be inferred from sulphate concentrations, $\delta^{34}S\text{-}SO_4^{2-}$, $\delta^{34}S\text{-}S_{org}$, and C/S ratios of dissolved organic matter. There are significant differences between upland and wetland streams in all of the parameters within the catchment.

Trends in sulphate concentrations and $\delta^{34}S\text{-}SO_4^{2-}$ in the Harp and Plastic catchments are similar to those seen in other studies of forested catchments on the Canadian Shield.

The $\delta^{34}S\text{-}SO_4^{2-}$ and sulphate concentrations of most samples in the Harp Lake catchment do not reflect those of present precipitation. Samples taken from shallow groundwater and upland streams (Harp 4-21) appear to have an influence from historical sulphur deposition. Wetland streams show a large seasonal variability in both $\delta^{34}S\text{-}SO_4^{2-}$ and sulphate concentrations, which is mainly driven by hydrology.

Sulphate concentrations and $\delta^{34}S\text{-}SO_4^{2-}$ in the Plastic swamp are also variable throughout the hydrologic year. Sulphur cycling at this site is controlled by hydrology and ultimately, climate; Oxidation-reduction conditions within the wetland affect the amount of sulphate reduced by DSR and the mineralization of peat in the wetland.

Harp Lake is the only sample which is similar in sulphate concentrations and $\delta^{34}S\text{-}SO_4^{2-}$ to precipitation, despite a large input of sulphate of high concentration and enriched $\delta^{34}S\text{-}SO_4^{2-}$ from streams draining sub-catchments. This could be explained by a large input of sulphate from precipitation and/or mineralization of DOS from allochthonous input. Concentrations of DOS in the lake are also less than the input from streams, indicating a loss of DOS in the lake. The $\delta^{34}S$-DOM in Harp Lake has a more enriched signal than any of the streams which input the lake. Mineralization of DOS could enrich the $\delta^{34}S$-DOM while adding enriched $\delta^{34}S\text{-}SO_4^{2-}$ to the lake.

Upland streams are similar in $\delta^{34}S$-DOM to the vegetation that the DOM was originally derived. This suggests $\delta^{34}S$-DOM is source-dependent, and therefore probably controlled by vegetation type.

The processes that affect sulphate and DOS in wetlands are on different time scales, and information from samples collected on the same day reflect these time scales. Varying hydrologic flowpaths in the wetland appear to alter the $\delta^{34}S$-DOM of the output of the wetland. The $\delta^{34}S$-DOM is enriched during spring and fall, which could reflect DOS derived from newly deposited plant material. Also, addition of sulphur to organic matter by reduction in the wetland is suggested by $\delta^{34}S$-DOM and C/S ratios.

It is evident that S cycling is extremely complex within the Harp and Plastic Lake catchments. Inorganic and organic S cycling appears to be linked in the catchment. Information from $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{34}S$-DOM and C/S ratios suggest interactions between inorganic sulphur and organic sulphur in both wetlands and Harp Lake.

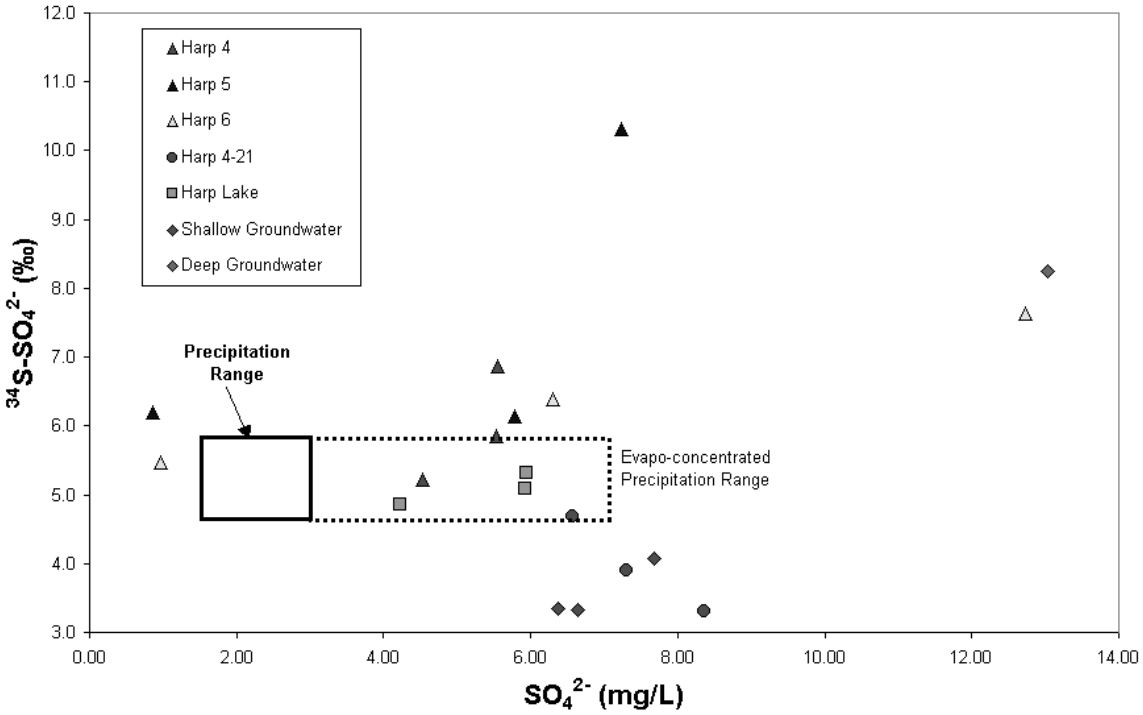

Figure 10. Environmental differences in inorganic S cycling within the Harp Lake catchment. Precipitation data taken from Eimers (2002); Evapo-concentration range is calculated using the difference in SO_4^{2-} concentration between PC1-08 and precipitation at Plastic Lake catchment.

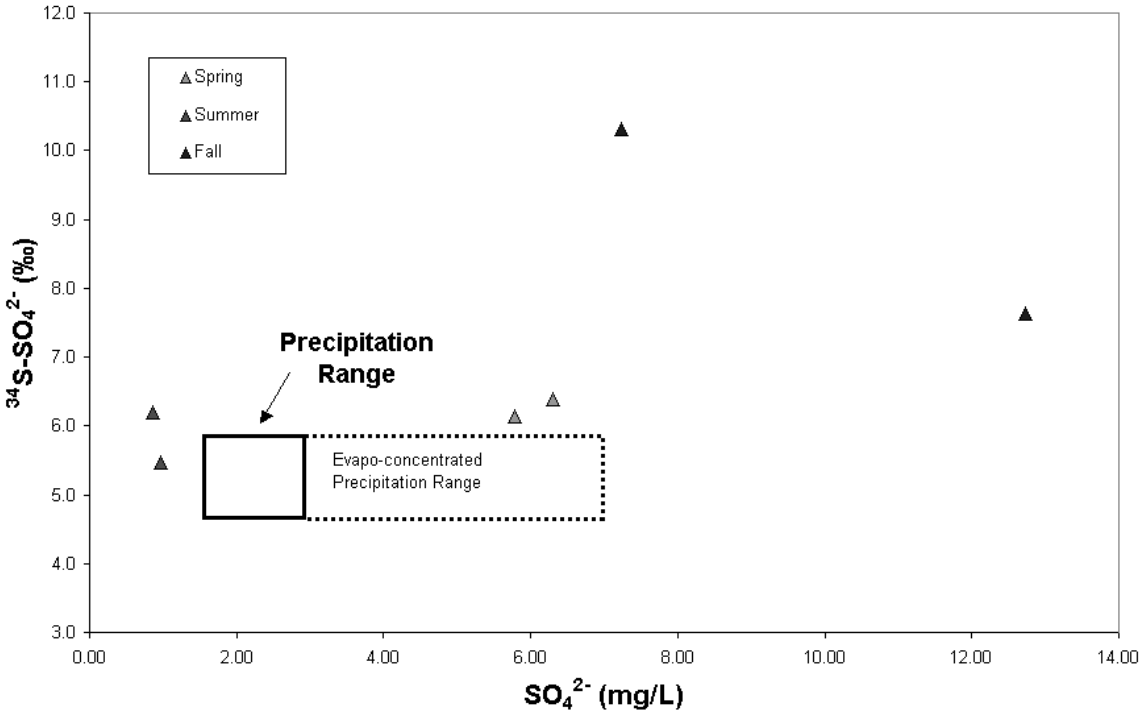

Figure 11. Wetland seasonal differences in sulphate in the Harp Lake catchment. These seasonal differences are attributable to hydrologic conditions in the wetland. See Figure 10 for details on precipitation range and evapo-concentrated precipitation range.

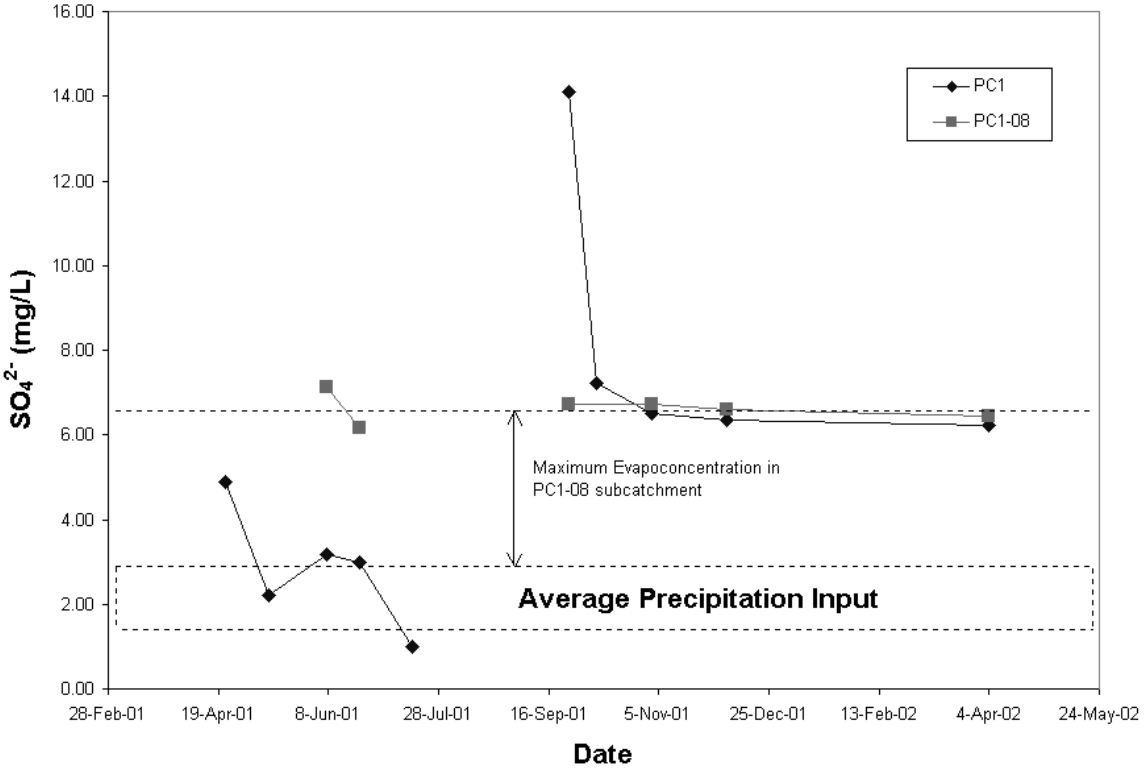

Figure 12. Sulphate concentrations for hydrologic year 2001-2 at Plastic Lake watershed. Average evapo-concentration in the catchment is calculated from the difference between precipitation and PC1-08. This estimate is a maximum, since PC-108 has been known to export SO_4^{2-}. Average precipitation data taken from Eimers (2002) and Ontario Ministry of Environment from 2001-2002.

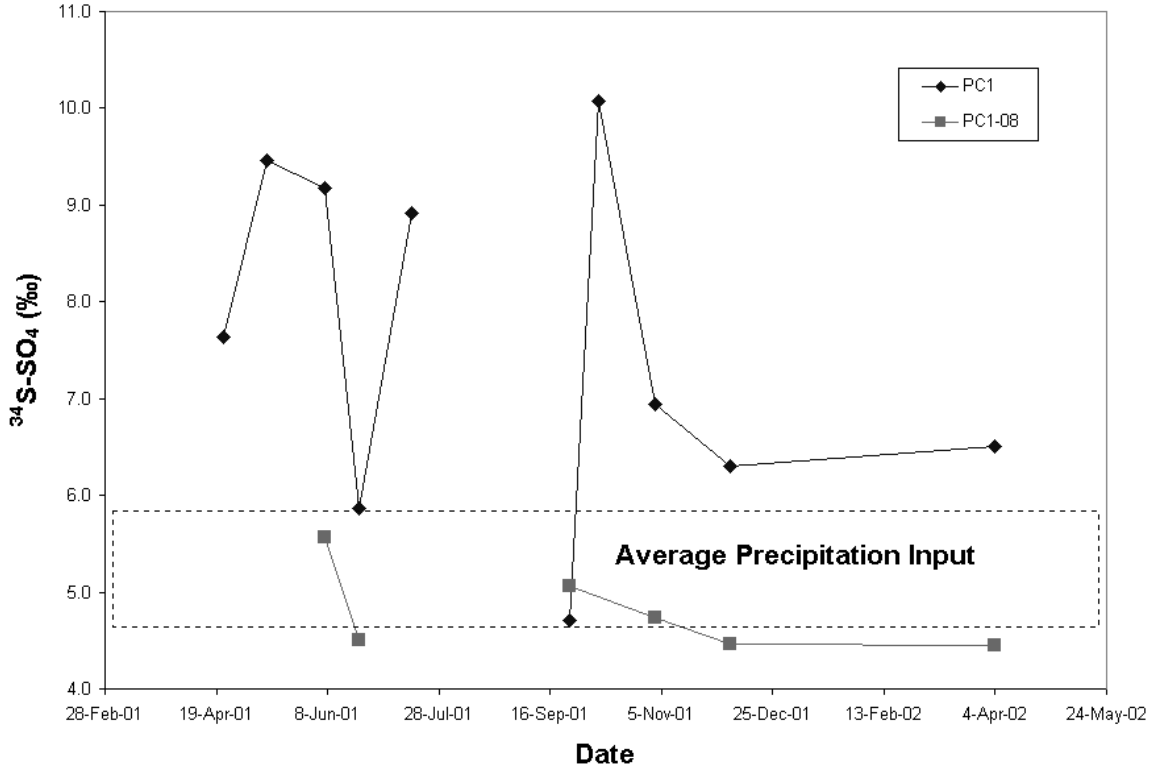

Figure 13. $\delta S\text{-}SO_4^{2-}$ for the hydrologic year 2001-2 at Plastic Lake watershed. Output $\delta S\text{-}SO_4^{2-}$ from the Plastic swamp (PC1) is higher and more variable that the input (PC1-08). Average precipitation data taken from Eimers (2002).

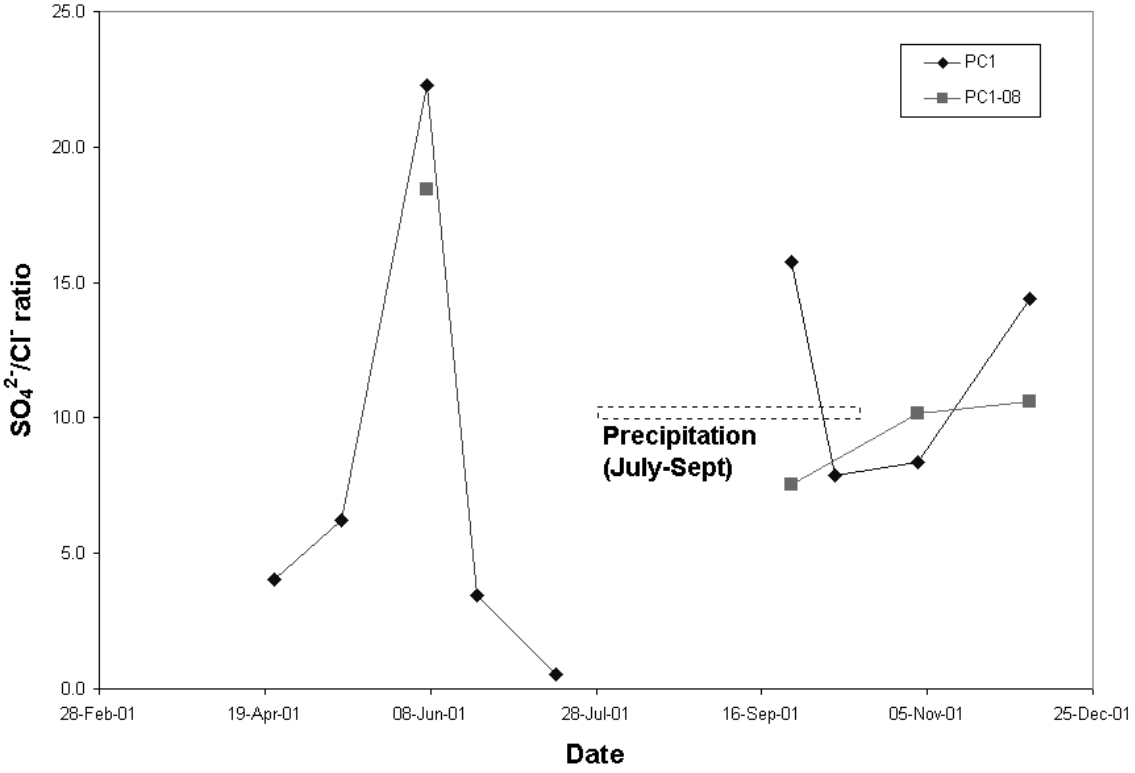

Figure 14. SO_4^{2-}/Cl^- ratios for the Plastic subcatchment. July to September precipitation data was taken as a combined sample.

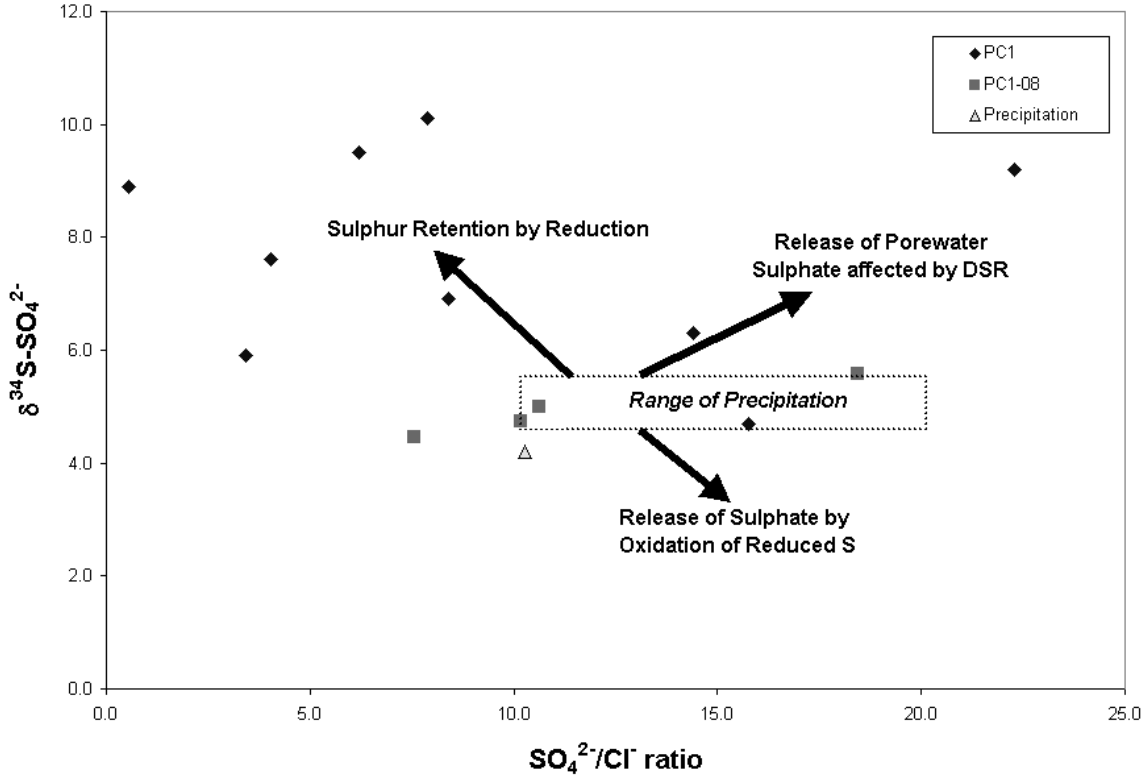

Figure 15. S dynamics in Plastic Lake catchment. Range of δS-SO_4^{2-} taken from Eimers (2002); range of SO_4^{2-} /Cl^- ratios taken from Ministry of Environment of Ontario in 2001-2002. Precipitation is a mixed sample from July-September.

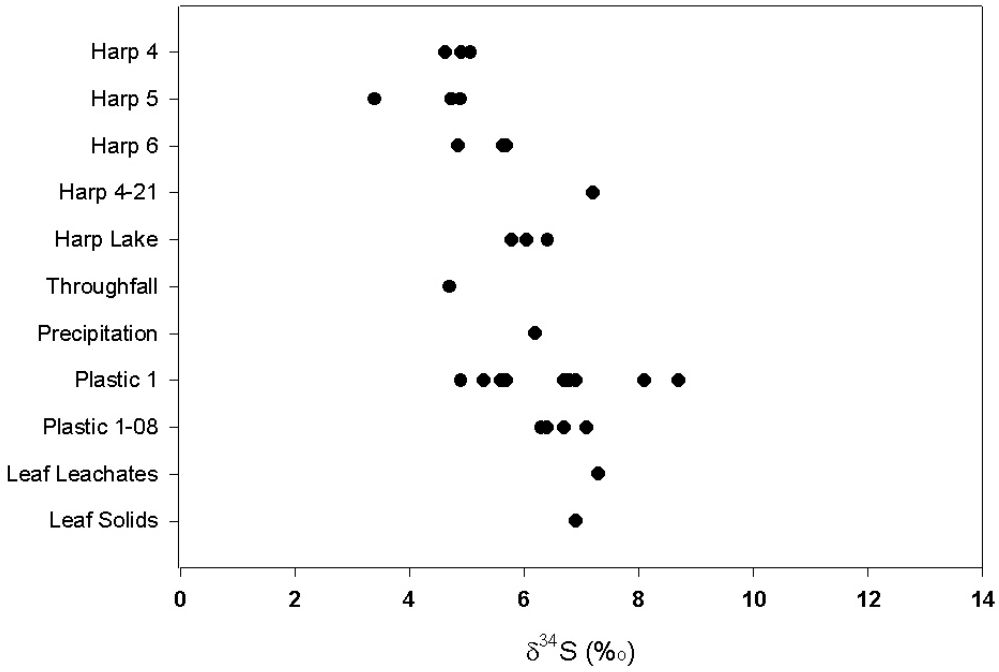

Figure 16. Distribution of $\delta^{34}S\text{-}S_{org}$ for DOM the Harp and Plastic watersheds.

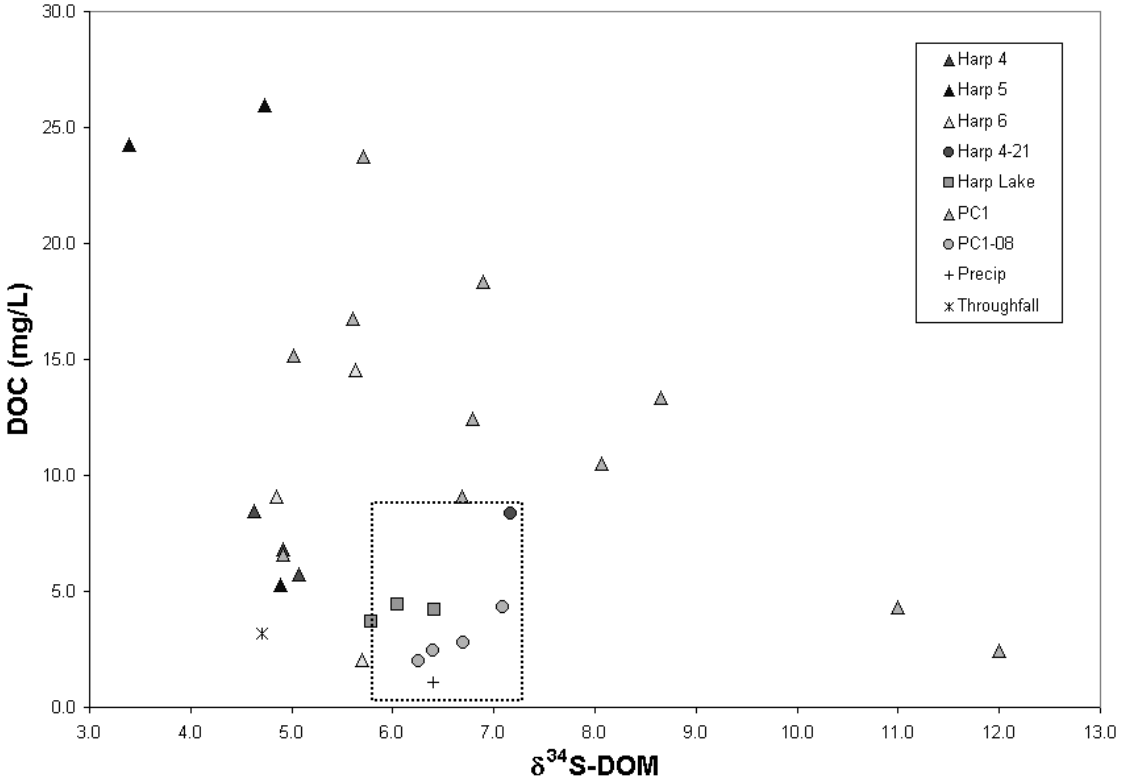

Figure 17. DOC concentrations and δ^{34}S-DOM by environment for the Harp and Plastic Lake catchments. Boxed area contains upland streams and Harp Lake.

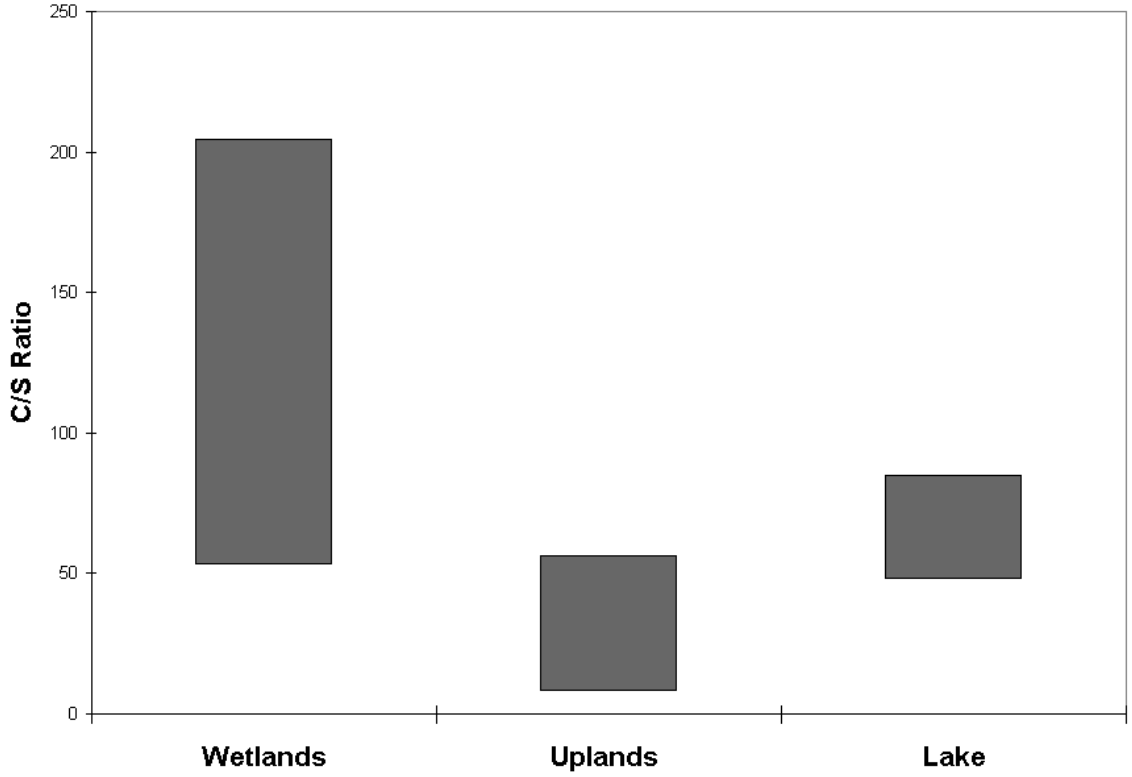

Figure 18. Variations in ranges of C/S ratios between wetland streams (PC1, Harp 5, 6), upland streams (PC1-08, Harp 4-21), and Harp Lake.

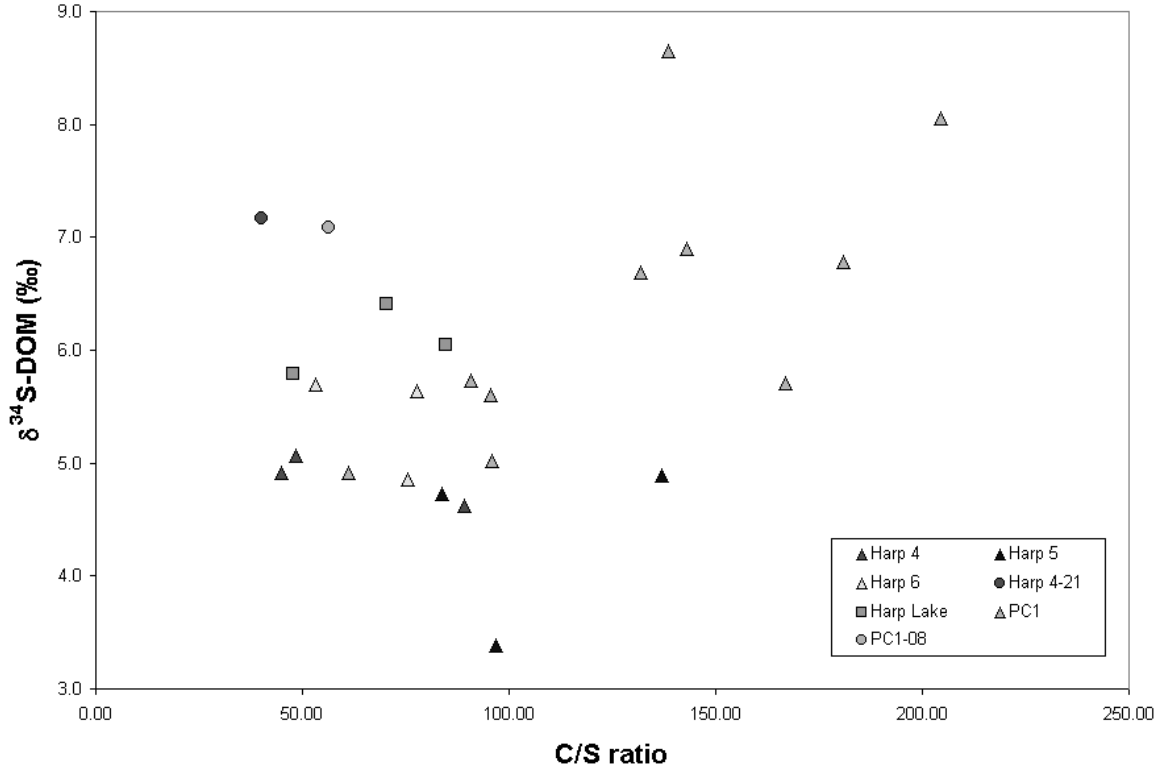

Figure 19. C/S ratios and δ^{34}S-DOM in the Harp and Plastic Lake catchments.

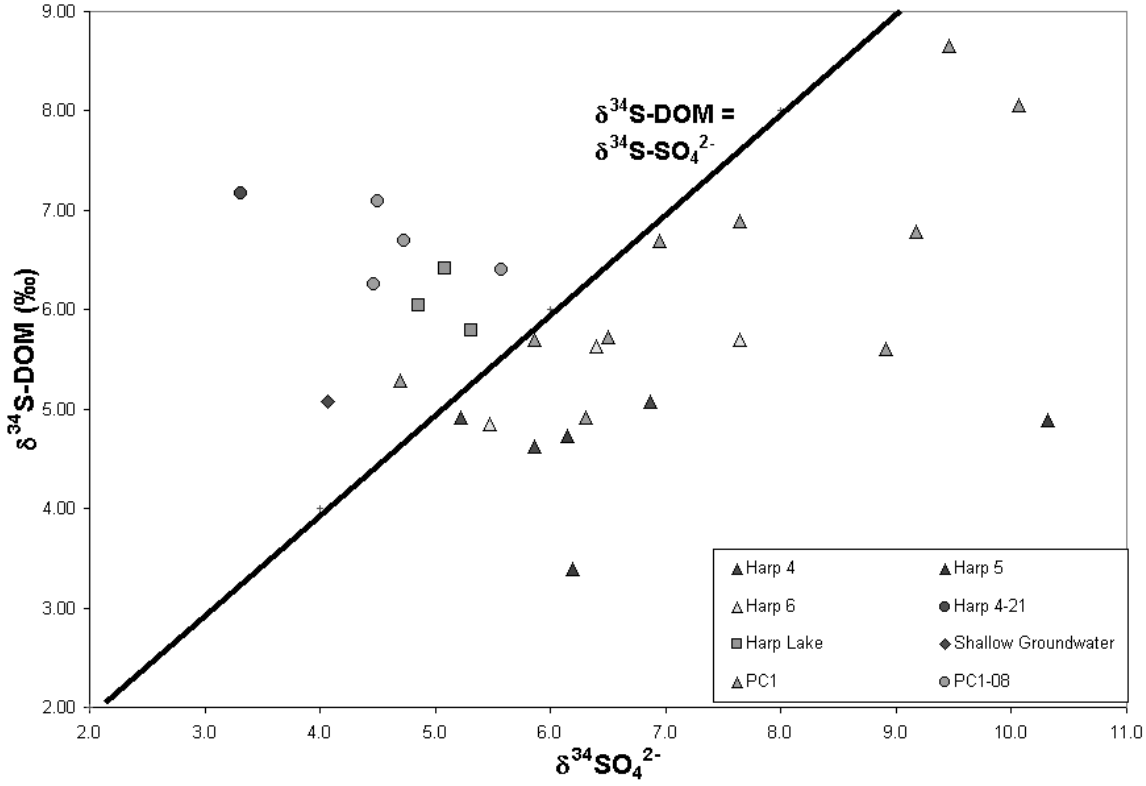

Figure 20. A comparison of $\delta^{34}S\text{-}S_{org}$ and $\delta^{34}S\text{-}SO_4^{2-}$ in the Harp and Plastic Lake catchments.

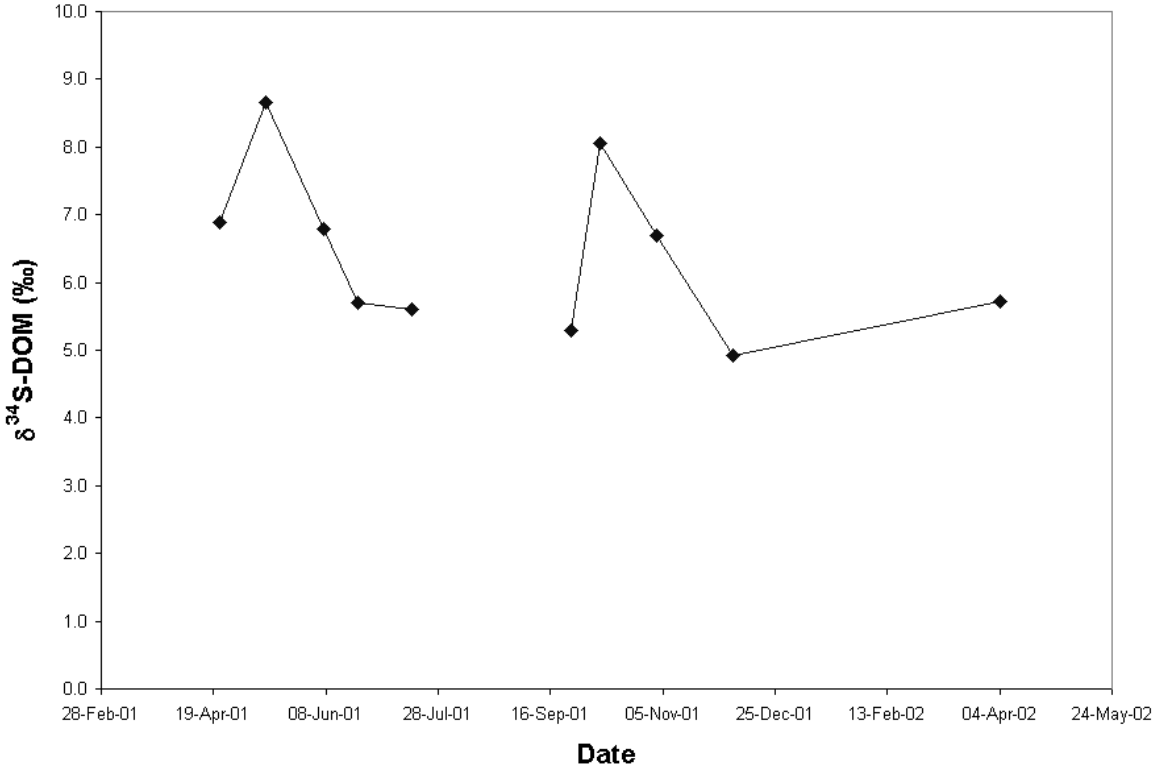

Figure 21. Time series of $\delta^{34}S\text{-}S_{org}$ shows the possible effects of different hydrologic flowpaths.

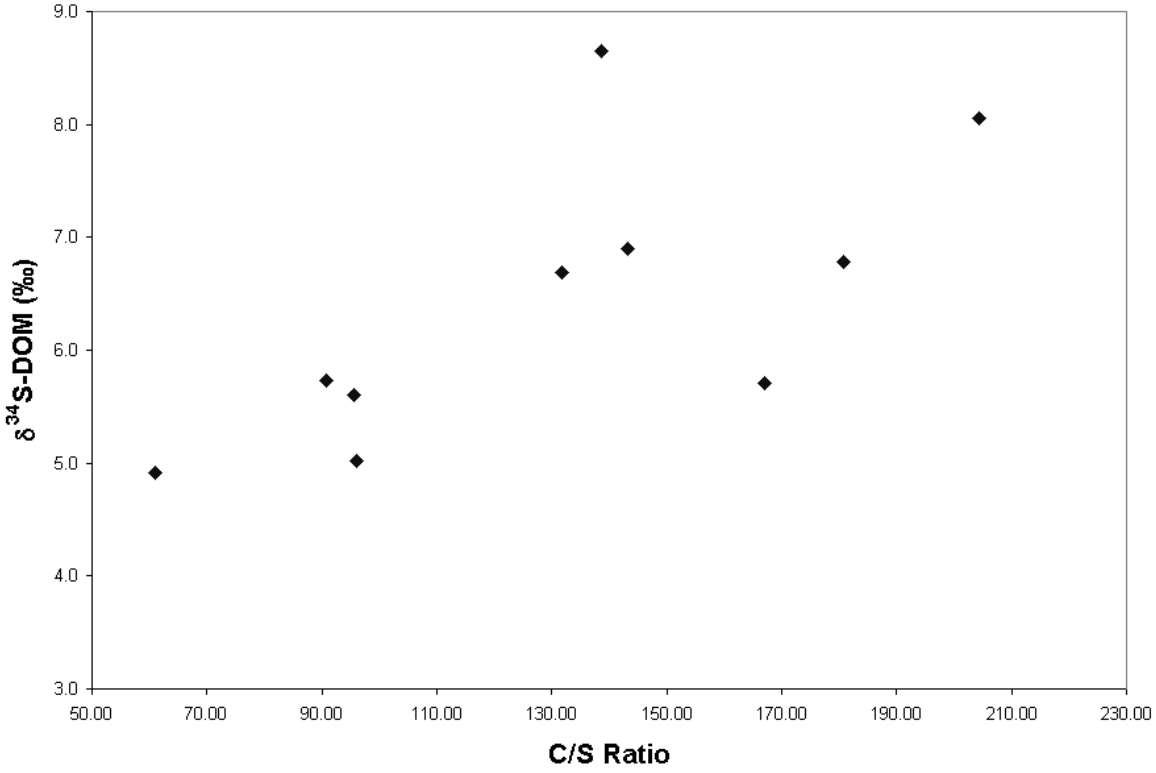

Figure 22. Relation between C/S ratios and $\delta^{34}S\text{-}S_{org}$ in the Plastic swamp.

Chapter 5: $\delta^{18}O$ in Dissolved Organic Oxygen from Forested Watersheds: Implications for DOM Alteration

5.1 Introduction

Dissolved organic matter (DOM) consists of a continuum of organic molecules ranging from small monomers such as sugars to large polymerized molecules such as humic substances. The composition of DOM in forested catchments is highly variable and differs both spatially and temporally within the catchment. The distribution of this continuum is dependent upon the original organic matter, the hydrologic flowpaths in the catchment, and the degradation conditions along these flowpaths. As DOM moves through the catchment, it can be subject to physical, biological, or chemical transformations, which change both the original chemical structure and composition of DOM.

Organic Oxygen

The major elements in DOM, listed in order of abundance, are carbon, oxygen, hydrogen, nitrogen and sulphur. Organic oxygen can constitute between 23 to 45% by weight of the DOM molecule (Thurman, 1985) and is ubiquitous in many functional groups in DOM (Drever, 1997). Oxygen accounting has been used to ascertain information about functional groups (Thurman, 1985). Other than oxygen accounting, few studies have been performed on organic oxygen in DOM, despite its abundance and importance in functional groups in DOM.

$\delta^{18}O$ in Organic Matter

There has been considerable research on organic oxygen within plant carbohydrates, mainly cellulose. The focus of these studies has been mainly for paleoclimatological research, and not for characterizing DOM. Paleoclimatic conditions can be inferred by the

$\delta^{18}O$ in carbohydrates. The $\delta^{18}O$ of the cellulose formed at the time of photosynthesis is determined by the isotopic ratio of water with a constant enrichment factor of 27‰ (Epstein, 1977, Sternberg, 1986). Aquatic cellulose and cellulose from tree rings are used to determine the $\delta^{18}O$ of the water at the time of photosynthesis which can provide insight into paleoclimatic conditions (Edwards et al., 1989, Wolfe et al., 1997, Anderson et al., 2002).

The fractionation of +27‰ during photosynthesis is consistent across all plant types (regardless of photosynthetic mode), terrestrial or aquatic, and does not deviate greatly (+26 to +28‰; Epstein, 1977, Sternberg et al, 1986, Farqhuar & Lloyd, 1993, Sauer et al, 2001). Sternberg et al. (1986) showed that enrichment occurs at the carbonyl hydration step where oxygen is fixed. Oxygen exchange occurs between the carbonyl oxygens in the carbohydrate and water during cellulose synthesis, resulting in a 27‰ difference between water and cellulose.

In addition to the +27‰ fractionation between cellulose and water, the water in terrestrial plants can undergo further fractionation due to evapotranspiration in the leaf (Sternberg, 1986, Farqhuar & Lloyd, 1993, Sauer et al., 2001). Evapotranspiration of water results in a kinetic isotope effect, preferentially enriching the water in $\delta^{18}O$ (Clark & Fritz 1997). This added fractionation of the water due to evapotranspiration gives rise to the differentiation between aquatic cellulose and terrestrial cellulose. Aravena & Warner (1992) determined that differences of $\delta^{18}O$ of cellulose from sphagnum result from variations in microclimate in peatlands in Ontario. Submerged sphagnum displayed a different $\delta^{18}O$ signature to sphagnum located on hummocks, due to fractionation of the water from evapotranspiration. From these differences in $\delta^{18}O$ signatures, allochthonously derived cellulose can be differentiated from autochthonously derived cellulose (Edwards & McAndrews, 1989, Wolfe & Edwards, 1997, Abbott et al, 2000, Sauer et al, 2001).

Naturally occurring DOM is derived from many types of organic matter, not simply cellulose. This is important because other fractions of organic matter may vary in $\delta^{18}O$ signatures.

Whole leaf tissue has been shown to vary from leaf cellulose $\delta^{18}O$; Barbour & Farquhar, (2000) state leaf tissue in cotton plants can be 4.2 to 9.2‰ more depleted than its cellulose. The $\delta^{18}O$ in leaf material can also vary diurnally. Cernusak et al. (2002), using dry leaf matter, found that $\delta^{18}O$ in different components of the leaf can vary almost ±6‰ above and below cellulose, attributing this to variations in evapotranspiration throughout the day.

Whole plant matter can also range in $\delta^{18}O$. For instance, lignin in tree rings has been shown to vary annually (Anderson et al., 2002, Barbour et al., 2002, Borella et al., 1999). Saurer et al. (1997) found stem cellulose of different species to range in $\delta^{18}O$. They concluded that the transfer of $\delta^{18}O$ signal in leaf water to whole plant material is damped and dependent upon species. Since $\delta^{18}O$ fractionation from evapotranspiration in terrestrial plants occurs at the leaf, the site of synthesis (leaf vs. stem) can be important in studying $\delta^{18}O$ of terrestrial plants (Sauer et al., 2001). Therefore, even though cellulose can be fractionated by a constant +27‰ or greater, other organic constituents may exhibit a range of $\delta^{18}O$ signatures (Sternberg, 1989, Cernusak, 2002).

$\delta^{18}O$ of Organic Matter during Decomposition

Although $\delta^{18}O$ ratios in organic matter have been studied fairly extensively, there have been few or no studies determining the decomposition the effect of organic matter on the subsequent $\delta^{18}O$ of DOM. When plant organic matter is first leached, easily degradable carbohydrates of low molecular weight are formed (Thurman, 1985). Saunders (1976) proposed that simple organic molecules (e.g. glucose, acetate) are broken down most rapidly by microbes, with turnover rates of less than one hour to several hours. These molecules are not transported past the upper soil horizons in the forest because of their high lability. The remaining dissolved organic matter is most likely subject to hydrolysis which breaks the bonds of the polymeric dissolved constituents (Thurman, 1985).

Thurman (1985) states that only 10% or less of the DOM are simple compounds and that microbes must hydrolyze more complex DOM as the pool of simple compounds is depleted.

Covalent bonds in the organic molecule can be broken by hydrolysis (Fig. 23 a, b). When this occurs, oxygen from water is added to the resulting polymers. Progressive hydrolysis would serve to lower the $\delta^{18}O$ of the DOM, because the $\delta^{18}O$ of water is much less than that of organic matter. Larger molecules with many functional groups would be subject to hydrolysis as further degradation occurs. Amon & Benner, (1996) determined a size-reactivity continuum in which the smallest molecules were the most degraded and recalcitrant. Therefore, degradation of DOM should result in fewer functional groups, smaller molecules, and lower $\delta^{18}O$.

5.2 Methods

Sample Collection

Samples were collected in 2001from the Harp and Plastic Lake catchments in Ontario, Canada, located approximately 200km north of Toronto.

The Harp Lake catchment was sampled on three different occasions, to investigate differences between spring, summer, and fall (April 22, July 6, October 8). Deep groundwater was collected on July 25/26, 2002. Sampling at Plastic Lake was performed more frequently to examine changes in DOC character over time. Collection of the samples occurred every 2-3 weeks from April to July, and on a monthly basis from September to December. No samples were collected at PC1 between July 16 and September 25, because of little to no stream flow. Samples from PC-108 were only collected on dates where sufficient volume for the RO procedure could be obtained. Precipitation was collected in precipitation buckets located in a clearing approximately 200m from the edge of the lake, 400m north of PC-1. The precipitation samples were combined from the months of July-September in order to ensure an adequate mass of DOM for the RO process. Throughfall samples were collected with a modified eavestroughing collector; samples were collected once a month from October to November and combined. Dry leaves were collected from the Harp 6 catchment after leaf fall in October 2002.

Laboratory Methods

Sample collection and processing followed those presented in Chapter 3. Briefly, the large volume samples were filtered using a Balston stainless steel aluminum 20 μm pre-filter followed by a Geotech 147mm inline filter containing a 0.7 μm precombusted glass fiber filter (Whatman GF/F, 0.7μm nominal size). Concentration of samples was performed by RO, using a membrane cutoff of 300 Daltons. Recovery of DOC within the RO membrane has an efficiency of 99%. Concentration factors of solutes in the retentate solutes ranged from approximately 8-20×, and sample volumes were reduced to 4 to 5 litres.

Concentrated samples were then subject to an isolation procedure to remove sulphate from the solution. This procedure consisted of a combination of the addition of HCl and BaCO3 to the solution, effectively precipitating SO_4^{2-} as $BaSO_4$. Steps were taken to ensure maximum recovery of organic matter.

A portion of the leaves collected from the Harp 6 catchment was progressively leached with deionized water (DI). Leaves were leached once with DI, drained, and then left for 2 days at 4°C. Subsequently, more DI was added. The second leach was then drained, and after a similar rest period, a third volume of DI was added. Then, the final leachate was drained, and samples of leachate are subsequently known as leaf leaches 1, 2, and 3.

Peat from Plastic swamp and zooplankton (48-500μm) from Harp Lake were used from previous studies in an attempt to quantify end-members representative of allochthonous and autochthonous organic matter, respectively (Elgood, unpublished data).

Analysis of Organic $\delta^{18}O$

Organic samples were run for $\delta^{18}O$ using a Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA 1108) with a high T combustion in the Environmental Isotope Laboratory (EIL), University of Waterloo. This apparatus has a detection limit of ±0.8‰ for $\delta^{18}O$.

The precision of the apparatus for δ^{18}O-DOM is ±1.2%. The reproducibility of δ^{18}O-DOM from isolation procedures is ±1.2‰, although more samples need to be duplicated in order to obtain a more accurate estimate.

Relative Molecular Weights of DOM

Original filtered samples were sent to Trent University for the determination of relative molecular weight by HPLC (Wu, unpublished data, 2002). Samples were processed according to Wu (2002), which followed procedures outlined by Chin et al. (1994), using UV absorption at 254nm. Data generated from this process included number-averaged molecular weight (M_n) and weight-averaged molecular weight (M_w). Data used in this thesis is weight-averaged molecular weight, since it is more representative of the bulk properties of the DOM molecules. Weight averaged molecular weight is determined by methods which depend on the masses of material in different factions (Aiken et al., 1985, USGS, 1994). Caution must be used when considering the HPLC determined average molecular weight by UV absorption. Her et al. (2002a) state the estimation of molecular weight by UVA detection to be inherently inaccurate because not all components of DOM absorb UVA at 254nm equally at 254nm. Thus, the absolute molecular weight will be biased towards these components that absorb UVA, the fulvic acid component of the sample. Despite this shortcoming, the weight-averaged molecular weight can be useful in showing relative differences in molecular weight if the fractions of DOM do not vary greatly between samples. Therefore, the weight-averaged molecular weight (M_w) determined in these samples will be referred to as a "relative weight averaged molecular weight".

Wu (personal comm., 2002) estimates precision of weight-averaged molecular weight to be 5-9%.

5.3 Results

Chemistry

The range of original DOC concentrations for uplands and wetlands differ dramatically for the Harp Lake catchment. Harp 4-21, an upland catchment, ranges from 2.02-3.67 mg/L. Shallow groundwater, which feeds Harp 4-21, exhibited a narrow low range of 0.42-1.23 mg/L. Harp 4 ranges from 5.7-8.4 mg/L. Wetland streams showed a much higher and larger range of DOC: Harp 5 ranged from 8.3-25.9 mg/L and Harp 6 ranged from 5.3-14.5 mg/L. Harp Lake stayed relatively constant, and ranged from 3.7-4.4 mg/L.

Plastic Lake catchment also exhibits the same differences between uplands and wetlands. PC1-08, an upland stream, ranged from 2.0-3.2 mg/L while PC1, draining the swamp, ranged from 9.06-23.8 mg/L.

Precipitation showed a DOC concentration of 1.1mg/L, while throughfall was 3.2mg/L.

Ranges for the SO_4^{2-} concentrations also differ dramatically between uplands and wetlands in the Harp Lake catchment. Harp 4-21 ranges from 6.57-8.37 mg/L; Harp Beaver Pond (Harp 4 catchment) was 4.61 mg/L in April; Harp 4 ranged from 4.53-5.55 mg/L. Wetland streams showed a larger range in sulphate: Harp 5 ranged from 0.87-7.24 mg/L; Harp 6 ranged from 0.96-12.72 mg/L; Harp Lake ranged from 5.91-5.94 mg/L and shallow groundwater ranged from 6.37-7.68 mg/L.

In the Plastic Lake catchment, PC1-08 ranged from 6.15-7.12 mg/L; PC1 ranged from 0.99-14.09 mg/L; Combined precipitation was 2.84 mg/L; and LFH water was 9.88 mg/L.

In all samples, concentrations of sulphate in RO retentates are too high for successful analysis of δ^{18}O-DOM, therefore SO_4^{2-} must be removed prior to analysis.

Organic $\delta^{18}O$

The distribution of $\delta^{18}O$ in the Harp and Plastic Lake catchments is variable by environment. In the Harp Lake catchment, the lake and deep groundwater are most depleted (8.9-9.4‰, Table 12). The lake samples show little variability in $\delta^{18}O$, having an average $\delta^{18}O$ of 8.8‰ ±0.6‰. In contrast to the lake and deep groundwater, the wetland streams in Harp (5,6) are the most enriched and show the most variation (9.0-12.8‰). There is a large range in $\delta^{18}O$ in the wetland stream in Plastic (PC1; 8.4-14.4‰, average 11.6 ± 2.4‰, Table 13). There is little range in the upland stream (PC1-08; 9.5-10.1‰, average 9.7 ±0.3‰), except for the sample collected November 2 (5.1‰). Precipitation at Plastic Lake was 13.9‰, while throughfall was 13.1‰.

The sample collected from PC1-08 on November 2, 2001, had a significantly different signature to the $\delta^{18}O$ from PC1-08 collected on different dates (5.2‰ compared to 9.6-10‰). This sample had a larger amount of DOM pass through the membrane during the RO procedure relative to other samples (9.5%; Appendix C). This could mean that the molecules from this samples are smaller and less complex, passing through the 300Da membrane easily. Since this sample has a significantly different % DOM passing, it will be excluded from further analyses.

Leaves collected from the Harp 6A catchment have an $\delta^{18}O$ of 24.2‰. Progressive leaf leachates from the same leaves were 25.3‰, 23.6‰, and 23.4‰, within error. DOC in progressive leachates, however, was very different (173mg/L, 416mg/L, and 431mg/L for the first, second, and third leaches, respectively).

Peat from the centre of Plastic swamp (piezometers P15 and P16, (Devito & Hill, 1997, Eimers, 2002) ranges between 17.3 and 17.8‰. Peat approximately 5m from the edge of Plastic swamp (P17; Devito & Hill, 1997, Eimers, 2002) ranges 14.6 to 15.6‰. Zooplankton (50-500 μm) from Harp Lake had an $\delta^{18}O$ of 16.3 ± 0.4‰.

The $\delta^{18}O$ of the Harp 4-21 sample from July and shallow groundwater have not yet been analysed.

Table 11. $\delta^{18}O$-DOM for Harp Lake catchment

Sample	April 22, 2001	July 6, 2001	October 8, 2001	2002
Harp 4	11.4	11.3	11.5	-
Harp 5	9.0	12.2	12.8	-
Harp 6	9.5	11.7	10.0	-
Harp 4-21	10.5		9.0	-
Harp Lake	8.9	8.2	9.4	-
Well 55	-	-	-	9.0
Harp Leaf Leachate 1	-	-	-	25.3
Harp Leaf Leachate 2	-	-	-	23.6
Harp Leaf Leachate 3	-	-	-	23.4
Harp Leaves	-	-	-	24.5

Table 12. $\delta^{18}O$-DOM for Plastic Lake catchment time series.

Date	PC1	DOC (mg/L)	PC1-08	DOC (mg/L)
22-Apr-01	11.2	10.6	NF	NF
12-May-01	13.4	18.4	NF	NF
07-Jun-01	13.4	13.4	9.6	2.5
22-Jun-01	14.4	12.5	10.1	4.3
16-Jul-01	10.3	23.8	NF	NF
25-Sep-01	8.4	16.7	NF	NF
08-Oct-01	9.4	15.2	NF	NF
02-Nov-01	10.6	10.5	5.1	2.8
06-Dec-01	10.8	9.1	9.5	2.0
04-Apr-02	13.7	6.6	9.5	2.2

NF = No flow at weir

Relative Molecular Weight

Relative average molecular weight varies between different environments in Harp and Plastic Lake catchment: shallow groundwater in the Harp 4-21 catchment ranged from 2200 to 5500Da; Harp 4-21 ranged between 5400 and 6100Da; Harp 4 ranged between 6000 and 6300Da; Wetland streams Harp 5 and 6 ranged between 5700 and 6500Da; Harp Lake ranged

between 4200 and 5200Da; and Well 55 was 4800Da. Samples in the Plastic Lake catchment varied for the wetlands, but not for the upland: PC1 ranged between 5200 and 6400Da; and PC1-08 ranged from 4800 to 5600Da. Precipitation at Plastic Lake was 5800Da, and throughfall was 5400Da. Progressive leaf leachates had relative average molecular weights of 4200, 4900, and 5300Da, respectively.

The data for M_n and M_w were compared to investigate any differences between the two averages (Fig. 24). The two averages correlate well, but it should be noted that the molecular weights from some upland stream samples and groundwater do not fit as well to the relationship. The number-average tends to be lower in these samples, which is expected, since they are typically lower in the humic substances which have a high molecular weight. The slope of the plot is less than 1, which means there is a larger spread in the number averaged molecular weight for samples with smaller molecules. The weight-average molecular weight emphasizes the heavier molecular weight species in the sample (USGS, 1994). Therefore, the smaller molecules would be more dispersed for the number-average molecular weight (Fig. 24)

Both averages were compared to DOC concentrations (Fig 25a, b), differences in environment can be seen. The lower molecular weight molecules tend to have lower DOC concentrations, and the higher molecular weight molecules tend to have higher DOC concentrations.

5.4 Discussion

$\delta^{18}O$ in DOM Sources: Leaves, Leachates and Throughfall

According to Sternberg (1989), the $\delta^{18}O$ of terrestrial vegetation in Harp and Plastic catchments should be enriched by at least +27‰ from the groundwater in the region. Groundwater in Harp 4-21 studied by Hinton (1998) had an average $\delta^{18}O$ of 11.7‰ ± 0.5‰ in 1989, and groundwater from Harp 6 has a range of $\delta^{18}O$ from -11.8‰ to -12.4‰ (Schiff, unpublished data, 1996, 1997, 1998). The leaves collected from the Harp Lake catchment

are enriched +35.3‰ ± 1.0‰ from the $\delta^{18}O_{water}$ of groundwater. Thus, the evapotranspiration occurring at the Harp Lake catchment must result in an additional enrichment in the organic matter by approximately +8.3‰. Harp 6A is located on a hillslope, and this enrichment might be expected for this site; Saurer et al. (1997) found higher $\delta^{18}O$ in stem cellulose to occur in drier areas, due to increased evapotranspiration. This enrichment might not be representative of the leaves in Harp Lake catchment, since the leaves were sampled from a limited area on the hillslope at Harp 6A. Further research needs to be performed to quantify the enrichment in other areas of the Harp Lake catchment.

Another factor to consider when investigating bulk leaf $\delta^{18}O$ is that different components in the leaves may have different $\delta^{18}O$ signatures. Photosynthesis is the only process which enriches the $\delta^{18}O$ in carbohydrates by +27‰. Subsequent oxygen addition to different components in the leaf would therefore be derived from water. These components would consist of macromolecules with a more depleted $\delta^{18}O$, since the water is relatively very depleted. Thus, the bulk leaf would be slightly depleted in $\delta^{18}O$ when compared to the carbohydrates formed in photosynthesis.

Leaf leachates represent a starting point of DOM in the forest, and initial leaf leaches would represent the first leaches of leaves on the forest floor. The $\delta^{18}O$ of leaf leachates from Harp 6A is similar to the leaves from which they were leached from, and are within the precision of analysis.

Typically, when leaves are initially leached, the small molecules such as sugars and simple carbohydrates are released first (Thurman, 1985). It is plausible these molecules are simple monosaccharides or disaccharides containing oxygen only fixed by photosynthesis. These molecules would then display an enriched $\delta^{18}O$ when compared to the original leaves. Subsequent microbial activity and leaches could mobilize the larger compounds from the leaf, thereby releasing molecules with a more depleted $\delta^{18}O$. This is supported by molecular weight data (Fig. 26).

As DOM is progressively leached from the leaves, it is possible that the relative proportions of small and large molecules will change with each leach. DOC concentrations from each successive leaf leachate exhibit substantial increases in the amount of DOM for each progressive leachate. This increased amount of DOM would probably consist of both small and large molecules, but a higher proportion of large molecules is released each subsequent leachate, as evidenced by the increase in molecular weight.

In fall, DOM is leached from accumulated litter after leaf fall. The expectation would be that this leachate would be relatively low in molecular weight, relatively enriched in $\delta^{18}O$ and high in DOC concentrations, similar to leaf leachates leached by DI. This was not the case, however, as it appears that the DOM is rapidly altered after leaching in the natural catchment. Thurman (1985) showed the DOM leached by precipitation to be much different to DOM leached by distilled water.

In a forested catchment, throughfall DOM can be derived from the leaching of organic material in the forest canopy by water from precipitation. The $\delta^{18}O$-DOM of precipitation is similar to throughfall (13.6‰ compared to 13.1‰), but concentrations of DOC in precipitation are 3x less than that in throughfall (1.1mg/L compared to 3.2mg/L). Therefore, there is another source of DOM in throughfall, caused by the leaching of organic matter in the canopy.

If the $\delta^{18}O$-DOM of throughfall is partly derived from leaching of the forest canopy, then it should show an $\delta^{18}O$-DOM similar to that of the vegetation. However, the $\delta^{18}O$-DOM of throughfall is much different than the $\delta^{18}O$ of either whole leaf material or the DOM from the successive leaf leachates. A factor in the difference in $\delta^{18}O$-DOM between throughfall and leaf leachates could be the different canopy type in Plastic Lake catchment. Regardless of different vegetation, the organic matter in Plastic Lake catchment should be greater than +15‰. Therefore, there appears to be some process which alters the $\delta^{18}O$-DOM after leaching of forest canopy.

$\delta^{18}O$ as an Indicator of DOM Alteration

The $\delta^{18}O$-DOM from different types of samples (lake, groundwater, upland, wetland) differs in both mean and standard deviation (Fig. 27).

The leaf leachates provide an upper limit of $\delta^{18}O$ from which to compare DOM in uplands, streams from streams, groundwaters, and lakes. In Harp and Plastic Lake catchments, the majority of DOM is derived from wetlands (Dillon & Molot, 1997, Schiff et al., 1990). When compared to both peat (14.6-17.8‰) and leaf leachates, the majority of the $\delta^{18}O$-DOM in the samples from Harp and Plastic are relatively depleted. This means that the DOM in these catchments is subject to some sort of alteration which would deplete the $\delta^{18}O$-DOM from its original organic matter. As mentioned previously, the mechanism for this alteration could be hydrolysis, which would serve to deplete the $\delta^{18}O$ in the molecule by adding $\delta^{18}O$ depleted oxygen from water (~-12‰). Thus, $\delta^{18}O$-DOM could be an indicator of progressive alteration (hydrolysis) of DOM.

The $\delta^{13}C$ of DOM also changes with increasing alteration, since $\delta^{13}C$ of DOM generally increases with depth along the soil profile and along the hydrologic flowpath (Schiff et al., 1990, Schiff et al., 1997). Schiff et al. (1990) suggest the $\delta^{13}C$ increase along the soil profile into the groundwater is due to preferential decomposition or sorption of selected compounds. In the Harp and Plastic catchments it appears that the samples with the more enriched $\delta^{13}C$ have the most depleted $\delta^{18}O$ (Fig. 28), which is consistent with the hypothesis that $\delta^{18}O$ is an indicator of progressive decomposition of DOM.

Changes in Relative Molecular Size, DOC, and $\delta^{18}O$ with Environmental Origin

Molecular size and DOC concentrations also change with the alteration of DOM. Amon & Benner (1996) showed a large portion of DOM with low molecular weight to be refractory in nature, because these molecules have been subject to substantial degradation. A significant relationship exists between $\delta^{18}O$ and relative molecular weight for the Harp Lake catchment (Fig. 29).

The alteration and decomposition of DOM typically lowers DOC concentrations. When δ^{18}O-DOM is compared with DOC concentrations for the Harp Lake catchment, environmental differences become apparent (Fig. 30). To assess whether δ^{18}O is an indicator of DOM decomposition, δ^{18}O-DOM, molecular size, and DOC concentrations will be discussed in context of sources of DOM and alteration along hydrologic flowpaths.

Upland Streams and Groundwater in Harp Lake catchment

Upland streams such as Harp 4-21 have a lower molecular weight lower δ^{18}O-DOM signature and lower DOC concentrations than wetland streams (Fig. 29, 30). The source of DOM in these streams is the forest floor and upper soil horizons. However, this DOM has been extensively reworked in the upper LFH horizon, and the groundwater contains DOM of low molecular weight (Schiff et al., 1990). Studies of δ^{14}C in DOM show that this DOM consists of "old" organic matter (Schiff et al., 1990), and is most likely refractory, since it is very degraded.

The deep groundwater is simply a flowpath continuation of shallow groundwater. Since deep groundwater is further along the hydrologic flowpath, it would consist of DOM which has been further degraded. The decrease in both δ^{18}O-DOM and molecular size (Fig. 29) are consistent with this further alteration of DOM.

Upland streams are fed principally by groundwater, but DOM may be added from shallow organic horizons depending on antecedent moisture and groundwater flowpaths. Hinton (1998) showed that most of the DOC export in the upland Harp 4-21 originated in the shallow organic-rich soils adjacent to the stream and dependent upon flow conditions. Since DOC concentrations in Harp 4-21 (2.02-3.67 mg/L) are elevated in comparison to shallow groundwater (0.42-1.23 mg/L), a portion of the DOM could be derived from these shallow organic horizons. This agrees with δ^{14}C results which show that δ^{14}C varies from old baseflow under dry antecedent conditions to new at high discharges (Schiff et al., 1997).

This could explain the enriched δ^{18}O-DOM and relative molecular weight of the Harp 4-21 April sample when compared to groundwater.

Wetland Streams in the Harp Lake Catchment

Most catchments in the Dorset area contain wetlands, which are the dominant source of DOM in the Harp Lake catchment (Dillon & Molot, 1997). Based on δ^{14}C studies, wetland DOM is derived from the first 50cm of peat in the wetlands, and usually consists of recently fixed "young" carbon (Schiff et al., 1990, Schiff et al., 1997). This DOM has typically undergone little alteration, and consists of a large portion of complex macromolecules such as humic substances (Thurman, 1985).

Concentrations of DOC from wetlands in catchments on the Canadian Shield are controlled by hydrologic flowpaths within the wetland (Schiff et al., 1997). DOM derived from surface of the wetland is generally less decomposed, while DOM from the lower layers of the wetland would be the opposite. Variable DOC concentrations in the wetland streams could indicate the sources of DOM within the wetlands in Harp Lake catchment are different (Fig. 30).

DOM from wetland streams has the highest and most variable in relative molecular weights, DOC concentrations, and δ^{18}O-DOM signatures in the Harp Lake catchment (Fig. 29). The δ^{18}O-DOM could be a measure of alteration or source of DOM.

Harp Lake

Harp Lake is a net sink for DOM (Dillon & Molot, 1997). Most of the DOM in Harp Lake is derived from wetland streams, which has a relatively enriched δ^{18}O-DOM and a higher relative molecular weight (the largest sub-catchments in the basin have wetlands, Devito et al., 1999).

There could also be a significant input of δ^{18}O-DOM from autochthonous DOM. If zooplankton were used as a proxy signal for δ^{18}O of autochthonous organic matter, then the

allochthonous input would have an $\delta^{18}O$ of +15‰. Also, it would predominantly comprise lower molecular weight compounds, since it is primarily produced from algae (Thurman, 1985). Therefore, autochthonous input to the lake would add enriched $\delta^{18}O$-DOM and low relative molecular weight. The $\delta^{18}O$-DOM in Harp Lake is relatively depleted despite receiving both allochthonous and autochthonous DOM with relatively enriched $\delta^{18}O$ (Fig. 29).

Harp Lake has a residence time of 2 years, and the DOM received from streams in the catchment is subject to prolonged alteration by UV decomposition and microbial degradation (Thurman, 1985). Photodegradation of DOM within lakes breaks bonds in the larger macromolecules to create smaller, more biologically labile compounds (Moran & Zepp, 1997). The photodegradation process, or subsequent biological degradation, results in a depletion of $\delta^{18}O$-DOM. These values of $\delta^{18}O$-DOM were the lowest observed in this study.

In general, $\delta^{18}O$-DOM and relative molecular weight seems to decrease from DOM source areas as a result of alteration/degradation. If $\delta^{18}O$-DOM can be a measure of the degree of alteration of DOM, then the most depleted signatures would be from environments containing the most altered DOM. Also, the most enriched signatures would be from the sources areas of DOM. The most depleted samples in the sample set are the deep groundwater and lake, and are environments that typically comprise the most altered DOM. The most enriched samples are derived from wetlands, environments that are large sources of DOM.

Plastic Lake Catchment

The upland stream of the Plastic Lake catchment (PC1-08) fits the $\delta^{18}O$ vs. molecular weight relationship observed in the Harp Lake catchment (Fig. 31). This is significant since vegetation at this site is very different from the Harp Lake catchment, consisting mainly of coniferous trees. Therefore, processes which occur to deplete $\delta^{18}O$-DOM in the Plastic Lake uplands are the same or similar to those in the Harp Lake catchment.

The samples from Plastic swamp are shifted toward both higher and lower $\delta^{18}O$ relative to the regression for the Harp Lake catchment (Fig. 31). The vegetation in the Plastic swamp is different than in wetlands at the Harp catchment; the swamp has increased sphagnum content and contains a large proportion of coniferous trees. This increased sphagnum content and different vegetation could export a different DOM than the Harp wetlands.

Additional insight into the differences between wetlands in Harp and Plastic Lake catchments can be attained from a temporal analysis of the Plastic Lake catchment (Fig. 32). Samples from Plastic swamp show a large seasonal component in $\delta^{18}O$-DOM; the lowest values occur in the summer and fall, while highest values occur in the spring.

The variations in $\delta^{18}O$-DOM could be explained by the differences in hydrological flowpaths in the Plastic swamp. During spring, spring melt causes high water tables in the swamp. This results in the release of DOM from the upper layers of the swamp, which is relatively "young", unaltered DOM (Schiff et al., 1990, Schiff et al., 1997). In summer, when water tables decrease, DOM is derived from lower layers in the wetland. These lower layers would consist of peat which is relatively older and its DOM would consist of more altered/decomposed molecules (Schiff et al., 1990, Schiff et al., 1997). In fall, the water levels rise because of decreased evapotranspiration and increased precipitation, and the hydrologic flowpath would be shallower, thereby releasing "newer", unaltered DOM.

Conceptual Model for $\delta^{18}O$-DOM

A conceptual model of $\delta^{18}O$ can be developed for the Harp and Plastic Lake catchments, incorporating leaf leachates and $\delta^{18}O$-DOM of PC1-08 (Fig. 33, 34).

As the DOM moves through different hydrologic flowpaths in the catchment, the $\delta^{18}O$-DOM reflects the alteration of DOM (Fig. 33). Environments that are sources of DOM (forest floor, wetlands) show the most enriched $\delta^{18}O$-DOM. The environments with the most depleted $\delta^{18}O$-DOM are those which typically contain the most altered/decomposed altered DOM (lake, groundwater). This is probably due to decomposition or some other alteration

process occurring in the various environments through which the DOM is transported, thereby alters the $\delta^{18}O$.

5.5 Summary and Conclusions

In Harp and Plastic Lake catchments, the $\delta^{18}O$-DOM varies both by environment and by season. Wetland streams show the largest range in $\delta^{18}O$-DOM, while uplands, groundwater, and Harp Lake are the least varied. The most depleted samples are from groundwater and Harp Lake.

The DOM from all samples in the Harp and Plastic Lake catchments has been subject to some sort of alteration. In the Harp Lake catchment, $\delta^{18}O$-DOM is highly correlated with relative molecular weight. It is possible $\delta^{18}O$-DOM could be an indicator of DOM alteration. Relative molecular size has been shown to decrease with increasing alteration, and $\delta^{13}C$ increases with increasing alteration. Changes in $\delta^{18}O$-DOM therefore could be a reflection of the magnitude of alteration. The $\delta^{18}O$-DOM from these samples is consistently lower than both leaf leachates and peat value (23.6-25.4‰, 14-17‰), supporting this hypothesis.

DOM from wetlands is the least altered, since it has a relatively enriched $\delta^{18}O$-DOM and high relative molecular weight. Uplands, groundwater, and Harp Lake show a depleted $\delta^{18}O$-DOM with lower molecular weights, indicating more altered DOM. The $\delta^{18}O$-DOM in Harp Lake is the most depleted, because of high residence times in the lake subjecting the DOM to prolonged UV decomposition and microbial degradation.

Hydrology of wetlands appears to have a large control on the $\delta^{18}O$-DOM of wetland streams. Results show $\delta^{18}O$-DOM from wetlands to be temporally variable, likely due to differing water levels in the wetland over the hydrologic year. Also, the $\delta^{18}O$-DOM from Harp and Plastic Lake wetlands appears to differ, with Plastic swamp showing a much higher $\delta^{18}O$-DOM. This difference could be a product of differing vegetation types.

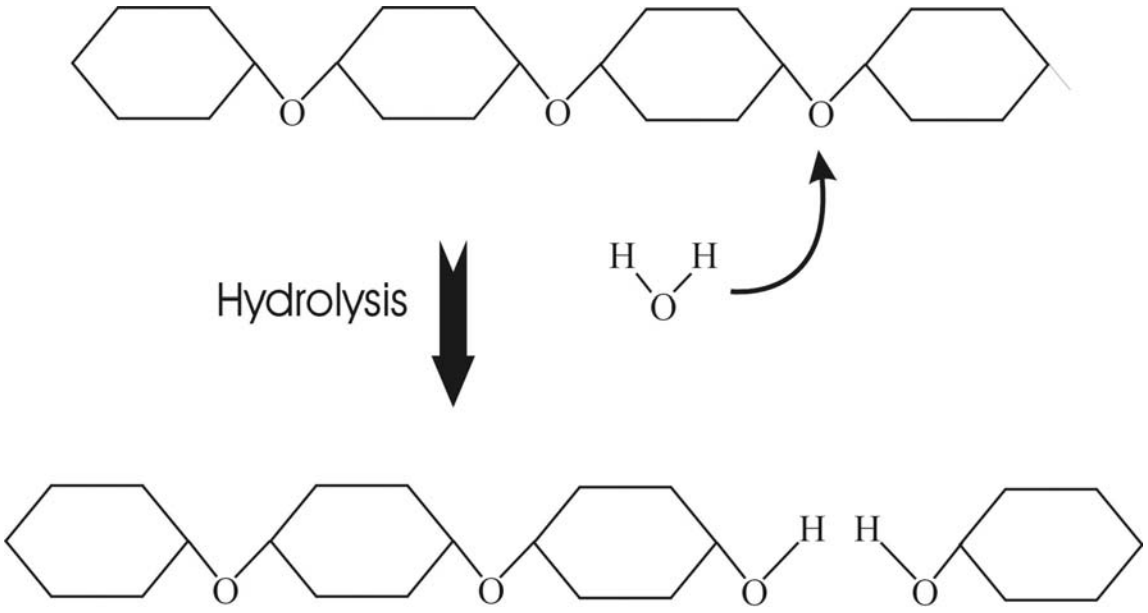

Figure 23a: Generalized diagram of the hydrolysis of a complex molecule.

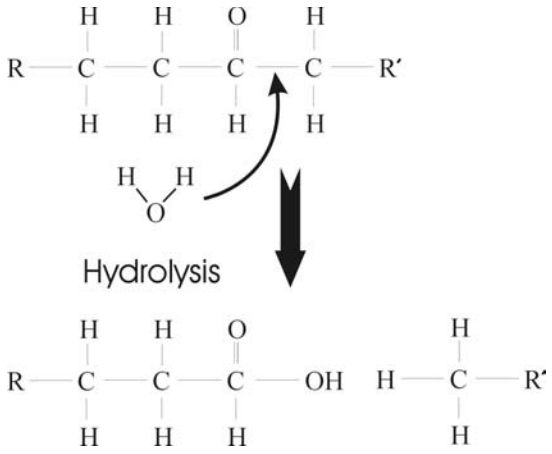

Figure 23b. Hydrolysis of carboxylic acids, which could be important in fulvic acids (Thurman, 1985).

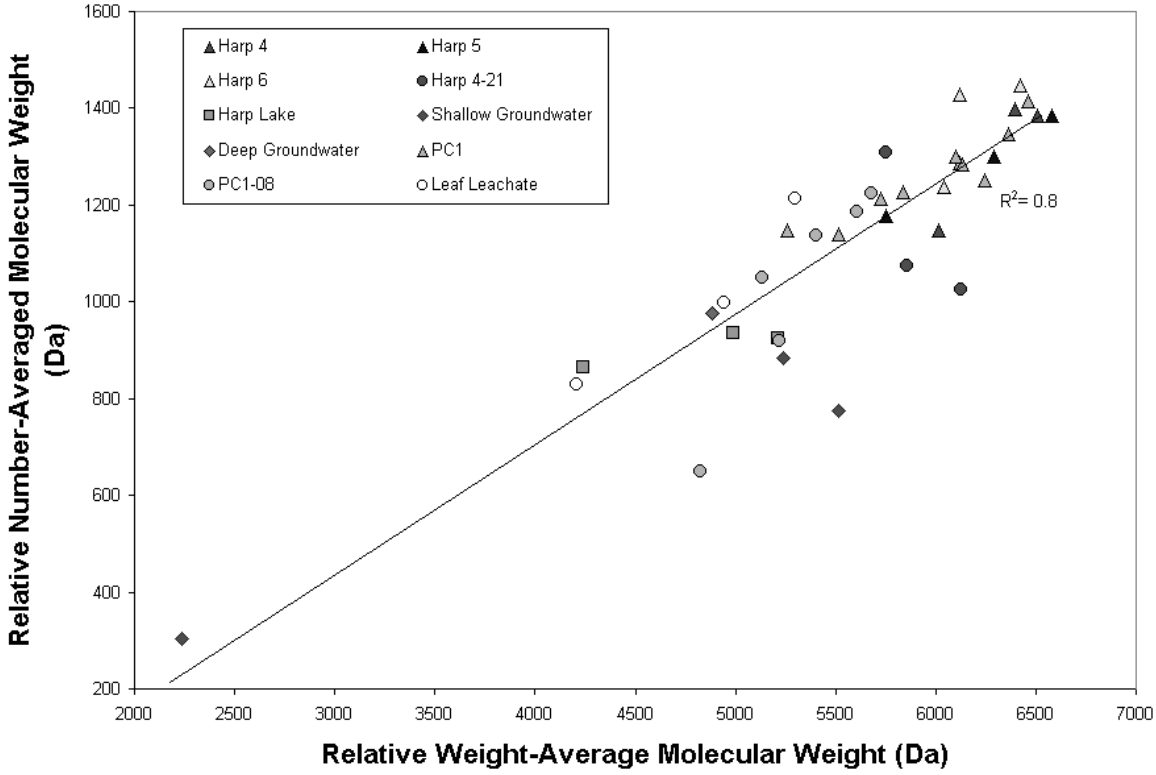

Figure 24. Comparison of Mn and Mw. The two averages are similar and show a good correlation. Relative weight averaged molecular weight from Wu (unpublished, 2002).

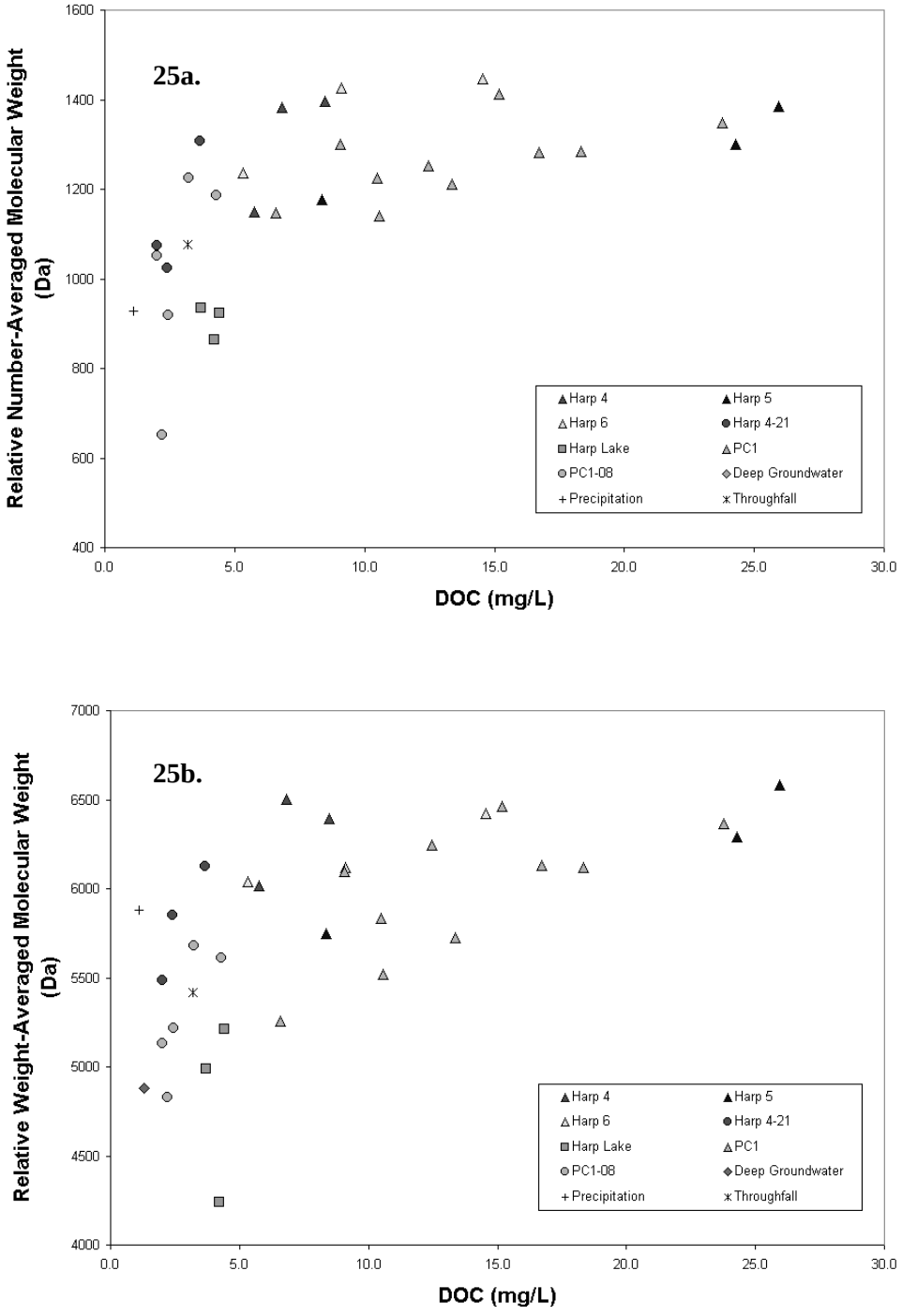

Figure 25a,b. Comparison of relative number-averaged molecular weight and weight-averaged molecular weight to DOC concentrations. Relative weight averaged molecular weight from Wu (unpublished, 2002).

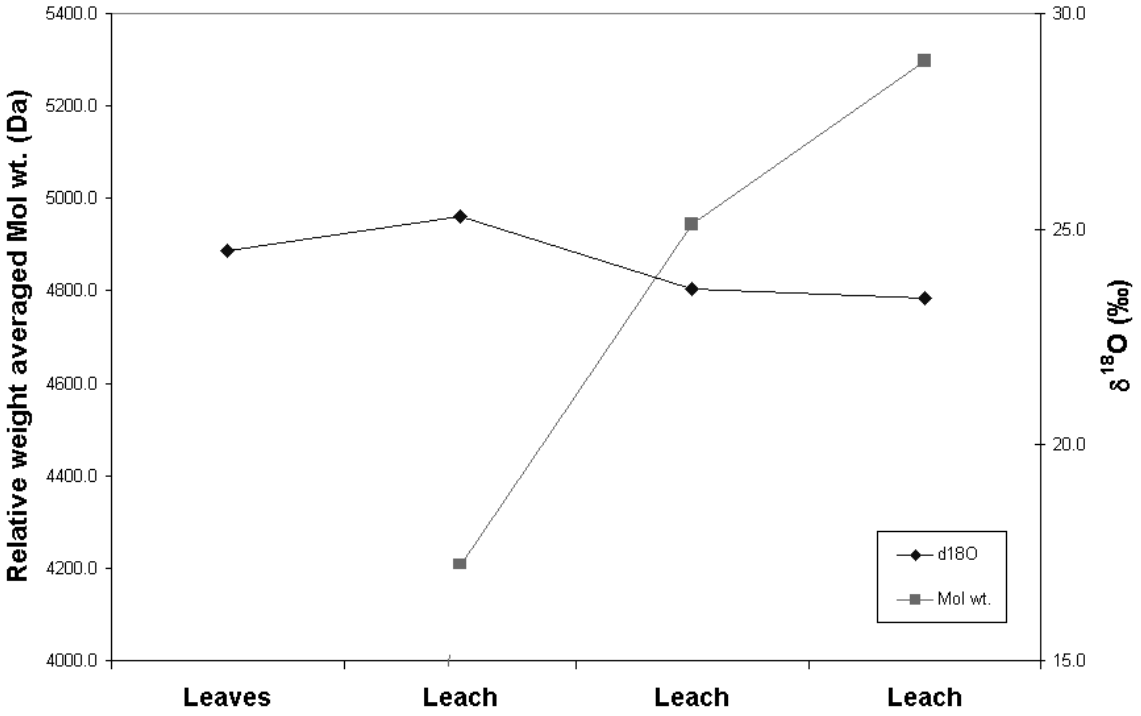

Figure 26. Progressive leaf leachates show decreased $\delta^{18}O$ values and increased relative molecular weights. Relative weight averaged molecular weight from Wu (unpublished, 2002).

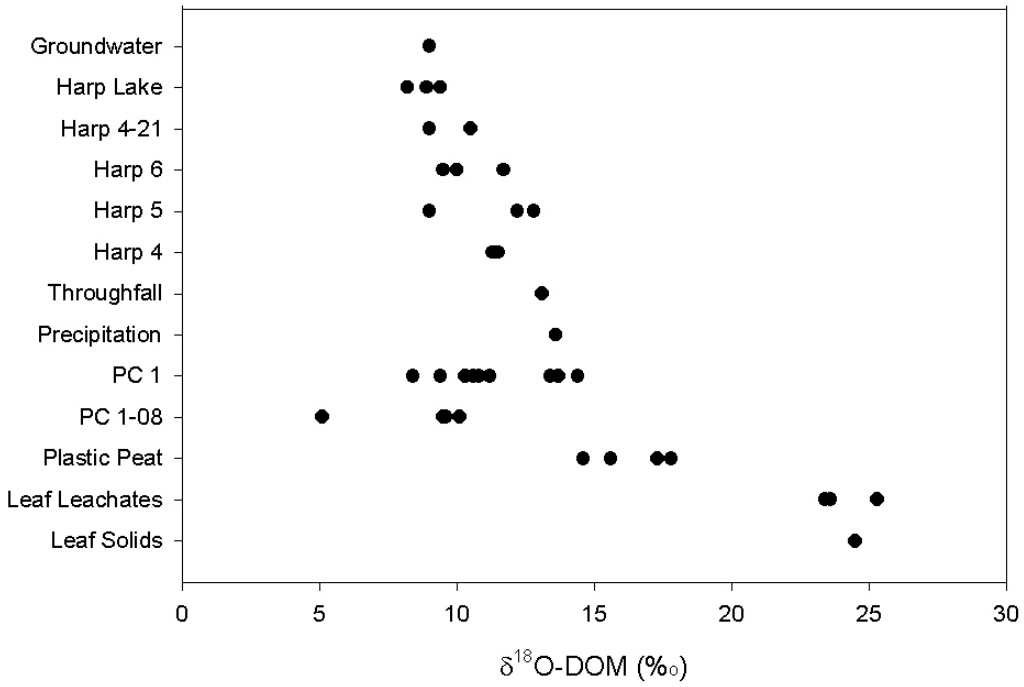

Figure 27. Values of $\delta^{18}O$ for the Harp and Plastic Lake catchments.

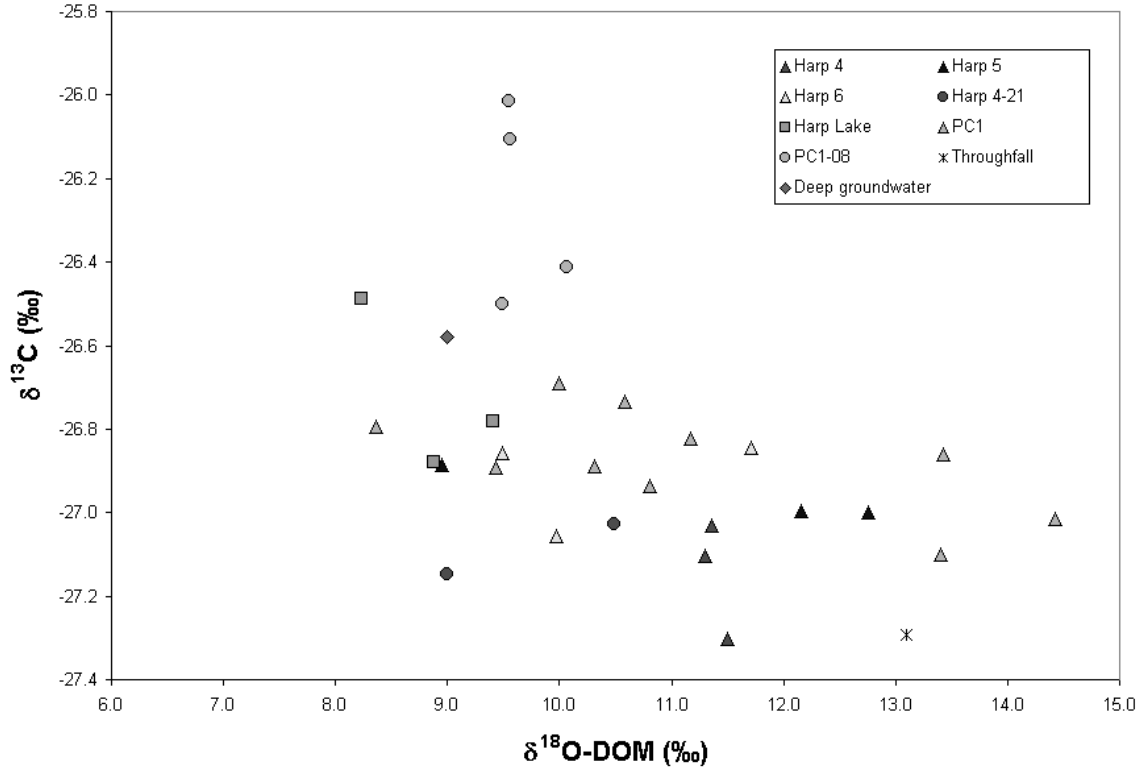

Figure 28. $\delta^{13}C$ and $\delta^{18}O$ for the Harp and Plastic Lake catchments. As $\delta^{13}C$ is depleted, ^{18}O is more enriched, supporting the hypothesis of

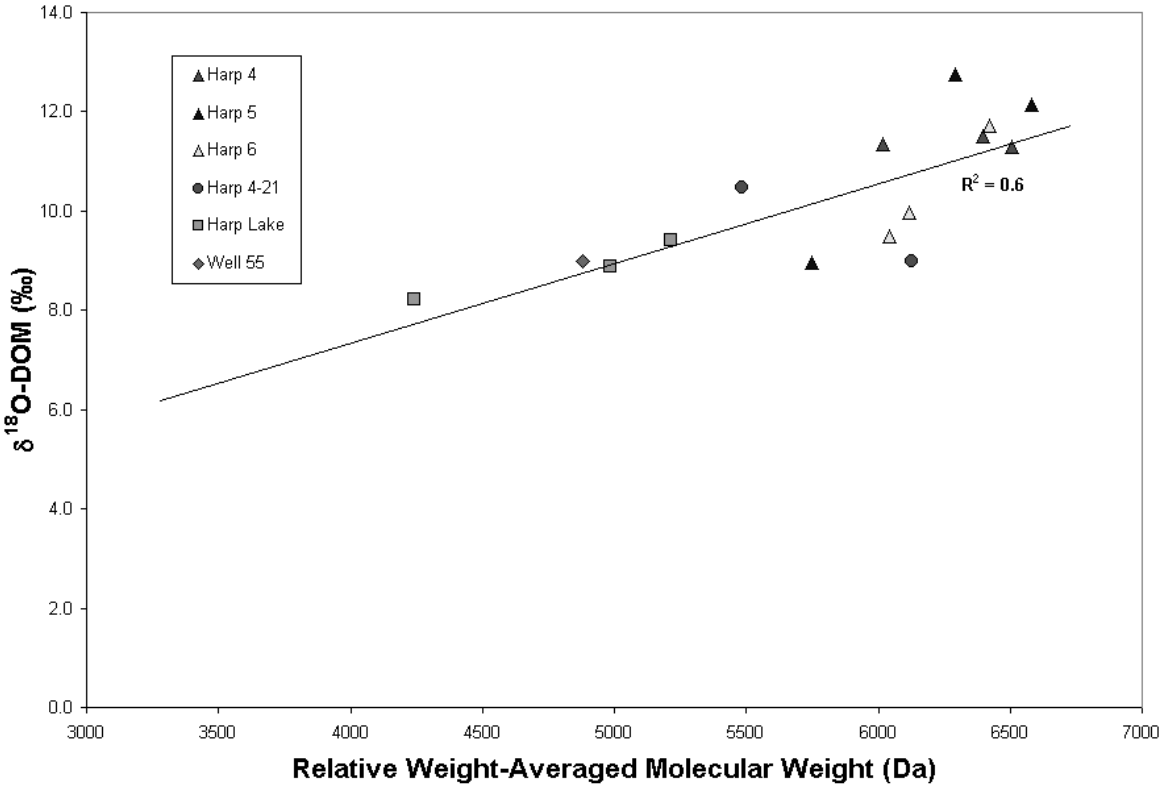

Figure 29. Relative molecular weights and $\delta^{18}O$ by sample in the Harp Lake catchment. The samples from this catchment are linearly correlated. Relative weight averaged molecular weight from Wu (unpublished, 2002).

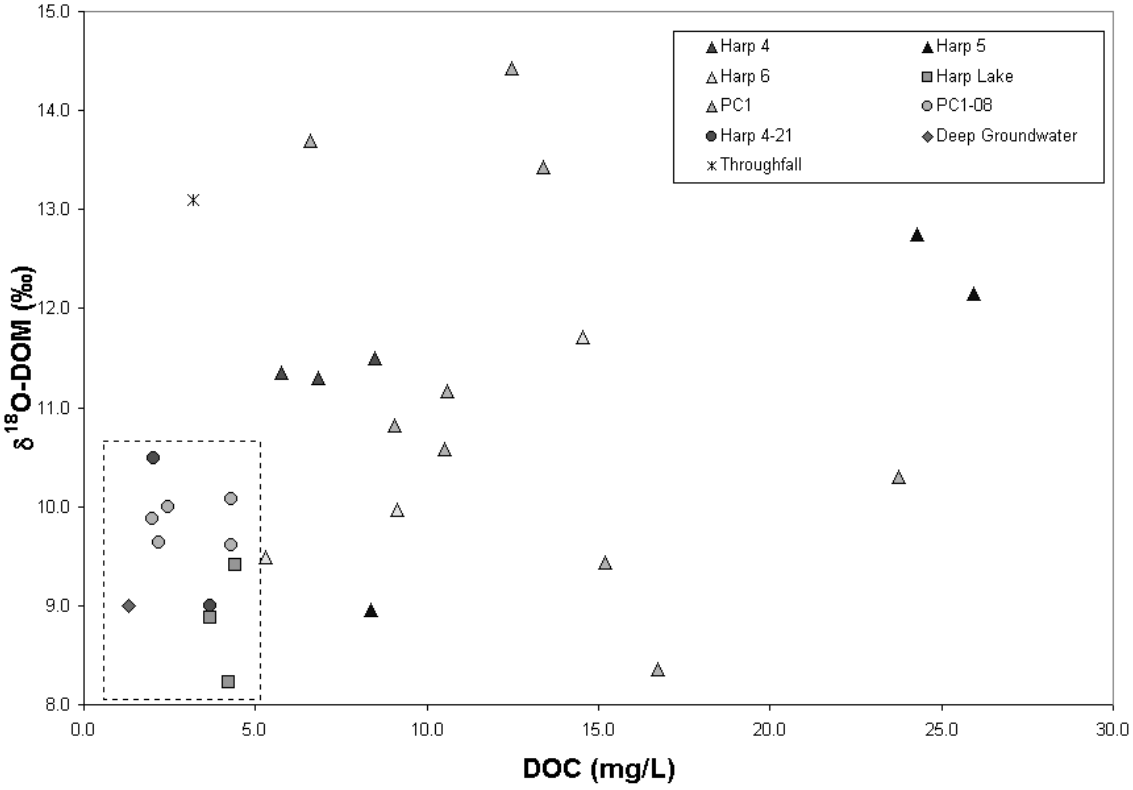

Figure 30. Environmental differences in δ^{18}O-DOM and DOC concentrations for the Harp and Plastic Lake catchments. Upland streams, groundwater, and Harp Lake vary little, plotting in the box, while wetland streams differ greatly.

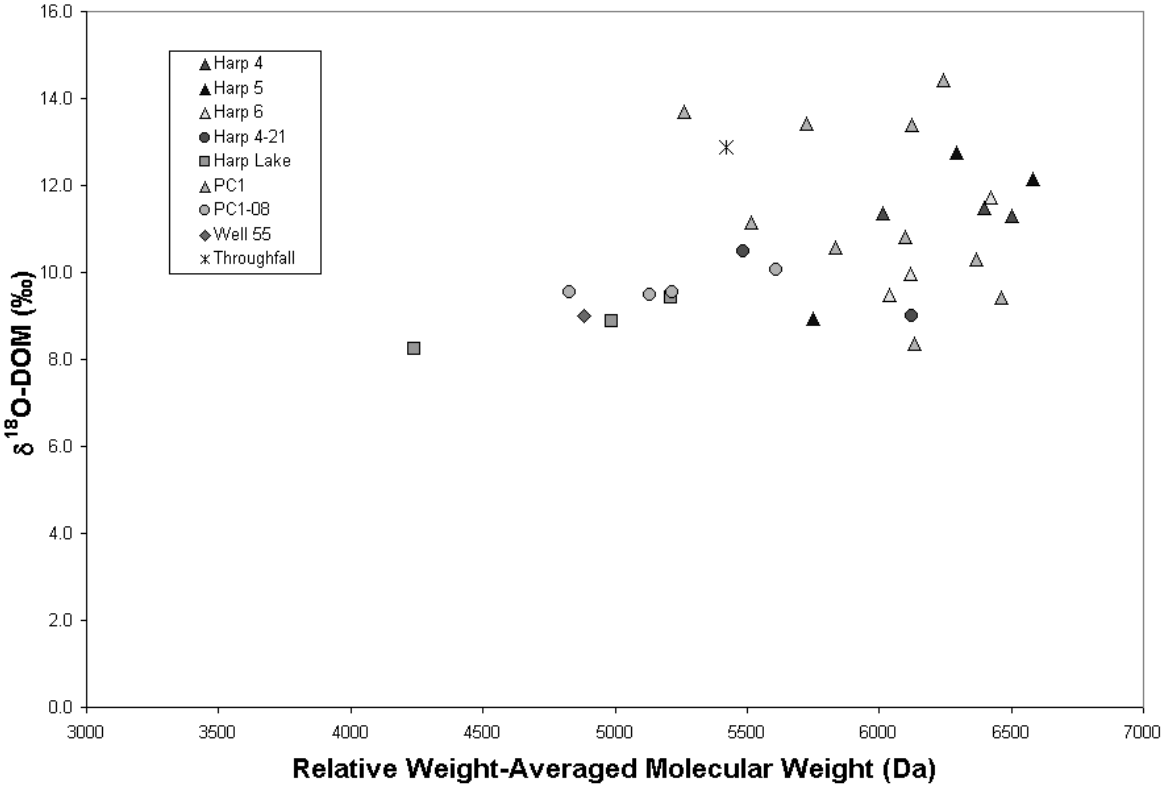

Figure 31. Relative molecular weights and $\delta^{18}O$ by sample for the Harp and Plastic Lake catchments. Samples from PC1-08 follow the regression from the Harp Lake catchment, while samples PC1 deviate from this regression. Relative weight averaged molecular weight from Wu (unpublished, 2002).

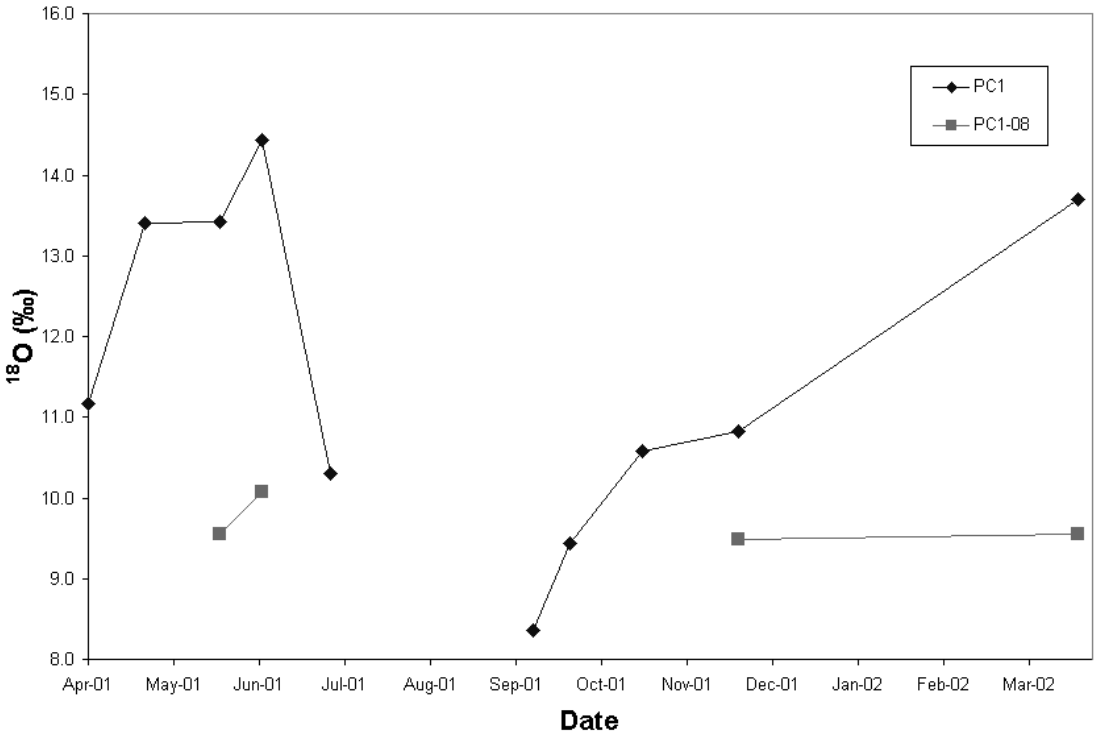

Figure 32. Seasonal δ^{18}O-DOM for the Plastic Lake catchment over the hydrologic year. Input (PC1-08) into Plastic swamp varies little, but output from the swamp is highly variable and appears to be dependent upon hydrological conditions.

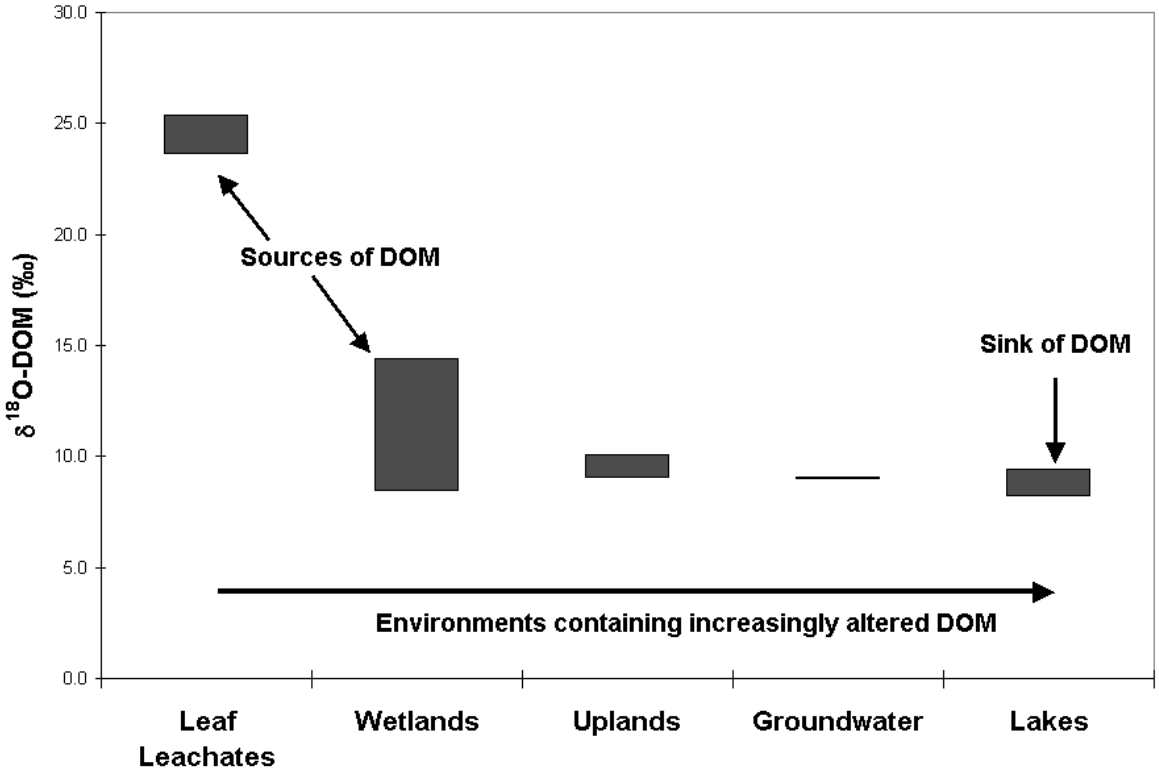

Figure 33. Conceptual model for δ^{18}O-DOM for the Harp Lake catchment and PC1-08 (excluding PC1-08 Nov 2/01). The δ^{18}O-DOM is much greater in sources of DOM such as leaf leachates and wetlands than in environments containing altered and reworked DOM, such as groundwater and lakes.

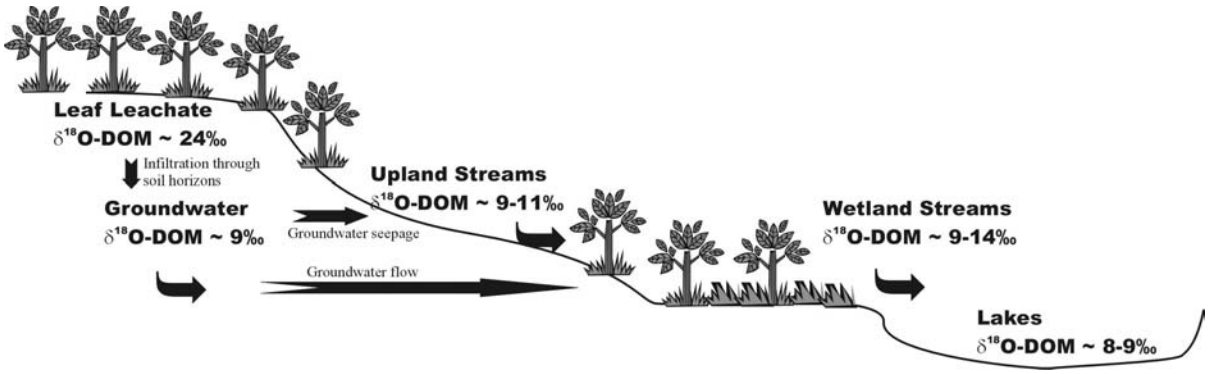

Figure 34. Generalized conceptual model of δ^{18}O-DOM for Precambrian Shield catchments. As DOM moves through the hydrologic flowpath, δ^{18}O-DOM is depleted in environments with the most altered DOM. Large differences occur along the hydrologic flowpath.

Chapter 6: Summary, Conclusions and Recommendations

6.1 Summary

Dissolved organic matter is present in all forested catchments, and can be important in binding metals, absorbing UV, and the transport of nutrients (C, N, S, O). Because of the heterogeneity in sources in the catchment and the number of constituent compounds, DOM is difficult to characterize. Therefore, knowledge of the processes that affect DOM composition is limited. Information from $\delta^{34}S$ and $\delta^{18}O$ in DOM in this research provides valuable insight into sources and sinks of DOM within the forested catchment.

New Techniques for the determination of $\delta^{34}S$-DOM and $\delta^{18}O$-DOM

Data generated for $\delta^{34}S$-DOM and $\delta^{18}O$-DOM in this thesis appears to be the first data reported in the literature for DOM. Since there was no data found in the literature hitherto, new techniques had to be developed in order to analyse for $\delta^{34}S$ and $\delta^{18}O$ in DOM. An isolation procedure was designed to isolate DOM from sulphate and nitrate, thereby enabling the removal of inorganic S and O from the sample. This procedure involved the concentration of organic matter by reverse osmosis and subsequent removal of sulphate by precipitation of barium sulphate. Nitrate (if present in appreciable quantities) is removed by dialysis. Steps were taken to ensure the maximum recovery of organic matter. Standards and duplicates were used to verify that there was no alteration of the original $\delta^{34}S$ and $\delta^{18}O$ in DOM.

Samples takes from the Harp and Plastic Lake catchments were subject to isolation procedures and analysed for $\delta^{34}S$-DOM and $\delta^{18}O$-DOM. In addition to $\delta^{34}S$-DOM and $\delta^{18}O$-DOM, C/S ratios, $\delta^{34}S$-SO_4^{2-}, and DOC and SO_4^{2-} concentrations were analysed for each sample.

Sulphur in Harp and Plastic Lake Catchments

In the Harp and Plastic Lake catchments, both inorganic and organic sulphur cycling are dynamic and complex. Information about various processes causing sulphur transformations within the catchment can be inferred from sulphate concentrations, $\delta^{34}S\text{-}SO_4^{2-}$, $\delta^{34}S\text{-}S_{org}$, and C/S ratios of dissolved organic matter.

The inorganic ($\delta^{34}S\text{-}SO_4^{2-}$) and organic S ($\delta^{34}S$-DOM) differs by environment in both catchments. Sulphate in the Harp Lake catchment in most samples is subject to some sort of cycling within the watershed, since $\delta^{34}S\text{-}SO_4^{2-}$ differs from precipitation. The $\delta^{34}S$-DOM appears to be dependent on the source of DOM and the subsequent alteration.

Streams draining upland catchments show both different inorganic and organic S signatures than wetland streams and Harp Lake. Harp 4-21 contains sulphate, which appears to be derived from historical sulphate deposition by precipitation. The depleted $\delta^{34}S\text{-}SO_4^{2-}$ and higher sulphate concentrations are likely due to groundwater residence times. The $\delta^{34}S$-DOM in upland catchments (both Harp 4-21 and PC1-08) seems to originate from $\delta^{34}S$ of vegetation. This vegetation forms the forest floor and organic matter in the upper horizons of the soil and is leached into the upland stream by interflow and/or groundwater flow.

In wetland streams, both sulphate and DOS appear to be controlled by hydrology. Wetland streams show a large seasonal variability in $\delta^{34}S$-DOM, $\delta^{34}S\text{-}SO_4^{2-}$, sulphate concentrations, and C/S ratios. Hydrologic flowpaths in the wetland affect the amount of sulphate subject to DSR in the wetland, in turn affecting $\delta^{34}S\text{-}SO_4^{2-}$. Varying hydrologic flowpaths in the wetland also appear to alter the $\delta^{34}S$-DOM of the output from the wetland. Higher water tables leach fresh organic material in the upper horizon of the wetland, resulting in enriched $\delta^{34}S$-DOM. DOM derived from porewater in the swamp during low flow conditions is depleted in $\delta^{34}S$-DOM, possibly from peat which is depleted.

Sulphate from samples in Harp Lake shows similar concentrations and $\delta^{34}S\text{-}SO_4^{2-}$ to precipitation, which contrasts with the rest of the samples taken from the catchment. This similarity in $\delta^{34}S\text{-}SO_4^{2-}$ to precipitation is despite input from streams which are enriched in $\delta^{34}S\text{-}SO_4^{2-}$. It is hypothesized that the lake could derive input $\delta^{34}S\text{-}SO_4^{2-}$ either from precipitation, or from DOS mineralization within the lake itself. The input from both of these sources would cause the $\delta^{34}S\text{-}SO_4^{2-}$ of the lake to be more similar to precipitation. Both $\delta^{34}S$-DOM and DOS concentrations suggest that mineralization in Harp Lake could occur, which would deplete the $\delta^{34}S\text{-}SO_4^{2-}$ in the lake.

Oxygen in Dissolved Organic Matter in Harp and Plastic Lake Catchments

The $\delta^{18}O$-DOM in Harp and Plastic Lake catchments varies both by environment and by season. Wetland streams show the largest range in $\delta^{18}O$-DOM, while uplands, groundwater, and Harp Lake are the least varied. The highest $\delta^{18}O$-DOM values are from sources of DOM such as leaf leachates (representative of forest floor litter) and wetlands. The most depleted samples are from groundwater and Harp Lake which typically contain highly altered DOM.

It is possible $\delta^{18}O$-DOM could be an indicator of DOM alteration. The $\delta^{18}O$-DOM in the Harp Lake catchment is highly correlated with relative molecular weight, which has been shown to decrease with increasing alteration. Therefore, the changes in $\delta^{18}O$-DOM by environment could be a reflection of the magnitude of alteration. The $\delta^{18}O$-DOM of samples in the Harp Lake catchment is consistently lower than both leaf leachates and peat value (23.6-25.4‰, 14-17‰), supporting this hypothesis.

The DOM from wetlands is the least altered, since it has a relatively enriched $\delta^{18}O$-DOM and high relative molecular weight. Uplands, groundwater, and Harp Lake show a depleted $\delta^{18}O$-DOM with lower molecular weights, indicating more altered DOM. The $\delta^{18}O$-DOM in Harp Lake is the most depleted, because of high residence times in the lake subjecting the DOM to prolonged UV decomposition and microbial degradation.

Hydrology of wetlands appears to have a large control on the δ^{18}O-DOM of wetland streams. Results show δ^{18}O-DOM from wetlands to be temporally variable, likely due to differing water levels in the wetland over the hydrologic year. Also, the δ^{18}O-DOM from Harp and Plastic Lake wetlands appears to differ, with Plastic swamp showing a more varied δ^{18}O-DOM. This difference could be a product of differing vegetation types.

6.2 Conclusions

The δ^{34}S-DOM and δ^{18}O-DOM can provide valuable information on sources of DOM and DOM alteration within the catchment. When δ^{34}S-DOM and δ^{18}O-DOM are compared (Fig. 35), samples can be separated by environment. The samples from the lake and uplands approximately range between 8‰ and 10‰ for δ^{18}O-DOM and between 5.8‰ and 7.2‰ for δ^{34}S-DOM.

Both δ^{34}S-DOM and δ^{18}O-DOM vary seasonally in wetlands, which is driven by hydrology within the wetland. Information from δ^{34}S-DOM and δ^{18}O-DOM in wetland streams can aid in the differentiation of sources of DOM within the wetland.

6.3 Recommendations for Research

This research has provided some insight into a new field of research, and could be taken forward in a number of directions. Recommendations for further study are divided into two parts. The first sets of recommendations are directly related to this study, and are suggestions to make the dataset more complete. The second set of recommendations consist of suggestions for areas of further study, and directions for future research.

Recommendations for Current Research

To fill in gaps in the data for this particular study, it is recommended that both δ^{34}S and δ^{18}O of vegetation from Plastic Lake should be determined. Samples of vegetation should consist of coniferous pine needles and Sphagnum from Plastic swamp (at a very minimum).

After these samples are collected, it is recommended that vegetation be leached, similar to that of the leaves from Harp 6A. The leachates should then be analysed for both $\delta^{34}S$ and $\delta^{18}O$.

Due to time constants, there were a number of samples which could not be analysed for both $\delta^{34}S$-DOM and $\delta^{18}O$-DOM, such as shallow groundwater. It is critical that these samples be run in order to complete the dataset. Also, additional samples of zooplankton and phytoplankton should be run for $\delta^{18}O$. This would be useful in determining any possible trophic effects in $\delta^{18}O$, and allow a better estimate of the $\delta^{18}O$ of autochthonous DOM.

The standards used in $\delta^{18}O$ analysis consisted of cellulose ranging from +20 to +30‰. Most $\delta^{18}O$-DOM samples in this research were below these standards (8-14‰). Therefore, the correction curve for $\delta^{18}O$ is extrapolated to determine the $\delta^{18}O$ of the samples in this study. The Environmental Isotope Laboratory at the University of Waterloo recently purchased organic standards for $\delta^{18}O$ and %O. Currently, these standards are currently being verified, and will possibly be used as standards in the future analyses, without the need for extrapolation of the correction curve.

Verification of seasonal and environmental trends of sites in this study is recommended. Samples of precipitation and deciduous throughfall are good places to start analysis, but it is recommended that other samples be collected as well to enable a wider scope.

Recommendations for Future Research

In this research, there were a number of problems which hindered the analysis of $\delta^{34}S$-DOM and $\delta^{18}O$-DOM. Excess salt in samples caused problems in the burning of samples in the Elemental Analyzer. An outcome of this problem was a shortened life of the tube in the machine, which led to problems with drift in the machine.

If possible, the salts added to the concentrated solution should be at a minimum. Ways to achieve this could be: 1) developing an improved organic precipitation step; 2) dialysis of

the sample after barium sulphate precipitation to try remove excess salt; or 3) utilization of a Parr bomb to remove salt.

If excess salt cannot be removed from samples, it is recommended that the interference of these salts with the δ^{34}S-DOM and δ^{18}O-DOM be quantified. This could be done by the addition of salts to organic δ^{34}S and δ^{18}O standards, and examining the burn of the standard. Another problem with δ^{34}S-DOM and δ^{18}O-DOM analyses is that there are little to no isotopic organic standards by which to compare samples. It is recommended, therefore, that these standards be created until such standards are available.

New field sites could also be investigated in the future, and their results could be compared with this study. For instance, forested catchments such as Turkey Lakes (containing a large amount of sugar maple) and the Experimental Lakes area (Boreal forest) have differing vegetation from catchments in the Dorset area. Thus, these catchments could potentially have different δ^{34}S-DOM and δ^{18}O-DOM than this study.

Further investigation of sulphur and oxygen in DOM will lead to an increased knowledge in the fields of sulphur cycling and DOM alteration in forested catchments.

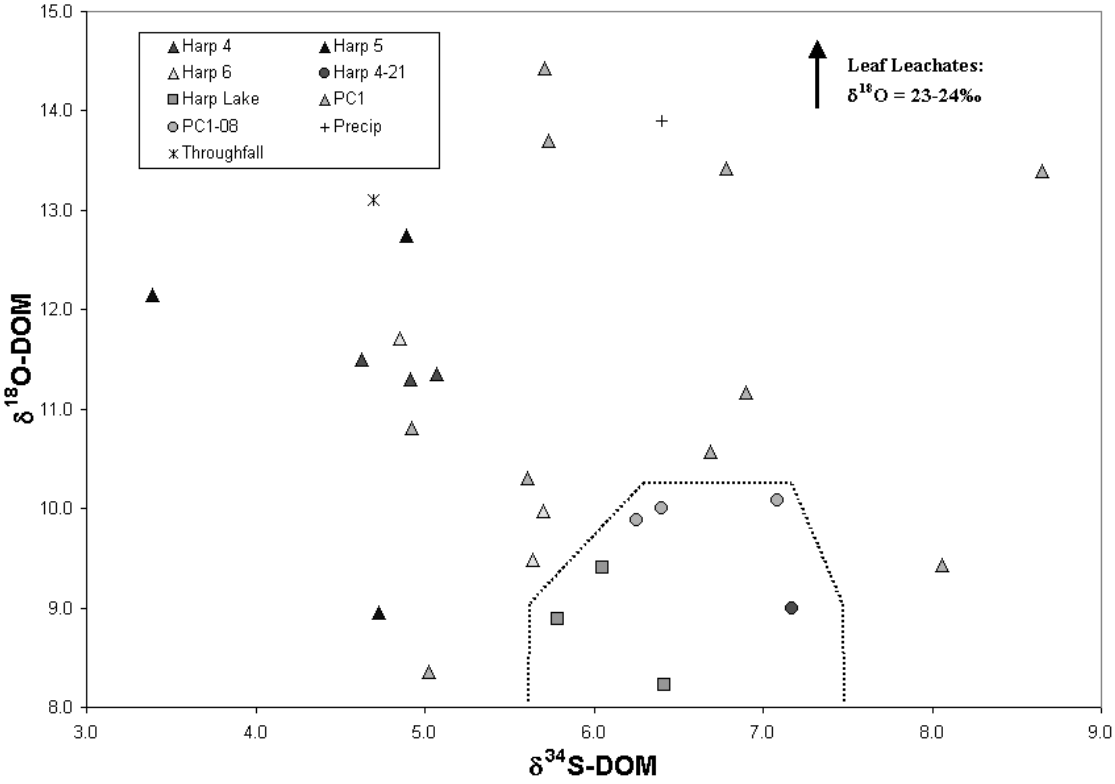

Figure 34. Environmental differences in DOM can be seen when plotting δ^{18}O-DOM with δ^{34}S-DOM. Boxed area contains upland streams and Harp Lake.

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Appendix A: Dialysis Experiments

Below is data used for Figure 5:

Experiment 1:

100D Spectra-por membrane, not washed with Extran

106ppm of SO_4^{2-} as K_2SO_4, mass = 5.8mg

Time (hrs)	SO_4^{2-} (mg/L)	Mass removed (mg)	% of orig.	Cum %
3.5	0.1	0.1	1.9	1.9
7.5	0.0	0.1	0.9	2.8
17.5	0.1	0.1	1.4	4.2
23.0	0.1	0.1	1.5	5.8
30.5	0.0	0.1	1.0	6.7
41.5	0.1	0.1	1.4	8.1
52.0	0.1	0.1	1.3	9.4
67.5	0.1	0.1	1.6	11.0
178.0	0.3	0.4	7.5	18.5
	Sum	1.1		

Retentate = 86mg/L, mass = 4.7mg (81% of original)

Experiment 2:

100D Spectra-por membrane, not washed with Extran

41ppm of SO_4^{2-} as K_2SO_4, mass = 2.07mg

Time (hrs)	SO_4^{2-} (mg/L)	Mass removed (mg)	% of orig.	Cum %
3.5	0.0	0.0	1.6	1.6
8.0	0.0	0.0	0.0	1.6
18.0	0.0	0.1	2.9	4.4
23.2	0.0	0.0	0.8	5.2
30.5	0.0	0.0	1.1	6.3
42.0	0.0	0.0	0.8	7.1
52.5	0.0	0.0	2.0	9.1
68.0	0.0	0.0	1.6	10.7
178.5	0.1	0.1	5.0	15.7
	Sum	0.33		

Retentate = 38.7mg/L, mass = 1.92mg (92.5% of original)

Experiment 3:

500D Spectra-por membrane, not washed with Extran

106ppm of SO_4^{2-} as K_2SO_4, mass = 6.92mg

Time (hrs)	SO_4^{2-} (mg/L)	Mass removed (mg)	% of orig.	Cum %
18.0	0.1	0.1	1.2	1.2
67.8	0.2	0.2	3.1	4.3
89.5	0.1	0.1	2.1	6.3
121.5	0.1	0.1	1.9	8.3
145.5	0.1	0.1	1.1	9.4
170.0	0.1	0.1	1.1	10.5
Sum		0.73		

Retentate = 88.5mg/L, mass = 5.76mg (83.3% of original)

Experiment 4:

100D Spectra-por membrane, washed with Extran

100.0ppm of SO_4^{2-} as K_2SO_4, mass = 5.58mg

Time (hrs)	SO_4^{2-} (mg/L)	Mass removed (mg)	% of orig.	Cum %
22.8	0.1	0.2	2.9	2.9
48.0	0.1	0.1	2.5	5.4
62.5	0.4	0.6	10.8	16.2
88.5	0.2	0.3	4.7	20.9
184.0	0.4	0.5	8.7	29.6
240.0	0.7	0.9	17.0	46.6
Sum		2.6		

Retentate = 46.9mg/L, mass = 2.62mg (46.9% of original)

Experiment 5:

500D Spectra-por membrane, washed with Extran

100.0ppm of SO_4^{2-} as K_2SO_4, mass = 5.58mg

Time (hrs)	SO_4^{2-} (mg/L)	Mass removed (mg)	% of orig.	Cum %
22.8	0.5	0.7	12.5	12.5
48.0	0.3	0.4	7.5	20.0
62.5	0.2	0.2	3.8	23.8
88.5	0.2	0.2	4.4	28.1
184.0	0.2	0.3	5.2	33.4
240.0	0.5	0.7	12.5	45.9
	Sum	2.6		

Retentate = 55.1mg/L, mass = 3.07mg (55.1% of original)

Experiment 6: Macrodialyzer (Spectra-por 500D membrane):

	SO_4^{2-} (mg/L)			
Sample	Original	Retentate	% in Retentate	% Removed
100 ppm K_2SO_4	100.0	88.5	88.5	11.5
40 ppm K_2SO_4	43.0	43.0	100	0
PC-1 Oct 2000	12.0	11.0	91.7	8.3

Appendix B: Experiments for Washing of BaSO4 Precipitate

Washing procedures using different types of salts (Fig 9):

Step	Procedure	Volume (mL)	DOC (mg/L)	Mass (mg)	% of orig
Original	-	100.0	106.0	10.6	100.0
Final after BaSO4 ppt	ppt removed	95.0	92.0	8.7	82.5
1st wash	DI	90.0	4.2	0.4	3.6
2nd wash	DI	89.0	0.5	0.0	0.4
3rd wash	NaOH	81.0	3.1	0.2	2.3
4th wash	HCl	82.0	8.5	0.7	6.6
5th wash	DI	81.0	0.7	0.1	0.6
Balance				0.4	

Typical amount of salts added during the above washing procedure:

		Concentration (mg/L)		Mass (mg)	
Salt	Vol (ml)	Low	High	Low	High
BaCO3	-	-	-	50	400
BaCl2	-	-	-	20	20
HCl	40	43405.4	43405.4	1736.2	1736.2
NaOH	90	4000	20000	360	1800

Examples of 1st wash recoveries of DOC with DI from $BaSO_4$ precipitate

Sample	Orig DOC (mg/L)	Volume	DOC- 1st wash (mg/L)	Volume	% recovery
HP Beaver Pond 1st wash	79.25	323	6.6	150	3.9
HP Lake 6 Jul 1st wash	69.2	371	9.7	150	5.7
PC 1 6 Dec 1st wash	82.62	321	6.3	150	3.5
PC 1 Sept 25 1st wash	176.8	194	10.3	150	4.5
PC 1 June 22 1st wash	135.55	200	9.7	150	5.4
Harp 5 7 Oct 1st wash	235.02	121	7.0	150	3.7
Harp 6 7 Oct 1st wash	131.92	199	12.0	150	6.8

Appendix C: DOC Recovery for Reverse Osmosis Procedure

The following table includes DOC recovery and DOC lost in the RO membrane.

Calculations of recovery in the permeate are done according to:

$$\%\,\text{Lost in Permeate} = \frac{\text{DOC in Permeate}}{\text{Original DOC}} \times 100\%$$

$$\%\,\text{Recovery in retentate} = 100\% - \%\,\text{Lost in Permeate}$$

Sample	Date	Detect?	Value	Orig DOC (mg/L)	% DOC lost	% Recovery
Harp 4	April 22, 2001	nd	0.00	5.74	0.00	100.00
Harp 4	October 7, 2001	nd	0.00	8.48	0.00	100.00
Harp 4	July 6, 2001	nd	0.00	6.83	0.00	100.00
Harp 4-21	July 6, 2001	nd	0.00	2.40	0.00	100.00
Harp 4-21	October 7, 2001	nd	0.00	3.67	0.00	100.00
Harp 4-21	May 1, 2001	nd	0.00	2.02	0.00	100.00
Harp 5	October 7, 2001	nd	0.00	24.28	0.00	100.00
Harp 5	July 6, 2001	nd	0.00	25.95	0.00	100.00
Harp 5	April 22, 2001	nd	0.00	8.35	0.00	100.00
Harp 6	October 7, 2001	nd	0.00	9.11	0.00	100.00
Harp 6	April 22, 2001	nd	0.00	5.31	0.00	100.00
Harp 6	July 6, 2001	nd	0.00	14.54	0.00	100.00
Harp beaver	April 22, 2001	nd	0.00	7.35	0.00	100.00
Harp Lake	July 6, 2001	nd	0.00	4.23	0.00	100.00
Harp Lake	April 22, 2001	nd	0.00	3.69	0.00	100.00
Harp Lake	October 7, 2001	nd	0.00	4.42	0.00	100.00
LFH	June 22, 2001	nd	0.00	2.30	0.00	100.00
PC1	May 12, 2001	nd	0.00	18.35	0.00	100.00
PC1	April 22, 2001	nd	0.00	10.57	0.00	100.00
PC1	June 7, 2001	nd	0.00	13.38	0.00	100.00
PC1	June 22, 2001	nd	0.00	12.47	0.00	100.00
PC1	July 16, 2001	yes	0.06	23.76	0.26	99.74
PC1	September 25, 2001	yes	0.16	16.73	0.96	99.04
PC1	October 8, 2001	yes	0.01	15.20	0.04	99.96
PC1	November 2, 2001	yes	0.29	10.49	2.75	97.25
PC1	December 6, 2001	yes	0.06	9.06	0.61	99.39
PC1	April 4, 2002	nd	0.00	6.60	0.00	100.00
PC1-08	June 7, 2001	yes	0.06	2.45	2.63	97.37
PC1-08	April 4, 2002	yes	0.03	2.20	1.23	98.77
PC1-08	December 6, 2001	nd	0.00	2.01	0.00	100.00
PC1-08	November 2, 2001	yes	0.26	2.76	9.48	90.52
PC1-08	June 22, 2001	nd	0.00	4.30	0.00	100.00
PC1-08	September 25, 2001	nd	0.00	3.23	0.00	100.00
Precipitation	July 1, 2001	nd	0.00	1.10	0.00	100.00
Precipitation	November 1, 2001	nd	0.00	1.60	0.00	100.00
SGW	October 1, 2001	nd	0.00	0.97	0.00	100.00
SGW	April 22, 2001	nd	0.00	0.42	0.00	100.00
SGW	July 1, 2001	nd	0.00	1.23	0.00	100.00
Throughfall	November 1, 2001	nd	0.00	3.20	0.00	100.00

Appendix D: SO_4^{2-} and DOC Concentrations for Reverse Osmosis

The table includes original and final concentrations for SO_4^{2-} and DOC during the Reverse Osmosis procedure:

The concentration of each solute can be calculated by:

$$\text{Concentration of Solutes} = \frac{\text{Final Concentration of Solute}}{\text{Original Concentration of Solute}}$$

		SO_4^{2-} (mg/L)		DOC (mg/L)		Concentration (%)	
Sample	**Date**	**Original**	**Conc.**	**Original**	**Conc.**	**SO_4^{2-}**	**DOC**
Harp 4	April 22, 2001	5.6	67.2	5.7	75.1	12.1	13.1
Harp 4	July 6, 2001	4.5	62.1	6.8	93.9	13.7	13.7
Harp 4	October 7, 2001	5.5	83.5	8.5	115.4	15.1	13.6
Harp 4-21	May 1, 2001	7.3	126.0	2.0	35.2	17.3	17.4
Harp 4-21	July 6, 2001	6.6	151.6	2.4	50.7	23.1	21.1
Harp 4-21	October 7, 2001	8.4	150.0	3.7	62.0	17.9	16.9
Harp 5	April 22, 2001	5.8	69.0	8.3	105.1	11.9	12.6
Harp 5	July 6, 2001	0.9	9.1	25.9	250.1	10.4	9.6
Harp 5	October 7, 2001	7.2	72.6	24.3	235.0	10.0	9.7
Harp 6	April 22, 2001	6.3	79.7	5.3	79.2	12.7	14.9
Harp 6	July 6, 2001	1.0	7.4	14.5	127.7	7.7	8.8
Harp 6	October 7, 2001	12.7	152.1	9.1	131.9	12.0	14.5
Harp beaver	April 22, 2001	4.6	41.1	7.3	69.0	8.9	9.4
Harp Lake	April 22, 2001	5.9	111.7	3.7	74.0	18.8	20.1
Harp Lake	July 6, 2001	5.9	90.6	4.2	69.2	15.3	16.4
Harp Lake	October 7, 2001	5.9	119.5	4.4	86.2	20.1	19.5
LFH	June 22, 2001	9.9	80.5	2.3	17.3	8.2	7.5
PC1	April 22, 2001	4.9	54.4	10.6	117.4	11.2	11.1
PC1	May 12, 2001	2.2	22.5	18.4	219.0	10.2	11.9
PC1	June 7, 2001	3.2	37.8	13.4	150.7	11.9	11.3
PC1	June 22, 2001	3.0	26.8	12.5	135.6	8.9	10.9
PC1	July 16, 2001	1.0	10.5	23.8	194.9	10.6	8.2
PC1	September 25, 2001	14.1	148.7	16.7	176.8	10.6	10.6
PC1	October 8, 2001	7.2	74.9	15.2	161.3	10.4	10.6
PC1	November 2, 2001	6.5	63.0	10.5	97.2	9.7	9.3
PC1	December 6, 2001	6.4	63.3	9.1	82.6	10.0	9.1
PC1	April 4, 2002	6.2	66.4	6.6	72.1	10.6	10.9
PC1-08	June 7, 2001	7.1	219.8	2.5	76.0	30.9	31.0
PC1-08	June 22, 2001	6.2	139.2	4.3	106.6	22.6	24.8
PC1-08	September 25, 2001	6.7	90.6	3.2	103.6	13.5	32.1
PC1-08	November 2, 2001	6.7	202.8	2.8	38.2	30.1	13.8
PC1-08	December 6, 2001	6.6	194.0	2.0	84.0	29.4	41.8
PC1-08	April 4, 2002	6.2	210.3	2.2	50.0	33.7	22.7
Precipitation	July 1, 2001	2.8	85.5	1.1	40.2	30.1	36.5
Precipitation	November 1, 2001	2.5	57.6	1.6	26.3	23.3	16.5
SGW	April 22, 2001	6.4	127.8	0.4	13.3	20.1	31.3
SGW	July 1, 2001	6.6	117.0	1.2	17.7	17.6	14.4
SGW	October 1, 2001	7.7	140.5	1.0	17.9	18.3	18.4
Throughfall	November 1, 2001	3.5	130.6	3.2	139.5	37.9	43.6
Well 55 (Deep)	July 25, 2002	13.0	547.2	1.3	37.0	42.0	28.4

Appendix E: Volumes for Reverse Osmosis

Initial and final volumes for samples in the Harp and Plastic Lake Catchments:

The % concentration of each sample can be calculated by volume:

$$\%\,\text{Concentration} = \frac{\text{Original volume}}{\text{Final volume}} \times 100\%$$

Sample	Date	Initial Volume (L)	Final Volume (L)	Concentration Factor
Harp 4	April 22, 2001	53	5	10.5
Harp 4	July 6, 2001	53	5	10.5
Harp 4	October 7, 2001	63	5	12.6
Harp 4-21	May 1, 2001	77	5	15.4
Harp 4-21	July 6, 2001	105	5	21.0
Harp 4-21	October 7, 2001	97	5	19.3
Harp 5	April 22, 2001	52	5	10.5
Harp 5	July 6, 2001	47	5	9.3
Harp 5	October 7, 2001	46	5	9.3
Harp 6	April 22, 2001	51	5	10.3
Harp 6	July 6, 2001	33	5	6.5
Harp 6	October 7, 2001	65	5	13.1
Harp Beaver	April 22, 2001	40	5	8.0
Harp Lake	April 22, 2001	79	5	15.8
Harp Lake	July 6, 2001	78	5	15.6
Harp Lake	October 7, 2001	96	5	19.3
LFH	June 22, 2001	38	5	7.6
PC1	April 22, 2001	45	5	9.0
PC1	May 12, 2001	52	5	10.5
PC1	June 7, 2001	40	5	8.1
PC1	June 22, 2001	39	5	7.8
PC1	July 16, 2001	40	5	8.1
PC1	September 25, 2001	50	5	9.9
PC1	October 8, 2001	47	5	9.5
PC1	November 2, 2001	48	5	9.5
PC1	December 6, 2001	45	5	9.0
PC1-08	June 7, 2001	144	5	28.9
PC1-08	June 22, 2001	94	5	18.7
PC1-08	September 25, 2001	67	5	13.3
PC1-08	November 2, 2001	141	5	28.1
PC1-08	December 6, 2001	133	5	26.6
Precipitation	July-Sept	160	5	32.1
Precipitation	Oct/Nov	116	5	23.2
SGW	April 1, 2001	169	5	33.9
SGW	July 1, 2001	146	5	29.2
SGW	October 1, 2001	151	5	30.2
Throughfall	Oct/Nov	161	5	32.3
Well 55 (Deep)	July 25, 2002	213	5	42.6