MONITORING CHANGES IN THE SULPHUR ISOTOPIC COMPOSITION AND CONCENTRATION OF TRANSPLANTED PENDULOUS EPIPHYTIC LICHENS

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MONITORING CHANGES IN THE SULPHUR ISOTOPIC COMPOSITION AND CONCENTRATION OF TRANSPLANTED PENDULOUS EPIPHYTIC LICHENS

by

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A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science in Environmental Science

Environmental Science Program
Memorial University of Newfoundland

August 1999

St. John's Newfoundland

ABSTRACT

Little is known about the response of lichens to sudden changes in the concentration and source of sulphur in the atmosphere despite an interest in lichens as tools in environmental monitoring. A transplant study was performed to determine this response in two species of pendulous epiphytic lichens by monitoring changes in their sulphur isotopic composition and sulphur concentration.

Branches covered with the lichens, *Alectoria sarmentosa* and *Bryoria capillaris*, were taken from the Bonavista Peninsula and transplanted into the Memorial University of Newfoundland (MUN) Botanical Garden, St. John's. Two separate transplant experiments were performed between June 1997 and September 1998. Experiment I involved monthly sampling from June 1997 to June 1998. Experiment II involved weekly sampling from June 1998 to September 1998.

The initial sulphur isotopic compositions and concentrations of the local lichens from the Bonavista Peninsula and St. John's were significantly different, providing an ideal opportunity to observe significant changes. The lichens in the Bonavista area had high isotopic compositions (~ +15‰) from seaspray sulphur and lichens in St. John's exhibited low isotopic compositions (~ +5-6 ‰) from anthropogenic sources.

The results from this study showed that the transplanted lichens, *A. sarmentosa* and *B. capillaris*, acquired sulphur isotopic compositions and concentrations approaching those of the atmosphere surrounding the MUN Botanical Garden over the one year study period. For all transplants at all sites, the sulphur isotopic composition decreased while the sulphur concentration increased. Experiment I determined that one year is an

insufficient amount of time to allow the transplanted lichens to achieve exactly the same sulphur isotopic composition and sulphur concentration as the local lichens. It is estimated that a minimum additional six months would provide enough time for the lichens to completely equilibrate with their new surrounding environment. Experiment II showed that natural variation in isotopic composition and concentration occurred on a weekly basis and three months is insufficient to reveal any significant patterns.

This investigation is the first attempt to monitor lichen response to changing atmospheric conditions using stable sulphur isotopes. It has provided essential information for further lichen studies and sulphur isotopic studies in particular. It also has important implications for environmental monitoring and assessment.

ACKNOWLEDGEMENTS

There are numerous people whom I would like to take this opportunity to thank for their assistance.

First, I would like to express my gratitude to my supervisor, Dr. Moire Wadleigh, and a member of my supervisory committee, Dr. Peter Scott. My supervisory committee has been a complete joy with which to work. Dr. Wadleigh provided constant enthusiasm, encouragement and patience. Dr. Scott was always willing to help at any time and provided witty remarks that always made me smile. The knowledge and memories I acquired from these two individuals will be with me on whatever path I take in the future. Their kindness and encouragement was greatly appreciated. Most importantly, they have allowed me to gain confidence in my ability to learn and conquer any task. I thoroughly enjoyed my time as a Master's student under the supervision of Dr. Wadleigh and Dr. Scott.

Next, I would like to thank the personnel at the MUN Botanical Garden, in particular Madonna Bishop. The MUN Botanical Garden provided an ideal location for my transplant experiment. Madonna and many other staff members were always accommodating and helpful with my "odd" requests.

I would like to thank a number of professors from across the country that contributed greatly to my research. The Dean of Science at St. Mary's University, Dr. David Richardson, taught a lichen field course I attended at the Humboldt Field Research Institute, Maine, U.S.A. From this course I gained valuable knowledge and memories that will not soon be forgotten.

Dr. Schneider, a professor of Biology at Memorial University of Newfoundland, contributed greatly to the statistics portion of this paper. I appreciate the time and effort he spent teaching me the necessary statistical methods needed to make my results accurate. He helped me build confidence in my research as well as my ability to learn.

Thanks are extended to Dr. Irwin Brodo at the Canadian Museum of Nature who helped identify the lichen samples utilized in this study.

Also, I would like to thank Dr. John Jacobs and Dr. Colin Banfield, professors of Geography at Memorial University of Newfoundland, for their help with the meteorological aspects of this study.

It is essential that I thank Alison Pye and Pam King for their unending support over the past two years. They both gave an enormous amount of their time to teach me about the mass spectrometer and the ion chromatograph. It was a pleasure to work with these individuals and to learn from their knowledge base of analytical instruments.

Throughout the past two years I have worked in the lab with some exceptional undergraduate and graduate students: Karen Wade, Alison Gallop, Lori Ennis, Christine Malloy, Michelle Miskell, Misuk Yun and Jocelyn Tucker. Whether they taught me specific lab procedures or simply made the never-ending lab work more bearable, their companionship was greatly appreciated.

To Jason Paterson I express my gratitude for his constant support over the past two years. He willingly set aside time from his busy schedule to help me set up the transplant experiments. Then, he faithfully helped collect the specimens monthly for a

year. His extensive computer knowledge made the presentation of this project a success. His friendship, patience and encouragement were greatly appreciated.

Finally, I would like to thank my family for their love and understanding. My parents, Jim and Lilas Wiseman, provided a home where I could excel. They provided endless support throughout my education. Also, thanks to my sisters Rhonda Lane and Roxanne Wiseman for making me believe that I could do anything!

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CHAPTER 1

INTRODUCTION

1.1 Scope and Objectives

The amount of sulphur in the atmosphere has been an issue of concern over the past two decades. Such environmental problems as acid precipitation and global warming are scientifically linked to the overall amount of sulphur present in the atmosphere. These environmental problems have, in turn, led to significant changes in ecosystems such as lake acidification, poor forest and agricultural production, and harm to human and animal health. Due to the continued public concern surrounding these potentially harmful environmental effects, much research within the scientific community has been directed toward the investigation of atmospheric sulphur.

In the past, a wide variety of methods has been used to perform comprehensive studies of atmospheric sulphur. Analysis of both wet and dry deposition gives a good representation of the sulphur in the atmosphere. The most commonly used techniques to monitor atmospheric deposition include precipitation samplers, air filters and various forms of biomonitoring.

In this particular study, lichens are used as bioindicators of atmospheric sulphur. Biomonitoring is defined by Sloof (1993) as "the use of properties of an organism or a part of it to obtain information on a certain quantity in a certain part of the biosphere". The characteristics of an ideal biomonitor include: (1) the capability of accumulating the element or compound of interest in detectable amounts, (2) availability in suitable quantities throughout the study area, (3) the possibility for sampling throughout the year,

(4) accumulation of substances must be directly related to exposure levels, (5) cost effective sampling, (6) accumulation of elements must occur principally from atmospheric sources, (7) element accumulation should be unmediated by biological processes (Puckett, 1988; Sloof, 1993). Consideration of the above criteria indicates that lichens are ideal organisms for atmospheric deposition monitoring.

The analysis of sulphur stable isotopes in lichens has provided interesting results concerning atmospheric sulphur (for example: Krouse, 1977; Case & Krouse, 1980; Krouse *et al.*, 1984; Wadleigh *et al.*, 1996; Wadleigh & Blake, in press). Sulphur isotopes have been recognized as a powerful tool for tracing sulphur sources to the atmosphere because each source has a specific isotopic signature that is not significantly changed during transport through the atmosphere (Forrest & Newman, 1977). For example, the sulphur isotopic composition of seawater today is constant at 21‰ (section 1.2.1) (Rees *et al.*, 1978). For central and eastern North America, typical anthropogenic $\delta^{34}S$ (section 1.2.1) values range from +3‰ to +6‰ (Jamieson, 1996).

The use of vegetation, in particular lichens, has been a tool in sulphur isotopic ratio analysis for many years. Recently, a study by Wadleigh & Blake (in press) analysed the sulphur stable isotopic composition of lichen samples throughout Newfoundland. It was found that lichens along the coastline displayed high isotopic compositions (~ +15‰) while the lichens sampled around point-sources of anthropogenic sulphur had low isotopic compositions (~ +4‰). These findings led to the conclusion that along the coastline the sulphur in the atmosphere is mainly from sea salt while the results around industrial point-sources are consistent with anthropogenic sulphur. It is evident that the

epiphytic lichens assume the sulphur isotopic signature of the surrounding atmosphere whether the contributing sources are natural or anthropogenic. However, little is known about the rate at which lichens respond to changes in source and concentration of atmospheric sulphur.

The objective of this study is to determine the response time of epiphytic pendulous lichens to changes in the isotopic signature of the atmospheric sulphur. This objective was achieved by transplanting lichens from the Bonavista Peninsula where the sulphur isotopic composition is quite high (~ +15‰) to the MUN Botanical Garden in the city of St. John's where the isotopic composition is low (~ +5-6‰). Transplanted lichens were sampled at specific time intervals and the rate at which pendulous epiphytic lichens accumulate sulphur was determined.

1.2 Background

1.2.1 Sulphur

Sulphur is found globally throughout the lithosphere, biosphere, hydrosphere and atmosphere. It occurs in the oxidized state as sulphate in oceans and evaporite rocks, in the reduced state as sulphides from biological and non-biological origin and in the native state in cap rock of salt domes and associated with rocks of active volcanic regions (Faure, 1986). There are four stable isotopes associated with the element sulphur: ^{32}S, ^{34}S, ^{33}S, ^{36}S; the natural abundance of each isotope is 95.02%, 4.21%, 0.75% and 0.02% respectively (Trust & Fry, 1992).

Various processes lead to the separation or partitioning of isotopes due to the differences in mass. This process is known as fractionation and will be discussed in more detail below (section 1.2.1.1).

The two most abundant isotopes, ^{32}S and ^{34}S, are the ones most frequently used in stable isotopic analysis (Trust & Fry, 1992). Sulphur isotopic compositions are expressed in the standard δ notation as shown by the following equation:

$$\delta^{34}S = \left[\frac{^{34}S/^{32}S_{sample}}{^{34}S/^{32}S_{standard}} - 1 \right] X\ 1000$$

Values are expressed in units of per mil which is parts per thousand (‰). Samples are compared to the international standard, CDT (Trust & Fry, 1992). This standard is an iron sulphide (FeS) from the Canyon Diablo meteorite and is believed to represent the primordial value with the ratio of S^{34}/S^{32} being 1/22.22 (Hoefs, 1987). Positive $\delta^{34}S$ values mean that the samples are enriched in ^{34}S relative to the meteoritic troilite, while negative $\delta^{34}S$ values mean that the sample has less ^{34}S than the meteoritic standard (Krouse *et al.*, 1984).

1.2.1.1 Fractionation Mechanisms

In the early 1930's, small differences in the chemical properties of isotopes were discovered by Urey and his coworkers (Faure, 1986). These differences arise from the

differences in mass and are collectively termed 'isotope effects'. The laboratory measurement of isotope effects has developed into a useful tool for investigating the mechanisms of chemical reactions (Thode, 1991). In nature, samples will have variable isotopic compositions that reflect differences in their chemical, biological and geological histories (Thode, 1991).

Isotope fractionation is a function of the relative masses of reacting molecules. It has been found that heavier isotopes (i.e., ^{34}S) have lower zero-point energies so they tend to be bonded more strongly and thus react less readily than lighter isotopes (i.e., ^{32}S) (Hoefs, 1987). Fractionation is represented by the symbol alpha (α) and defined by the following equation:

$$\alpha_{A\text{-}B} = \frac{R_A}{R_B}$$

where R_A and R_B are the ratios of the major isotope to the minor isotope (i.e., $^{34}S/^{32}S$) in substances A and B respectively. In chemical systems, isotope fractionation may occur during either equilibrium or kinetic processes (Thode, 1991).

Isotopic exchange is an example of an equilibrium isotope effect. It involves the exchange of isotopes between substances, phases or molecules in a system at chemical equilibrium (Jamieson, 1996). An example of isotopic exchange can be seen between sulphur dioxide (SO_2) and hydrogen sulphide (H_2S):

$$H_2{}^{34}S + {}^{32}SO_2 \longleftrightarrow H_2{}^{32}S + {}^{34}SO_2$$

The equilibrium constant K may be expressed as follows:

$$K = \frac{[^{34}SO_2] / [^{32}SO_2]}{[H_2{}^{34}S] / [H_2{}^{32}S]}$$

This expression shows that when K is not unity, the ratio $^{34}S/^{32}S$ will not be the same in the two equilibrated phases (Thode, 1991). Therefore, the extent to which K differs from unity is a measure of the equilibrium isotope effect (Thode, 1991). For the system above, the equilibrium constant or α is 1.0064 at 800K. Thus, at 800K under equilibrium exchange conditions, the $\delta^{34}S$ value of SO_2 will be 6.4‰ heavier than that of H_2S (Thode, 1991).

Unidirectional processes typically produce a kinetic isotope effect. Isotopic fractionation in a unidirectional process results from differences in reaction rates of the different isotope species (Thode, 1991). The isotopic fractionation factor between the instantaneously generated product and the remaining reactant is simply given by the ratio of rate constants for the two competing isotopic reactions (Thode, 1991). The ratio of rate constants (k_{32}/k_{34}) or α for the following competing reactions of sulphate to hydrogen sulphide is 1.022 at room temperature (Thode, 1991).

$$^{32}SO_4^{2-} \xrightarrow{k_{32}} H_2{}^{32}S$$

$$^{34}SO_4^{2-} \xrightarrow{k_{34}} H_2{}^{34}S$$

Since $^{32}SO_4^{2-}$ reacts faster than the $^{34}SO_4^{2-}$, the H_2S produced at any instant is depleted in ^{34}S by about 22‰ relative to the remaining SO_4^{2-} (Thode, 1991). Since lighter isotopes react more quickly, reactions tend to give products depleted of the heavy isotopes (Hoefs, 1987).

1.2.1.2 Atmospheric Sulphur Cycle

As a result of both natural and anthropogenic processes, various sulphur compounds; such as, hydrogen sulphide (H_2S), dimethyl sulphide (CH_3SCH_3), carbonyl sulphide (COS), carbon disulphide (CS_2), sulphite (SO_3^{2-}), sulphur dioxide (SO_2) and sulphate (SO_4^{2-}) enter the atmosphere (Ryaboshapko, 1983).

Many authors have tried to quantify the fluxes of sulphur between various reservoirs including the oceans, continents and atmosphere on a global scale. However, this task has proven to be complicated leading to a wide range of proposed estimations. Figure 1.1 illustrates the atmospheric sulphur cycle as estimated by Brimblecombe *et al.* (1989).

Sulphur isotopic compositions have been measured from -65‰ to +90‰, however, the majority of samples fall between -40‰ to +40‰ (Nielson 1979; Hoefs, 1987). Figure 1.2 illustrates the isotopic compositions for a number of sources that contribute sulphur to the atmosphere.

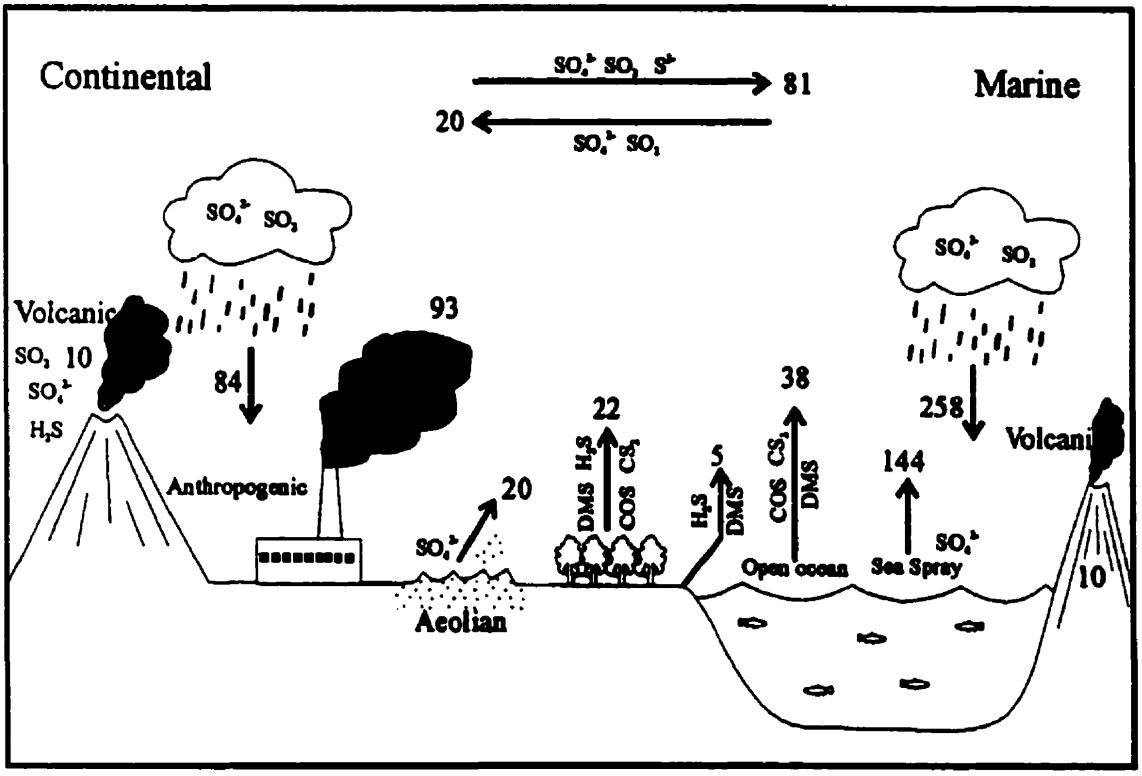


Figure 1.1 Atmospheric sulphur cycle (from Jamieson, 1996 modified from Brimblecombe *et al.*, 1989). All fluxes in $Tg(S)a^{-1}$ ($Tg=10^{12}g$).

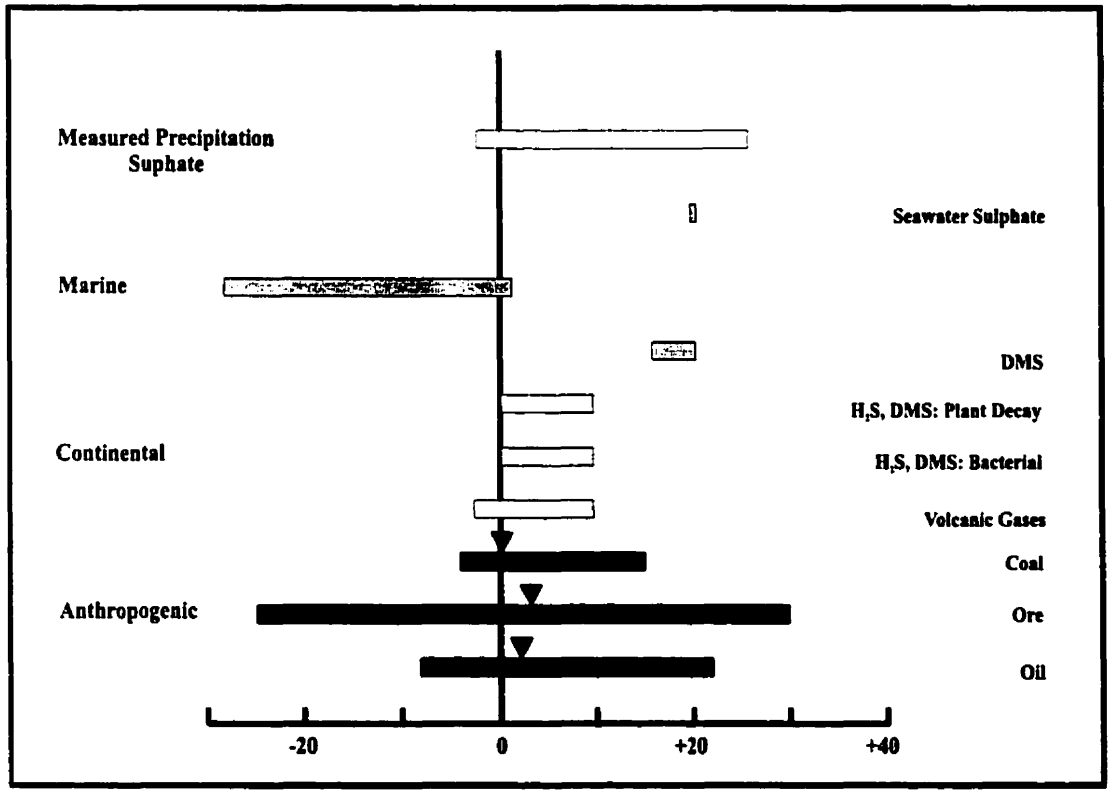


Figure 1.2 $\delta^{34}S$ values of various atmospheric sulphur sources (compiled by Jamieson, 1996).
Inverted triangles (▼) represent the majority of values.

1.2.1.2.1 Natural Sources

Natural inputs into the global sulphur cycle result from three main sources – marine, biogenic and volcanic (Meagher, 1980; Ryaboshapko, 1983). Other localized episodes such as aeolian weathering (i.e., wind-raised dust) and biomass burning also contribute to the amount of natural sulphur in the atmosphere (Ryaboshapko, 1983). These natural sources have a wide range of isotopic signatures (Figure 1.2). It is important to note that uncertainty still surrounds some of these isotopic compositions due to the difficulty associated with direct $\delta^{34}S$ measurement of natural sources globally.

The marine environment has a substantial impact on the global sulphur cycle. The oceans contain a vast amount of sulphate (1.3×10^{15} metric tons in total; Faure, 1986). A significant direct source of particulate sulphur to the marine atmosphere is from the production of sea salt aerosol at the ocean surface (Andreae, 1985). These particles are produced from droplets that are formed when air bubbles burst at the surface (Andreae, 1985). Under very high wind conditions, these droplets can be taken from the surface of the water into the surrounding atmosphere (Andreae, 1985). Most sea salt entering the atmosphere is deposited back into the ocean, however, it is estimated that as much as 10% may be carried over continents and deposited on land near the coastline (Andreae, 1985). The sulphur isotopic composition of seawater today is constant at 21‰ (Rees *et al.*, 1978). It is believed that only a small amount of fractionation occurs during the formation of sea salt particles, thus, it is expected that these particles should maintain the same isotopic composition as the seawater from which they originated (Luecke & Nielsen, 1972).

Biogenic sulphur production refers to the forms of reduced sulphur released by biological organisms from both oceanic and continental areas. For example, it has been shown that a main source of reduced sulphur over the open ocean is dimethyl sulphide (DMS) released during the assimilation and reduction of sulphate by phytoplankton (Andreae, 1985). Isotopic compositions for DMS have not been measured directly, however, a study by Calhoun *et al.* (1991) measured the isotopic composition of sulphate aerosols, which are believed to represent the sulphate produced by the oxidation of DMS, to be +15.6 ± 3.1‰ (standard deviation (1σ)) (Calhoun *et al.*, 1991).

On land, soil and vegetation emit reduced sulphur, often in the form of hydrogen sulphide that contributes to the total atmospheric sulphur (Andreae, 1985; Ryaboshapko, 1983). $\delta^{34}S$ estimates for biological sulphur emissions are estimated to range from –2 to +3‰ (Nriagu *et al.*, 1987).

Volcanoes and geothermal areas emit a number of sulphur gases as well as sulphate aerosol during both eruptive and noneruptive phases. Gases emitted include SO_2, H_2S, COS and CS_2 (Andreae, 1985). Also, it has been found that particulate sulphate emitted by volcanoes contributes to the global sulphur cycle (Andreae, 1985). The isotopic compositions of volcanic emissions are extremely variable. They generally cluster around 0‰ but may differ by as much as ± 15‰ (Nielson *et al.*, 1991; Castleman *et al.*, 1974).

Finally, sporadic events of aeolian weathering and biomass burning also contribute to sulphur in the atmosphere. It is very difficult to quantify these events because of their localized nature, however, it is important to specify that these sources are

contributing on a small scale to the total natural sulphur in the atmosphere. The $\delta^{34}S$ values of these events reflect the composition of the source rock and are generally enriched with ^{34}S.

In general, the natural sources of sulphur emissions to the atmosphere are substantial. The approximate percentage contributions of the main sources of natural sulphur emissions are given in Table 1.1. An estimation of the contribution of seaspray is excluded from Table 1.1 because approximately 90% of seaspray sulphate is cycled back into the oceans (Whelpdale, 1992). It is important to note that the fluxes of sulphur from natural sources exhibit high spatial and temporal variability. Therefore, many uncertainties arise when extrapolating from a few point measurements to the global scale (Whelpdale, 1992).

1.2.1.2.2 Anthropogenic Sources

The present day biogeochemical cycle of sulphur is significantly affected by anthropogenic processes (Brimblecombe *et al.*, 1989). Brimblecombe *et al.* (1989) estimated the flux of anthropogenic sulphur emissions (SO_2, SO_4^{2-}) into the atmosphere to be $93TgSa^{-1}$ (Figure 1.1).

The main human activities resulting in sulphur emissions to the atmosphere are the combustion of fossil fuels for the production of energy, smelting of ferrous and non-ferrous ores, oil processing and production of sulphuric acid for industrial use (Ryaboshapko, 1983).

Table 1.1 Percentage contributions of the main sources of natural sulphur emissions to the atmosphere (from Whelpdale, 1992).

NATURAL SOURCES	*PERCENTAGE CONTRIBUTION TO ATMOSPHERE*
Open ocean biogenic production	46%
Volcanoes	18%
Aeolian weathering	16%
Terrestrial plants and soils	13%
Biomass burning	4%
Coastal zone and wetland biogenic sources	3%

Sulphur occurs in all fossil fuels but its content varies. For example, natural gas is regarded as the "cleanest" fuel with respect to sulphur having a low average sulphur concentration of 0.05% (by weight). However, there are exceptions such as the sources in Alberta that contain 80% hydrogen sulphide in gas (Ryaboshapko, 1983). African oils have sulphur concentrations between 0.3-0.5% by weight whereas Venezuelan oils often exceed 5% (Ryaboshapko, 1983). The sulphur content of coals averages 2.2% (by weight) throughout the world (Ryaboshapko, 1983).

The isotopic composition of anthropogenic sulphur displays a wide range of $\delta^{34}S$ values. It is expected that the $\delta^{34}S$ values resemble those of the source from which the sulphur emissions originated. For example, the sour-gas wells of Alberta are used to produce elemental sulphur. The sulphur emissions from this process display a range of $\delta^{34}S$ values from +5 to +30‰ (Ryaboshapko, 1983). The combustion and refining of oil and gas produces $\delta^{34}S$ values that range from –5 to +30‰. From previous studies, it was found that the majority of $\delta^{34}S$ values for oil fall between 0 and +10‰ with an average of +5‰ (Krouse *et al.*, 1991a). For central and eastern North America typical anthropogenic $\delta^{34}S$ values range from +3 to +6‰ (Jamieson, 1996).

1.2.1.3 Atmospheric Sulphur Processes

1.2.1.3.1 Sulphur Transport

Once sulphur is released into the atmosphere, its subsequent transport is affected by several different scales of motion in the atmosphere, each scale dominated by different physical processes (Whelpdale, 1992). On a global scale, transport is largely dependent on the general circulation of the atmosphere; the westerlies of the mid-latitudes and the easterlies of the subtropics (Whelpdale, 1992). On the next smaller scale, transport is influenced by synoptic disturbances such as various low and high pressure systems (Whelpdale, 1992). Finally, at increasingly smaller scales; such as, regional, local and micro, the transport depends on the orography and nature of the underlying surface (Whelpdale, 1992). Thus, due to the numerous factors acting on the sulphur in the atmosphere, it is possible to have both long-range and short-range transport. The specific amount of time that a sulphur compound can stay in the atmosphere is determined by the Mean Residence Time (MRT). The MRT is defined by the following equation:

$$MRT = Mass / Flux$$

where flux may be either the input to or loss from the reservoir (Schesinger, 1991). Thus, the distance of transport ultimately depends on the efficiency of the mechanisms, wet and dry deposition, to remove sulphur from the atmosphere. Table 1.2 estimates the residence times of the most prominent sulphur compounds released into the atmosphere.

Table 1.2 Residence times (τ) and transport distances for different sulphur compounds in the atmosphere (compiled by Jamieson, 1996).

	Planetary Boundary Layer		Free troposphere		
	τ (days)	transport distance (10^3km)	τ (days)	transport longitudinal (10^3km)	distance latitudinal (10^3km)
H_2S	1	0.5	2	4	1
DMS	<0.5	<0.2	<1	<2	<0.5
COS	500	Global	500	Global	Global
CS_2	10	5	20	Global	5
SO_2	1	0.5	10	Synoptic	3
SO_4^{2-}	4	2	15	Global	4

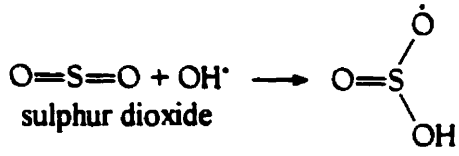
1.2.1.3.2 Sulphur Transformations

The atmosphere is a system with oxidative properties, thus the majority of reduced sulphur compounds become oxidized to SO_2 and then sulphate (Ryaboshapko, 1983). A variety of factors, including humidity, temperature and pH influences the nature and rate of the reactions. Sulphur dioxide may follow one of two pathways: the homogeneous (gas phase) pathway or the heterogeneous (aqueous phase) pathway.

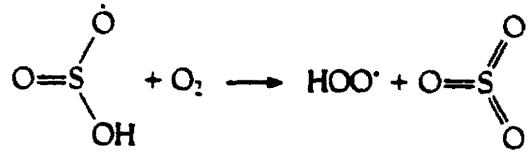
The sulphate ion is the ultimate end-product of the oxidation of sulphur dioxide, however, depending mainly on pH, many intermediate products such as bisulphite ion, bisulphate ion, sulphite ion and sulphurous acid may result.

When the sky is relatively clear with only a few clouds in the troposphere, the predominant mechanism for the conversion of SO_2 to H_2SO_4 is the homogeneous (gas phase) pathway (Baird, 1995). The mechanism for conversion consists of the following sequential steps:

1) addition of the hydroxyl radical to the sulphur dioxide molecule (SO_2)



2) removal of hydrogen atom by oxygen molecule (O_2) to form sulphur trioxide (SO_3)



sulphur trioxide

3) combination of sulphur trioxide molecule (SO_3) with gaseous water molecule to form sulphuric acid ($H_2SO_{4(g)}$)

4) the sulphuric acid molecule ($H_2SO_{4(g)}$) reacts with water (mist; water vapour) to form droplets of aqueous sulphuric acid ($H_2SO_{4(aq)}$)

The sequence of steps from gaseous SO_2 to aqueous H_2SO_4 is summarized below:

$$SO_2 + OH^{\cdot} \longrightarrow HSO_3^{\cdot}$$

$$HSO_3^{\cdot} + O_2 \longrightarrow SO_3 + HOO^{\cdot}$$

$$SO_3 + H_2O \longrightarrow H_2SO_4(g)$$

$$H_2SO_4(g) \xrightarrow{H_2O} H_2SO_4(aq)$$

The heterogeneous (aqueous phase) pathway occurs when there is a significant amount of cloud, fog or mist content in the air (Baird, 1995). Since sulphur dioxide is soluble in water, the following reaction occurs:

$$SO_{2(g)} + H_2O_{(aq)} \longleftrightarrow H_2SO_{3(aq)}$$

The dissolved sulphur dioxide is oxidized to sulphate ion by trace amounts of the well-known oxidizing agents, hydrogen peroxide (H_2O_2) and ozone (O_3), that are present in the airborne droplets (Baird, 1995). Dissolved hydrogen peroxide oxidizes dissolved sulphur dioxide by attacking all three of the species, H_2SO_3, HSO_3^- and SO_3^{2-} (Figure 1.3) (Baird, 1995).

1.2.1.3.3 Sulphur Removal Processes

Sulphur compounds in the atmosphere can be brought to the earth's surface by two main processes: wet deposition or dry deposition.

Wet deposition processes use precipitation as the delivery mechanism (Whelpdale, 1992). Sulphur dioxide gas can dissolve in cloud and rain drops or adsorb onto frozen precipitation elements (Whelpdale, 1992). Sulphate particles, on the other hand, are efficient condensation nuclei and are incorporated into precipitation by nucleation or as a result of in-cloud or sub-cloud scavenging (Whelpdale, 1992). The efficiency of wet deposition as a sulphur removal process depends largely on the form of sulphur present, and the type, duration, intensity and frequency of the precipitation in a given location (Whelpdale, 1992).

Dry deposition processes do not involve precipitation. Gases such as sulphur dioxide absorb on the surface of particles and simply fall from the atmosphere due to gravitational pull.

Overall, the amount of fractionation of atmospheric sulphur due to transport, transformation and removal is very small. Theoretically, the expected fractionation of the

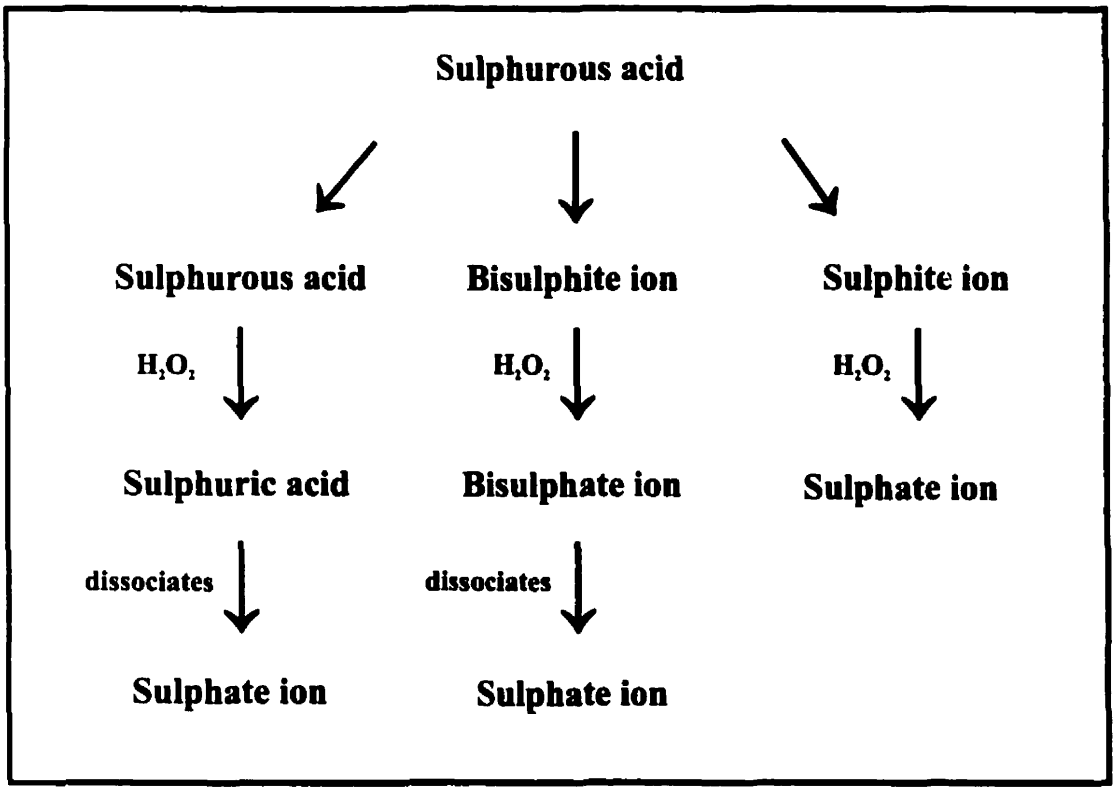


Figure 1.3 Heterogeneous aqueous phase pathway of sulphur oxidation.

homogeneous (gas phase) pathway leaves the resulting sulphate slightly depleted in ^{34}S. In contrast, the heterogeneous (aqueous phase) pathway results in the enrichment of sulphate in ^{34}S (Saltzman *et al.*, 1983). Based on the expected theoretical fractionation of the oxidation pathways, $\delta^{34}S$ values could change significantly, thus could not be used to delineate sources accurately in studies. However, the oxidation process is not reversible in the atmosphere and field measurements of SO_2 in a smelter plume show that sulphur isotopic compositions change by only 1‰ or 2‰ relative to their sulphur source (Forrest & Newman, 1977). In general, then, the sulphur isotopic composition of atmospheric sulphur compounds reaching vegetation has not changed significantly from that of the source where the sulphur compounds originated.

1.2.2 Lichens

Lichens have been found to inhabit a wide range of areas from the low tidal zone on seashores to the tops of high mountains, from hot deserts to the Arctic and Antarctic (Richardson, 1992; Hawksworth & Rose, 1976). A lichen is a symbiotic association between two organisms – a photosynthetic green alga, or less often a cyanobacterium, and a fungus (Richardson, 1992). The fungal partner of most lichens belong to a group known as the Ascomycetes (Richardson, 1992).

1.2.2.1 Nature of Symbiosis

Lichens display a very highly organized symbiosis. The fungal component (mycobiont) has successfully established a symbiotic relationship with the algal

component (phycobiont) (Hale, 1967). A mass of hyphae of the mycobiont form the vegetative thallus, while the phycobiont consists of a thin layer of algal cells just below the surface of the thallus (Hale, 1967).

Experiments have shown that the phycobiont "leaks" sugars and sugar derivatives to the mycobiont thus providing nutrition (Richardson, 1992). The mycobiont acts as a matrix in which the algae can survive in very severe environments (Hale, 1974). Many lichenologists prefer to view the lichen symbiotic relationship as mutualistic in nature, whereas others describe the relationship as a "controlled parasitism" of the lichen alga by the fungus (Richardson, 1974). Overall, it is evident that these very different organisms have evolved to live together and function as a single organism.

1.2.2.2 Morphology

Lichens are traditionally classified into three major growth forms: (1) crustose, (2) foliose and (3) fruticose.

Each growth form is characterized by a particular arrangement of fungal/algal cells which defines the overall shape of the organism. Also, each growth form has varying degrees of attachment to the substrate (Hale, 1967).

The two lichens chosen for this experiment, *Alectoria sarmentosa* and *Bryoria capillaris*, are fruticose lichens. They are characteristically pendant, hair-like, shrubby and very highly branched (Vitt *et al.*, 1988). The thallus of *A. sarmentosa* is yellow-green in colour. *A. sarmentosa* most commonly grows hanging from branches of coniferous trees in moist, coastal, montane and boreal regions (Vitt *et al.*, 1988). The

second lichen, *B. capillaris,* has a pale greenish-gray thallus and also grows most commonly on coniferous trees. The internal structure of these lichens is more or less radial with a dense outer cortex, a thin algal layer, a medulla and a hollow center (Figure 1.4) (Amhadjian, 1967). Most importantly, both *A. sarmentosa* and *B. capllaris* are epiphytic lichens and, because of this, it is thought that both *A. sarmentosa* and *B. capillaris* take nutrients directly from the atmosphere. (Sloof & Wolterbeek, 1993).

1.2.2.3 Biochemical Processes Utilizing Sulphur

All vegetation, including lichens, requires sulphur as an essential macronutrient to grow. There is a wide variety of sulphur-containing compounds in vegetation, however, the essential compounds include sulphur amino acids, glutathione, thiamine, vitamin B, biotin, ferredoxin, lipoic acid, coenzyme A and the sulpholipids of the chloroplasts (Krouse *et al.*, 1991b). It has been found that the sulphur amino acids (cysteine and methionine) contain approximately 90% of the sulphur in vegetation (Krouse *et al.*, 1991b). Thus, it is evident that vegetation must have the ability to convert sulphur from the surrounding environment to sulphur-containing amino acids needed by all organisms. This biochemical process by which inorganic sulphur compounds get reduced to organic sulphur compounds is called assimilatory sulphate reduction (Roy & Trudginer, 1970).

As was stated earlier, sulphur compounds are removed from the atmosphere by wet and dry deposition. Plants and lichens differ significantly in the method by which they accumulate sulphur. Vascular plants absorb most of their sulphur nutritional requirements through the root system. The aerial parts of higher plants are equipped with

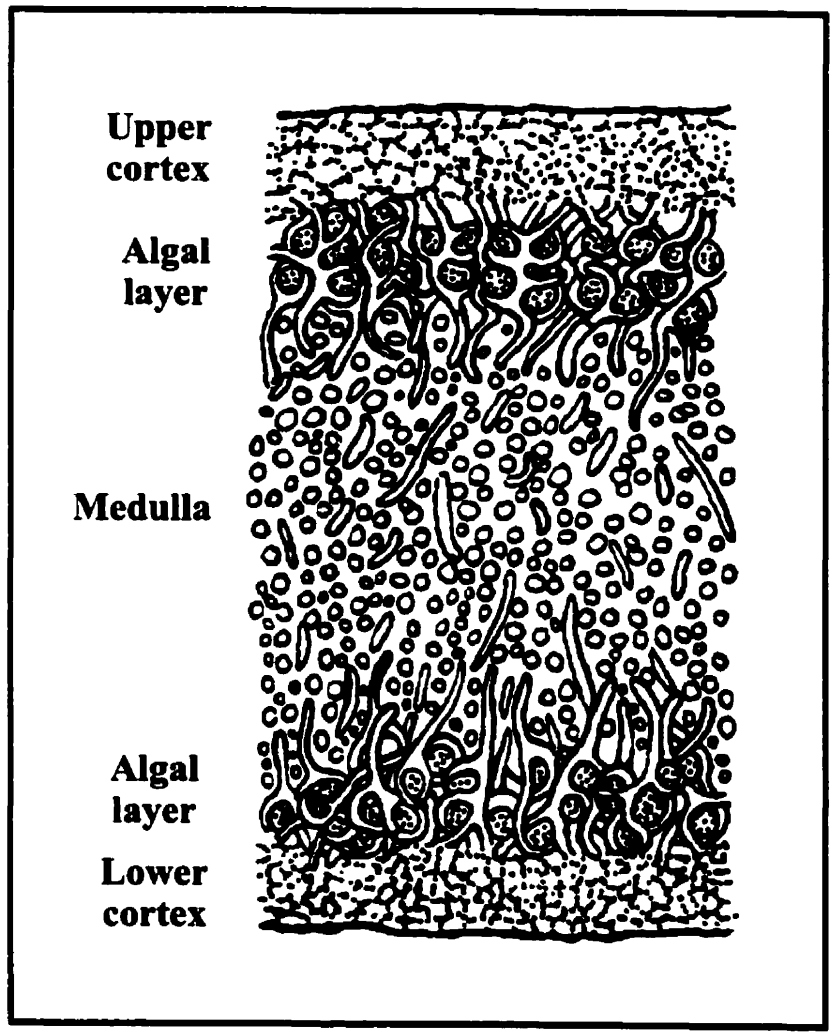


Figure 1.4 Cross-section of fruticose lichen thallus (from Ahmadjian, 1967).

protective mechanisms such as a cuticle and stomata that will only allow minimal amounts of sulphur into the organism (Marschner, 1995). Higher plants have the ability to control the amount of sulphur that enters the plant by utilizing a negative feedback control system (Marschner, 1995). Lichens, on the other hand, lack roots so surface absorption of rainfall is the primary mechanism of obtaining the required amount of nutritional sulphur. Sulphur in the air can enter lichen thalli both in solution (as sulphate, sulphite, bisulphite and sulphurous acid) and in gaseous form (sulphur dioxide and sulphur trioxide) (Hawksworth & Rose, 1976). Lichens are disadvantaged when sulphur is excessive because they cannot control the amount of sulphur that enters through the thallus.

A wide range of sulphur compounds in the atmosphere may be deposited on lichen surfaces. Most of these compounds become oxidized when they encounter water on the thallus and form sulphate. This sulphate then enters the cells where it is reduced and incorporated into useful organic compounds (Roy & Trudginer, 1970; Taiz & Zeiger, 1991).

The following sections detail the steps in the assimilatory sulphate reduction pathway. This process involves the formation of several intermediate sulphur compounds prior to the incorporation of sulphur into amino acids (Taiz & Zeiger, 1991).

1.2.2.3.1 Assimilatory Sulphate Reduction Pathway

Step #1: Activation of Sulphate

The cellular metabolism of sulphate begins by a series of activation reactions with adenosine triphosphate (ATP) (Ciba, 1980). Primarily, adenosine 5'-triphosphate (ATP) reacts with sulphate to form the first activated sulphur intermediate, adenosine 5'-phosphosulphate (APS), and pyrophosphate (PP_i):

$$ATP + SO_4^{2-} \xleftrightarrow[\text{ATP sulphurylase}]{} APS + PP_i$$

(energy unfavourable)

The adenosine 5-phosphosulphate (APS) then reacts with another adenosine triphosphate (ATP) to form the second activated sulphur intermediate, 3'-phosphoadenosine 5'-phosphosulphate (PAPS) and adenosine diphosphate (ADP):

$$APS + ATP \xleftrightarrow[\text{APS kinase}]{} PAPS + ADP$$

(energy favourable)

The first equation is not energetically favourable for product formation, however, this problem is overcome by coupling it to the second favourable equation. Essentially, the formation of PAPS drives the sulphate activation (Taiz & Zeiger, 1991). Another driving force is the constant hydrolysis of pyrophosphate (PP_i) to 2 moles of inorganic phosphate ($2P_i$):

$$PP_i + H_2O \longrightarrow 2P_i$$

(energy favourable)

Step # 2: Reduction of Sulphate to Sulphide

The next step in sulphur assimilation is the reduction of the activated form to sulphide (Taiz & Zeiger, 1991). Eight electrons are required to change the oxidation number of sulphur from +6 to –2 (Taiz & Zeiger, 1991). For some time, it was believed that the PAPS-activated form was the sole compound involved in the reduction of sulphate to sulphide (Muth & Oldfield, 1970), however, it has been found that assimilatory sulphate reduction exhibits two alternative paths: (1) utilizing APS as the activated sulphur intermediate or (2) utilizing PAPS as the activated sulphur intermediate (Taiz & Zeiger, 1991).

The first pathway utilizing APS as the activated sulphur intermediate is found in most oxygen-evolving photosynthesizers, including all eukaryotic algae, some prokaryotic blue-green algae and all higher plants (Ciba, 1980). The second pathway utilizing PAPS as the activated sulphur intermediate is prominent in bacteria and certain cyanobacteria, but is only of minor importance to higher plants (Ciba, 1980).

All plants utilize the first pathway (APS), thus, it will be examined in greater detail. First, PAPS is converted back to APS, which then serves as the substrate for reduction (Taiz & Zeiger, 1991). This occurs due to the hydrolysis of the 3′-phosphate groups of PAPS by the enzyme, 3′-phosphonucleotidase (Taiz & Zeiger, 1991). Therefore, it appears as though the formation of PAPS in plants simply occurs to drive the energetically unfavourable reaction of APS formation (Taiz & Zeiger, 1991).

In the reduction of APS to sulphide, reduced ferredoxin acts as an electron donor:

$$APS + 8Fd_{red} + 5H^{+} \longrightarrow \text{sulphide} + AMP + 8Fd_{ox} + 3H_2O$$

There is evidence that this overall reduction reaction may involve the production of sulphite as a protein-bound intermediate (Taiz & Zeiger, 1991). The reduction of APS can take place in the roots where light is not present, however, the reactions occur much faster in leaves in the presence of light. This stimulation by light could be due to the enhanced production of reduced ferredoxin by the photosystems (Taiz & Zeiger, 1991). In non-photosynthetic cells, NAD(P)H is the likely donor of electrons (Taiz & Zeiger, 1991).

Step # 3: Incorporation of Sulphide into Sulphur-containing Amino Acids

The sulphide produced by the reaction of APS does not accumulate in the plant cell, rather it is rapidly incorporated into the sulphur-containing amino acids (Taiz & Zeiger, 1991). Primarily, the sulphide reacts with a three-carbon acceptor (O-acetylserine) to form cysteine and acetate:

$$\text{O-acetylserine} + \text{sulphide} \longrightarrow \text{cysteine} + \text{acetate}$$

From this point, cysteine is used in the synthesis of most other sulphur-containing compounds such as coenzyme A, methionine, biotin, lipoic acid and glutathione (Trust & Fry, 1992).

Figure 1.5 is a complete diagram of the "assimilatory sulphate reduction" mechanism.

The majority of photosynthetic organisms, including lichens, mosses and higher plants, undergo assimilatory sulphate reduction (Figure 1.5) to meet their sulphur nutritional requirements (Taiz & Zeiger, 1991). However, due to the increasing amounts of atmospheric sulphur from anthropogenic activities, vegetation in general and lichens in particular are having to expend enormous amounts of energy simply converting inorganic sulphur compounds to useful organic compounds. The excessive amounts of sulphur in the atmosphere have changed from being beneficial, facilitating growth, to being detrimental by harming the chlorophyll in the organisms and in many cases leading to damage or death (Hale, 1983).

1.2.3 Lichens and Sulphur Stable Isotopes

1.2.3.1 Fractionation of Sulphur Stable Isotopes in Vegetation

By utilizing sulphur stable isotope studies, it has been found that there is little isotopic discrimination during sulphate assimilation and reduction by vegetation (Trust & Fry, 1992). Generally, plants have $\delta^{34}S$ values that average about 1.5‰ lighter than that of environmental sulphate (Trust & Fry, 1992). This information is pivotal for this particular study because it verifies the fact that the sulphur isotopic composition of vegetation remains virtually unchanged, thus reflecting the sulphur isotopic composition of atmospheric sulphur.

If each step of assimilatory sulphate reduction is examined with respect to isotopic fractionation of sulphur, only one part of the reaction process has the potential to

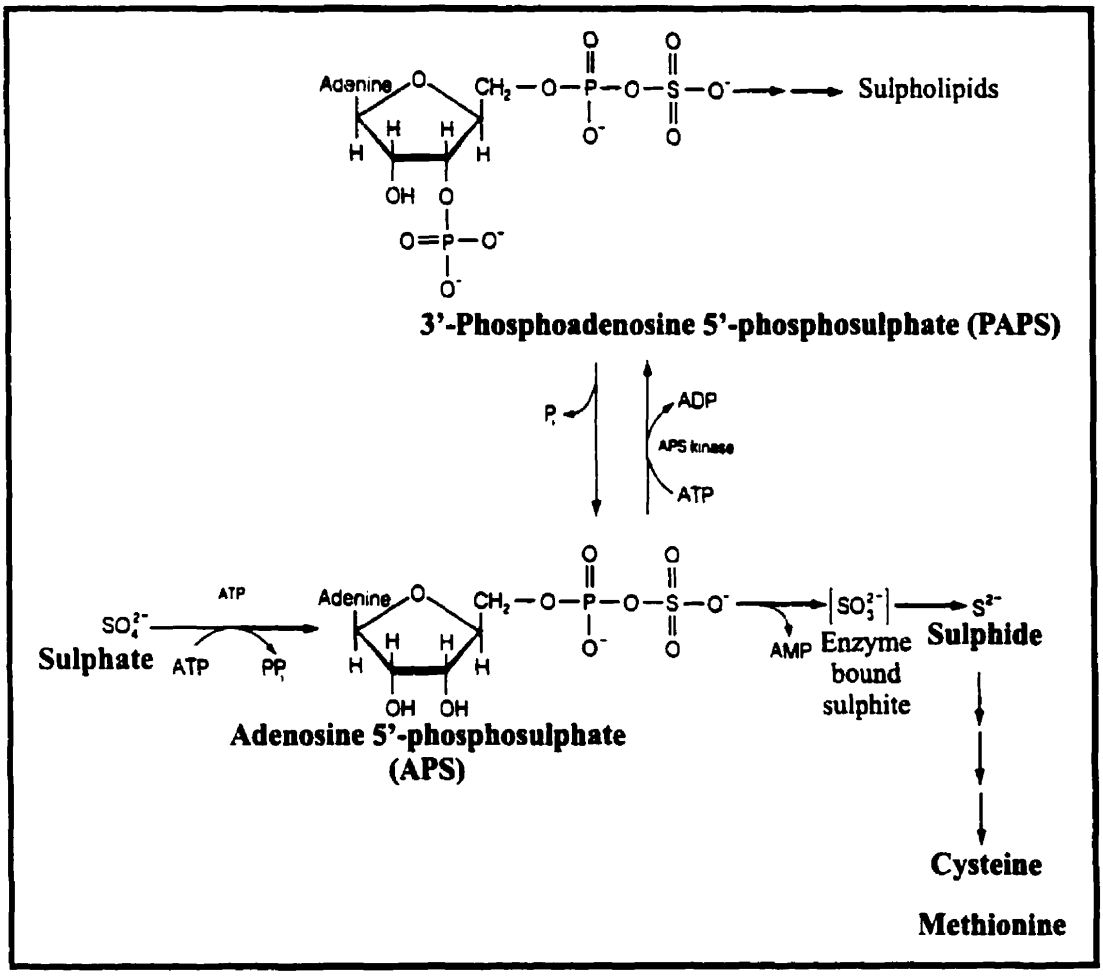


Figure 1.5 Complete diagram of the assimilatory sulphate reduction pathway (from Taiz & Zeiger, 1991).

allow any significant amount of fractionation (Trust & Fry, 1992). Very little fractionation is expected in the first and second steps of the reaction in which the sulphate is taken up by the plant and then activated by the ATP (Figure 1.5) (Trust & Fry, 1992). The third step, on the other hand, has the potential to produce large fractionations due to the actual reduction steps in which the sulphur/oxygen bonds are broken (Figure 1.5) (Trust & Fry, 1992). From numerous studies that will be examined later, it is evident that large fractionations do not occur (Krouse *et al.*, 1991b), thus, the rate and completion of reactions are responsible for the small amount of fractionation (Trust & Fry, 1992). Sulphate reduction in plants must be limited either by the uptake or the activation of sulphate with ATP (Trust & Fry, 1992). If the reaction were limited by the reduction step, larger fractionations would be observed (Trust & Fry, 1992). It is evident that higher plants allow only small amounts of sulphate to enter the system to be reduced at one time. Due to the energetically-favourable reactions discussed earlier, the small amount of sulphate completely reacts at a relatively quick pace, not allowing large amounts of fractionation to occur.

It is important to note that higher plants and lichens may emit isotopically light hydrogen sulphide and other reduced sulphur compounds in response to high sulphur loading, thus altering their isotopic signature (Trust & Fry, 1992). This release of H_2S is considered an important mechanism for the detoxification of sulphur dioxide (Marschner, 1995). In studies by Case & Krouse (1980) and Krouse *et al.* (1984) it was found that lichens and coniferous trees in an area with high industrial SO_2 emissions displayed $\delta^{34}S$ values considerably higher than those of available sulphur sources. Evidently, when

isotopically light H_2S was emitted, there was a subsequent increase in the isotopically heavy sulphur in the lichens and coniferous trees (Case & Krouse, 1980; Krouse *et al.*, 1984). This concept of hydrogen sulphide emission by vegetation should be taken into consideration when analysing isotopic results.

1.2.3.2 The Use of Higher Plants and Bryophytes in Sulphur Stable Isotope Studies

There is evidence available to support the concept that there is only a small amount of fractionation during sulphate assimilation and reduction by plants. An early study by Mektiyeva *et al.* (1976) demonstrated this concept. The sulphur content and isotopic composition of five perennial plants (linden, poplar, meadow-sweet, spruce, pine) were investigated from a region south of Moscow (Mektiyeva *et al.*, 1976). The plants were all grown in similar soil that had a $\delta^{34}S$ value of between +3.9‰ and +4.0‰ (Mektiyeva *et al.*, 1976). It was found that the total sulphur in the photosynthetic tissue of the plant (needles or leaves) was slightly depleted in S^{34} relative to the soil sulphate. The data indicated that the plants had practically the same sulphur isotopic composition as the sulphate in the surrounding medium - the soil (Mektiyeva *et al.*, 1976). In this study the influence of atmospheric sulphur was negligible because there was no industrial point source anywhere in the region (Mektiyeva *et al.*, 1976).

A more recent case study of the West Whitecourt Forest Ecosystem, Alberta, clearly displayed the interactions occurring among the four main components of the ecosphere (hydrosphere, atmosphere, lithosphere, biosphere). The objective of the study was to determine how the sulphur-gas emissions from the Amoco Canada Petroleum

Company Limited West Whitecourt Sulphur Recovery Gas Plant affected the boreal forest ecosystem as a whole (Krouse *et al.*, 1984). In this study, sulphur isotopes could be used as tracers because there was a large discrepancy between the $\delta^{34}S$ values of sulphur-gas emissions (+22‰) and the pre-industrial soil at a depth of 60 cm (0‰), representing the natural environmental background (Krouse *et al.*, 1984). The air samples confirmed that the West Whitecourt Gas Plant was the major source of sulphur-gas emissions to the forest ecosystem in the study area (Krouse *et al.*, 1984). The soil data indicated that industrial sulphur compounds penetrated the soil to a depth of one meter in exposed, dry areas lacking biological cover while, in the case of light forest cover, penetration to 60 cm had not occurred (Krouse *et al.*, 1984). The extent to which industrial sulphur had penetrated the soil, thus increasing the $\delta^{34}S$ values, was a function of a number of factors such as biological cover, location and distance from the sulphur-gas emission source (Krouse *et al.*, 1984). The $\delta^{34}S$ values of the sulphur compounds in the surface water approached a value of +22‰ (Krouse *et al.*, 1984), demonstrating that the sulphur-gas emissions affected the hydrosphere as well. Finally, the vegetation (jack pine needles and moss) displayed $\delta^{34}S$ values slightly lower than those obtained from the sulphur-gas emissions (Krouse *et al.*, 1984). Evidently, the vegetation was receiving sulphur from the atmosphere as well as inputs from the root system. The moss, *Polytrichum juniperinum*, clearly depicted how each part of the organism was isotopically affected by the source of surrounding sulphate (Figure 1.6). The upper portion of the moss had $\delta^{34}S$ values near +20‰ showing the direct influence of sulphur-gas emissions (Krouse *et al.*, 1984). The humus surrounding the rhizoids was much

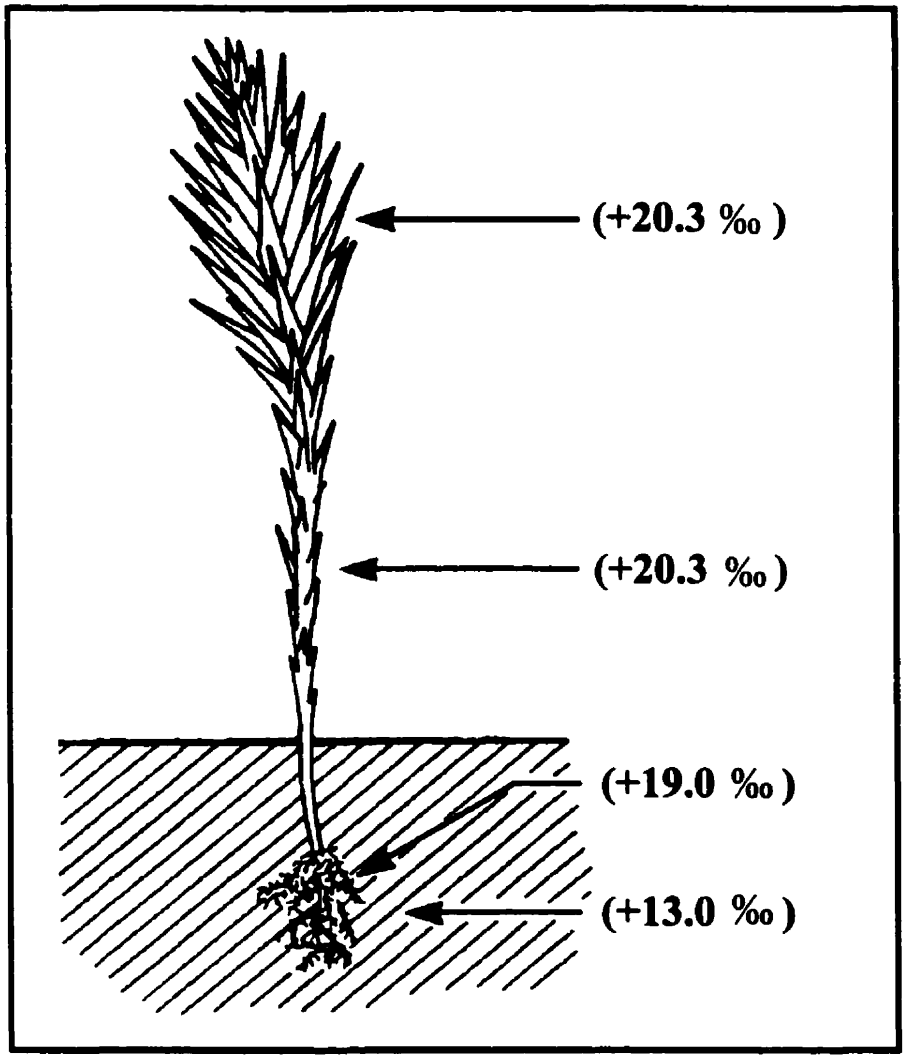


Figure 1.6 $\delta^{34}S$ values for different portions of a moss, *Polytrichum juniperinum* (from Krouse *et al.*, 1984).

lighter isotopically at 13‰. Thus, the value obtained for the rhizoids (+19‰) indicated a mixing of the two contributing mediums – soil and air (Krouse *et al.*, 1984). Essentially, each part of the plant displayed $\delta^{34}S$ values very close to their surrounding environment. This evidence supported the concept that sulphur stable isotope fractionation is very limited during the assimilation and reduction of sulphate in vegetation, due to the fact that the vegetation retained $\delta^{34}S$ values close to that of the surrounding sulphur in the environment.

1.2.3.3 The Use of Lichens in Sulphur Stable Isotope Studies

The previous examples of isotopic studies utilized bryophytes and higher plants as a means to determine sulphur isotopic composition, however, these are not the only types of vegetation that have been used in stable sulphur isotopic studies. Recently, the use of lichens as a tool by which to determine the extent of pollutant sulphur in the surrounding environment has emerged (Trust & Fry, 1992).

Lichens represent a specialized group of organisms that can be utilized very effectively in sulphur stable isotopic composition studies. In general, lichens have been demonstrated to be effective bioindicators of pollution, being sensitive to both sulphur and trace metal concentrations in the atmosphere (Richardson, 1992). They have a very slow growth rate and effectively absorb soluble and insoluble mineral nutrients from the air and precipitation. Sulphur isotopic measurements made on epiphytic lichens, such as *Alectoria*, match those of dissolved sulphate in precipitation from the same location (Evans, 1996).

In a study by Krouse (1977), it was found that lichens had an isotopic composition that coincided closely with that of SO_2 in the atmosphere. This showed that lichens take up sulphur from the air directly (Krouse, 1977). Also, it was demonstrated that pine needles (*Pinus contorta*) had isotopic compositions approximately 10‰ lighter than the $\delta^{34}S$ values of air and lichens (Krouse, 1977). This large discrepancy between the pine needles and lichens was explained by the fact that trees have a root system that transports sulphate upwards from the soil, as opposed to solely atmospheric uptake (Marschner, 1995). The uptake of sulphur from a combination of sources produces isotopic ratios in the pine needles that are weighted averages of the isotopic ratios of the individual sources (Trust & Fry, 1992). Thus, because pine needles derive sulphur from both soil and air, intermediate $\delta^{34}S$ values are obtained (Krouse, 1977). The sulphur isotopic variations among air, lichens and pine needles can be seen in Figure 1.7.

A study by Case and Krouse (1980) investigated variations in stable sulphur isotopic composition and sulphur content of vegetation near a sulphur dioxide source at Fox Creek, Alberta. Again, it was confirmed that lichen samples displayed higher $\delta^{34}S$ values than the pine needles. This supported the concept that lichens derive most of the sulphur from the atmosphere while pine needles derive sulphur from two sources; air and soil. Total sulphur content and $\delta^{34}S$ values for epiphytic lichens clearly revealed the trend of increasing values closer to the gas plant, while pine needles did not display this trend (Case & Krouse, 1980). In this particular case, the soil remained untouched by the SO_2 emissions due to the great amount of forest cover. Evidently, the pine needles absorbed most of their sulphur from the soil by the root system (Case & Krouse, 1980).

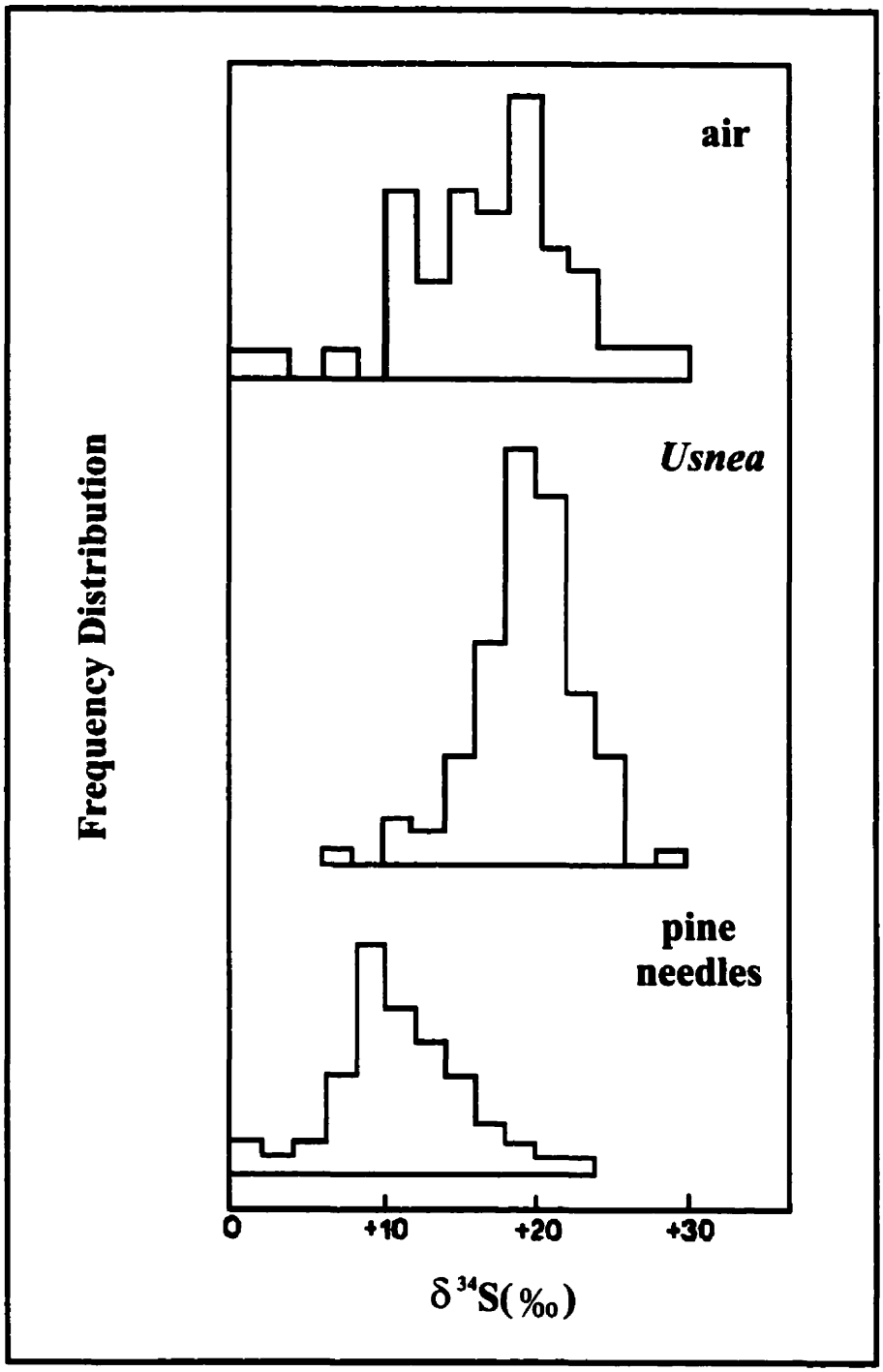


Figure 1.7 Sulphur isotope variations in air, lichens and pine needles collected near a sour-gas producing area of Ram River, Alberta (from Krouse, 1977).

A series of experiments throughout Newfoundland has provided additional isotopic data on lichens. Samples of the epiphytic lichen, *Alectoria sarmentosa*, were taken from sites throughout the entire island and analyzed for their sulphur isotopic composition and sulphur concentration (Wadleigh & Blake, in press). It was found that *A. sarmentosa* across Newfoundland had isotopic compositions ranging from +3.7‰ to +16.6‰ (Wadleigh & Blake, in press). Generally, the compositions increased as the coast was approached. Throughout the island, there were areas that showed the influence of sulphur from anthropogenic sources and areas of little anthropogenic influence. For example, the lichens sampled around the Come By Chance Oil Refinery had very low (~ +4‰) sulphur isotopic compositions consistent with anthropogenic sulphur (Wadleigh & Blake, in press). On the other hand, the lichen samples collected from along the coastline displayed higher (~ +15‰) compositions, leading to the conclusion that along the coastline sulphur is mainly from sea salt (Wadleigh & Blake, in press). In this experiment there was a correlation between sulphur concentration and sulphur isotopic compositions in lichens. The sulphur concentrations were highest (>600 ppm) close to the point sources and lowest (<400 ppm) far away from them. However, sulphur concentrations were also high at sites along the coast (400-600 ppm). Therefore, it is important to use both isotopic compositions and concentrations as a good indicator of sulphur impact (Wadleigh & Blake, in press).

Based on the above research, this study was undertaken. It is known that epiphytic lichens assume the sulphur isotopic signature of the surrounding atmosphere whether the contributing sources are natural or anthropogenic, however, the rate at which

lichens acquire sulphur from the atmosphere remains vague. It is not clear from the sulphur isotopic literature if the signature of lichens gives the isotopic composition at a particular moment in time or if the isotopic signature accumulates over a period of time. If the latter is the case, it is important to know if the response time of *A. sarmentosa* and *B. capillaris* is weeks, months or years.

1.2.4 Lichen Transplant Studies

The examples discussed previously all utilized indigenous organisms, which are those plants that are already present in the field and act as biomonitors (Powell, 1997). This section will discuss a second method of biomonitoring where organisms are collected from one location and transplanted to another. In contrast to the previous studies, this transplantation system allows for the exposure time to be controlled, so that both temporal and spatial environmental changes can be determined (Powell, 1997). The objective of this study was to determine the rate at which the lichens, *A. sarmentosa* and *B. capillaris,* accumulate sulphur from the atmosphere, thus a transplant procedure was chosen for this particular study.

Lichen transplant studies have long been used as a method of assessing atmospheric pollution in a certain area. Transplant studies involve the relocation of lichen samples to sites of interest. Usually a sample of lichen is collected and analysed initially to determine element concentrations. Samples are then collected at specific time intervals for the time period of interest. The majority of transplants provides a way of studying the effects of current air pollution on lichen survival or to assess what

substances are being emitted at different times of the year (Richardson, 1992). At the end of a transplantation period, it is also possible to perform a variety of physiological tests. The most common tests include electrolyte leakage, ash content and chlorophyll content (Richardson, 1992).

In addition to the above-mentioned physiological tests, numerous transplant studies have measured the accumulation of air-borne mineral elements by lichens (Sloof, 1995; Garty *et al.*, 1996; Garty *et al.*, 1997 Loppi *et al.*, 1998; Bennett *et al.*, 1996; Makholm & Bennett, 1998).

One such example is a lichen transplant study performed in The Netherlands by Sloof (1995). The lichen, *Parmelia sulcata*, was exposed to the atmosphere for periods of up to 12 months. Also, bulk (wet and dry) deposition was collected on a monthly basis. This study yielded accumulation factors for cobalt, scandium and zinc. The lichens were determined to be effective biomonitors because they reflected the metal content of the bulk deposition. Temporally, the results indicated that at least 12 months of exposure was required to distinguish the concentration of the exposed lichen from the starting concentration of the material used in this study.

Another study by Loppi *et al.* (1998) in central Italy measured a variety of metals (Cd, Cr, Cu, Pb, and Zn) in transplanted samples of the lichen, *Evernia prunastri*. Lichen samples were moved from an unpolluted area into an urban environment. The objective of this study was to determine the amount of time the transplanted lichens needed to accumulate the metals. It was found that after only 2 months, concentrations of all elements were statistically higher in transplanted lichens than in control samples. It was

suggested that motor traffic was the main source of Cr, Cu and Pb. The Cd and Zn, on the other hand, were believed to originate from the use of fertilizers and pesticides in the surrounding farmlands.

Finally, a study by Makholm & Bennett (1998) measured the accumulation of mercury in transplanted samples of the lichen, *Hypogymnia physodes*, at varying distances from a chlor-alkali plant in central Wisconsin. Lichen samples were tested quarterly for 1 year. It was found that the lichens effectively accumulated mercury. The concentrations of mercury decreased with increased distance from the chlor-alki plant. Elevated tissue concentrations of mercury were detected after 3 months at all distances. The mercury concentration continued to increase over the one-year study period at the sites closest to the chlor-alki plant. It is unclear from the data whether or not mercury would continue to accumulate beyond the duration of this study in transplanted lichens. It was suggested that if the lichens were exposed longer than 12 months, they might have accumulated higher levels of mercury.

All of the above transplant studies measured metal content. Literature involving the measurement of sulphur in transplant studies is not as abundant as literature measuring metal content.

A lichen transplant study by Cañas *et al.* (1997) measured the chemical response of *Parmotrema austrosinense* and *Parmotrema conferendum*. Samples were moved from a non-polluted area and transplanted to a downtown site in Córdoba, Argentina. A variety of chemical responses including pigments, malondialdehyde, hydroperoxy-conjugated dienes and sulphur was measured over an exposure period of six months.

Changes were observed throughout the exposure period in both species and in both sites. In particular, the sulphur concentration in the thalli of *P. austrosinense* showed a large increase in the urban site during the sixth month of exposure. However, the statistical analysis of the sulphur levels did not determine if the change in sulphur levels was due to the transplantation site or the exposure period.

A study by Bennett *et al.* (1996) measured elemental concentrations in the lichen *Hypogymnia physodes* transplanted along Lake Michigan. The study was performed to determine whether the air quality has improved enough so that lichens could recolonize in Indiana Dunes National Lakeshore Park. Samples of *H. physodes* were transplanted from Door County, Wisconsin to the national park and three other sites along the western shore of Lake Michigan. The lichens were sampled for 3 years and tissue concentrations of 20 chemical elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Zn) were measured. It was found that all but two elements (K and B) increased in concentration as the national park was approached from north to south. The greatest increase occurred in the third year of the experiment. Of particular interest is the element sulphur that increased 91% from north to south in the third year.

A study by Garty *et al.* (1997) measured the elements S, V and Ni in local and transplanted lichen samples to determine the environmental impact of pollutants on vegetation emitted by combustion of heavy fuel oil. The site of the study was the town of Ashdod in southwestern Israel that was polluted by the Eshkol heavy oil-fueled power plant and oil refineries, as well as other industrial sources. Samples of the lichen, *Ramalina duriaei,* from the peripheral region of the town and the HaZorea Forest located

100 km away from the town, were analysed and compared. Also, lichen thalli were taken from the HaZorea Forest and transplanted into the Ashdod region for a 10 month period. At the end of the study period, the elemental contents as well as other tests were performed on the local and transplanted lichens. High concentrations of S, V and Ni were found in the Ashdod region that corresponded with previous measurements of SO_2 and V in the Ashdod region. It was found that 10 months was sufficient time to observe a significant increase in the three measured elements of the transplanted lichens.

Finally, a transplant study by Palomäki *et al.* (1992) determined the rate of accumulation of sulphur and fluoride by measuring the concentrations of these elements. For this experiment, healthy lichens were collected from unpolluted environments and transplanted near a fertilizer plant and a strip mine (Palomäki *et al.*, 1992). This transplant experiment had two main objectives. First, it aimed to assess the rate of accumulation of sulphur and fluorides in *Hypogymnia physodes* thalli. Secondly, it aimed to study the relationship between the accumulation of toxic elements and the formation of visible and ultrastructural injuries in lichens (Palomäki *et al.*, 1992). The transplanted lichens were collected periodically and analysed for total sulphur and fluorides. It was found that after 5-6 months, the transplanted lichens had the same sulphur and fluoride contents as the original lichens growing at the same sites. There was a definite increase in the rate of accumulation of sulphur as industrial point-sources were approached. Also, injuries appeared in all samples when the sulphur content had increased by 200-300 ppm from the control level (Palomäki *et al.*, 1992).

As can be seen from the above examples, the literature gives a wide range of exposure times that a variety of transplanted lichen species need to accumulate sulphur. Thus, the purpose of our transplant experiment was to determine the rate at which the pendulous lichens, *Alectoria sarmentosa* and *Bryoria capillaris,* acquire sulphur from the surrounding atmosphere in the lichen thallus by monitoring the changing sulphur stable isotopic signatures and sulphur concentration. There was no relevant literature available measuring these parameters using the transplant technique.

1.3 Meteorological Considerations

This study also incorporated meteorological data to help explain the change in isotopic compositions throughout the period of the experiments. Lichens have evolved as a successful group of organisms mainly due to the fact that they can survive very harsh conditions. For example, during very dry periods, lichens become dormant by slowing their metabolic rates significantly. During these dormant periods, elemental accumulation is reduced greatly. Reports indicate that lichens accumulate sulphur efficiently only when they are moist (Palomäki *et al.*, 1992). Therefore, it is important to incorporate meteorological data into this study to determine periods of extreme dryness and wetness that may help explain why sulphur is accumulated at different rates at different times.

CHAPTER 2

METHOD

2.1 Transplant Sites and Techniques

Lichen samples of *A. sarmentosa* and *B. capillaris* were taken from the Bonavista Peninsula and transplanted into the MUN Botanical Garden in St. John's. Previous isotopic studies by Wadleigh & Blake (in press), Jamieson (1996) and Evans (1996) demonstrated that both rainwater and lichen samples from the Bonavista Peninsula displayed significantly elevated sulphur isotopic compositions (~ +15‰), whereas the rainwater sulphur isotopic composition from the city of St. John's was significantly lower (~ +7‰). Due to the large difference in atmospheric isotopic compositions, the Bonavista Peninsula and St. John's were the two sites chosen for this experiment (Figure 2.1). Theoretically, the range between the isotopic compositions of the two locations provided an ideal situation in which to see a definite change in sulphur isotopic composition of the lichen samples.

2.1.1 Bonavista Peninsula

The Bonavista Peninsula is located on the eastern portion of the island (Figure 2.2). It is bounded on the west by Bonavista Bay and on the east by Trinity Bay. The peninsula is about 95 km in length and less than 16 km in width. It was an ideal location from which to collect the lichens for the transplant experiment for three main reasons: 1) the sulphur isotopic composition of the lichens was high as a consequence of the abundance of seaspray sulphate present in the atmosphere; 2) the area consisted of

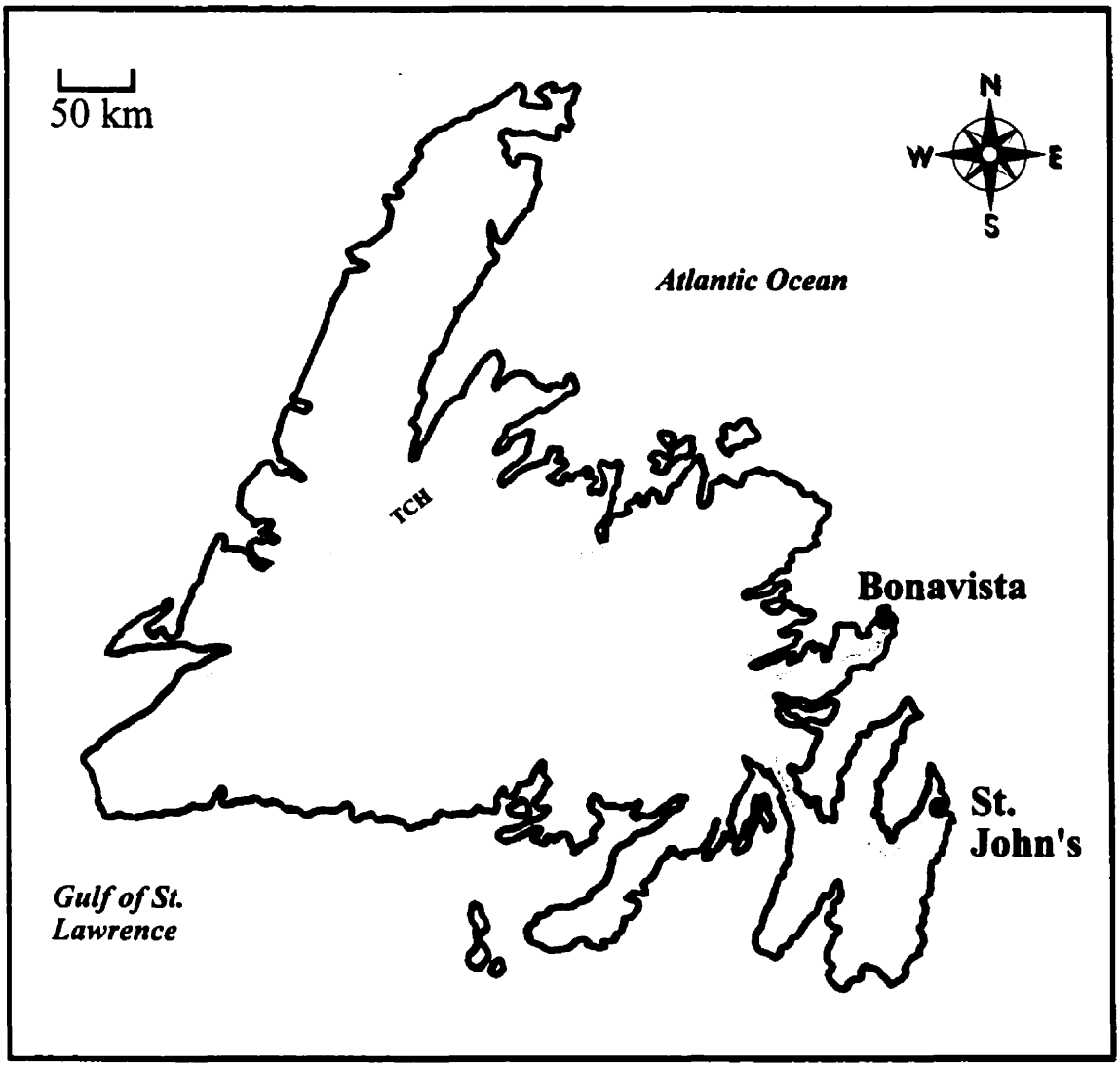


Figure 2.1 Map of Newfoundland showing the location of the two sites utilized in the transplant experiment (TCH= Trans Canada Highway).

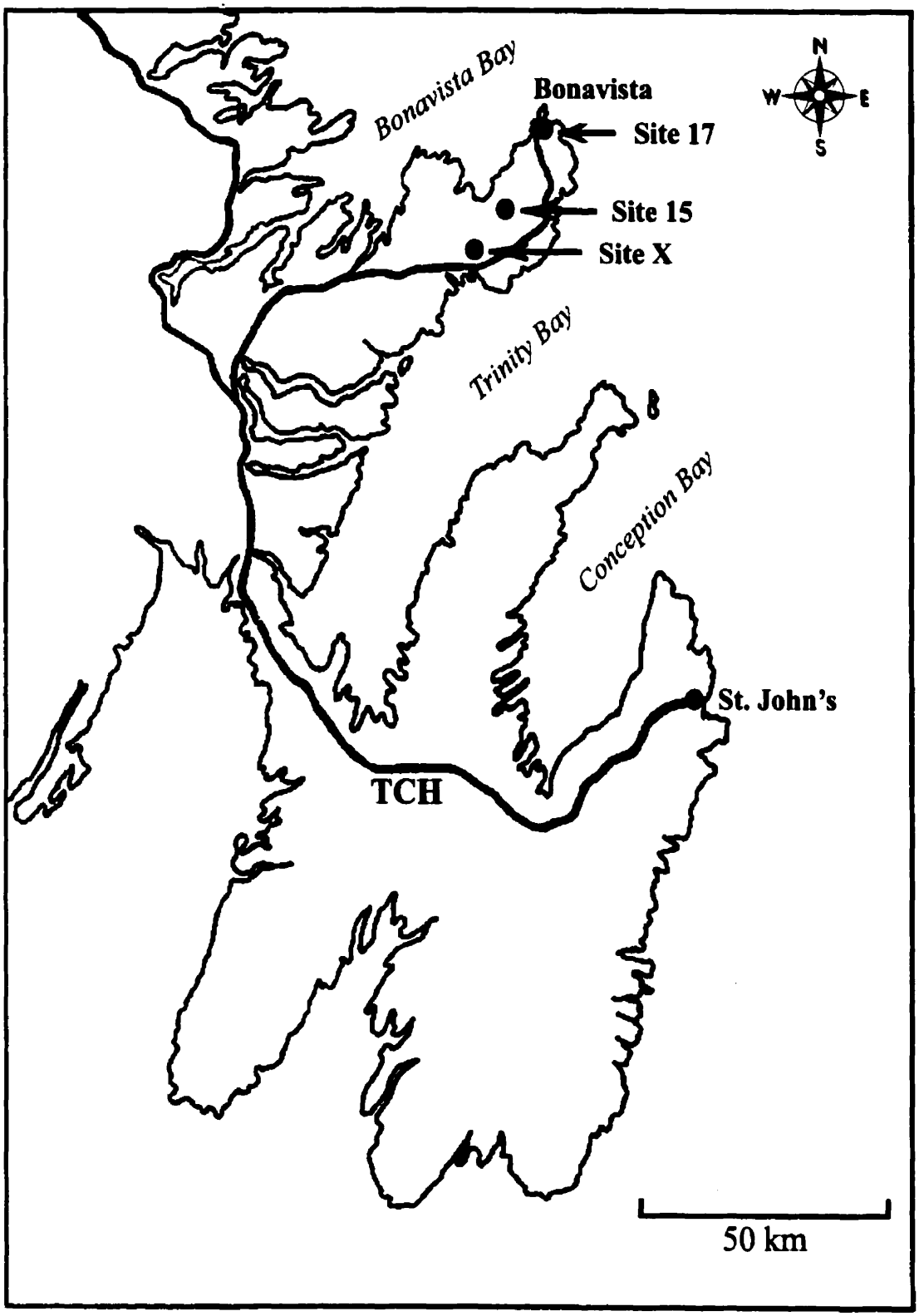


Figure 2.2 Map of Bonavista and Avalon Peninsulas, Newfoundland showing the sites from which the lichens were removed and the site to which they were transplanted.

numerous coniferous trees bearing the lichens, *A. sarmentosa* and *B. capillaris*, in great abundance; and 3) the Bonavista Peninsula was accessible throughout the year.

Three sampling sites (X, 15 and 17) were chosen along the Bonavista Peninsula using information from forest inventory maps produced by the provincial Department of Forestry (Figure 2.2). Each site demonstrated the following criteria:

- numerous coniferous trees (balsam fir (*Abies balsamea*), black spruce (*Picea mariana*)) between 40-60 years of age
- *A. sarmentosa* and *B. capillaris* present in great abundance
- located at least 50 m from the nearest road
- located in a non-sheltered environment in order to intercept a natural amount of atmospheric deposition
- no industrial point sources in the vicinity

Site 17 was located in the town of Bonavista, while sites X and 15 were located off the main highway along the Bonavista Peninsula.

In June 1997 and June 1998, approximately 100 branches of balsam fir and black spruce (10-20mm diameter) containing *A. sarmentosa* and *B. capillaris* were detached and placed in large clear plastic storage bags for transport. To reduce the number of variables affecting the results, branches with attached lichen were only taken from living trees. To prevent contamination of the lichen, non-powdered latex gloves were worn. The branches containing the lichen were transported to the MUN Botanical Garden, St. John's, for transplantation.

2.1.2 MUN Botanical Garden, St. John's

St. John's, the capital city of Newfoundland, is located on the Avalon Peninsula (Figure 2.2). The population of the city of St. John's is approximately 102,000. As with most eastern North American cities, the atmosphere surrounding the city is anthropogenically-influenced (Jamieson, 1996).

The MUN Botanical Garden was considered to be an ideal location in which to transplant the lichens (Figure 2.3). The isotopic composition of the atmosphere is significantly different from that of the Bonavista Peninsula. Also, the MUN Botanical Garden was located very close to Memorial University of Newfoundland, thus it was accessible throughout the time period of the experiment for collection at specified time intervals. Finally, there was a great abundance of pendulous epiphytic lichens already growing in the Botanical Garden, therefore it was deduced that this location was suitable for growth of the transplanted lichens.

Three sites along the trails of the Botanical Garden (A,B and C) were chosen for this experiment (Figure 2.4). The three sites chosen at the Botanical Garden contained local *A. sarmentosa*, thus the sites were suitable habitats to ensure growth and minimize the amount of stress on the transplanted lichens.

The branches containing lichen from the Bonavista sites were hung with string on the trees present in each site at the Botanical Garden.

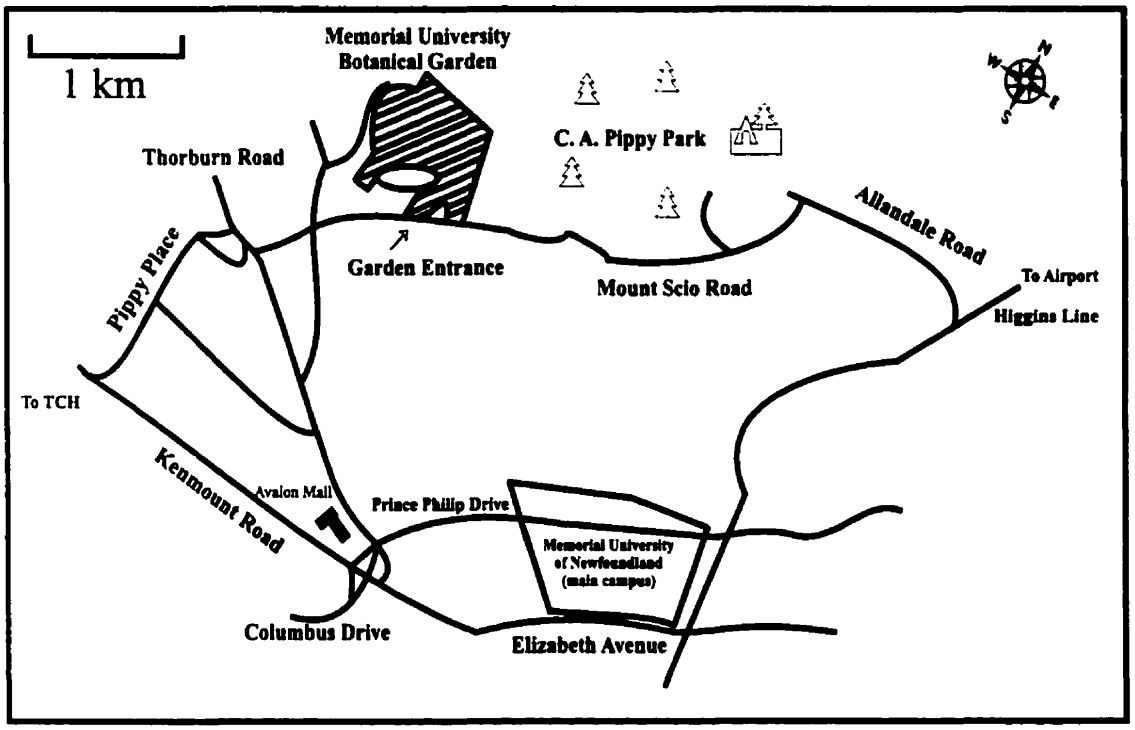


Figure 2.3 Map of St. John's, Newfoundland study area (modified from MUN Botanical Garden Web Page, used with permission).

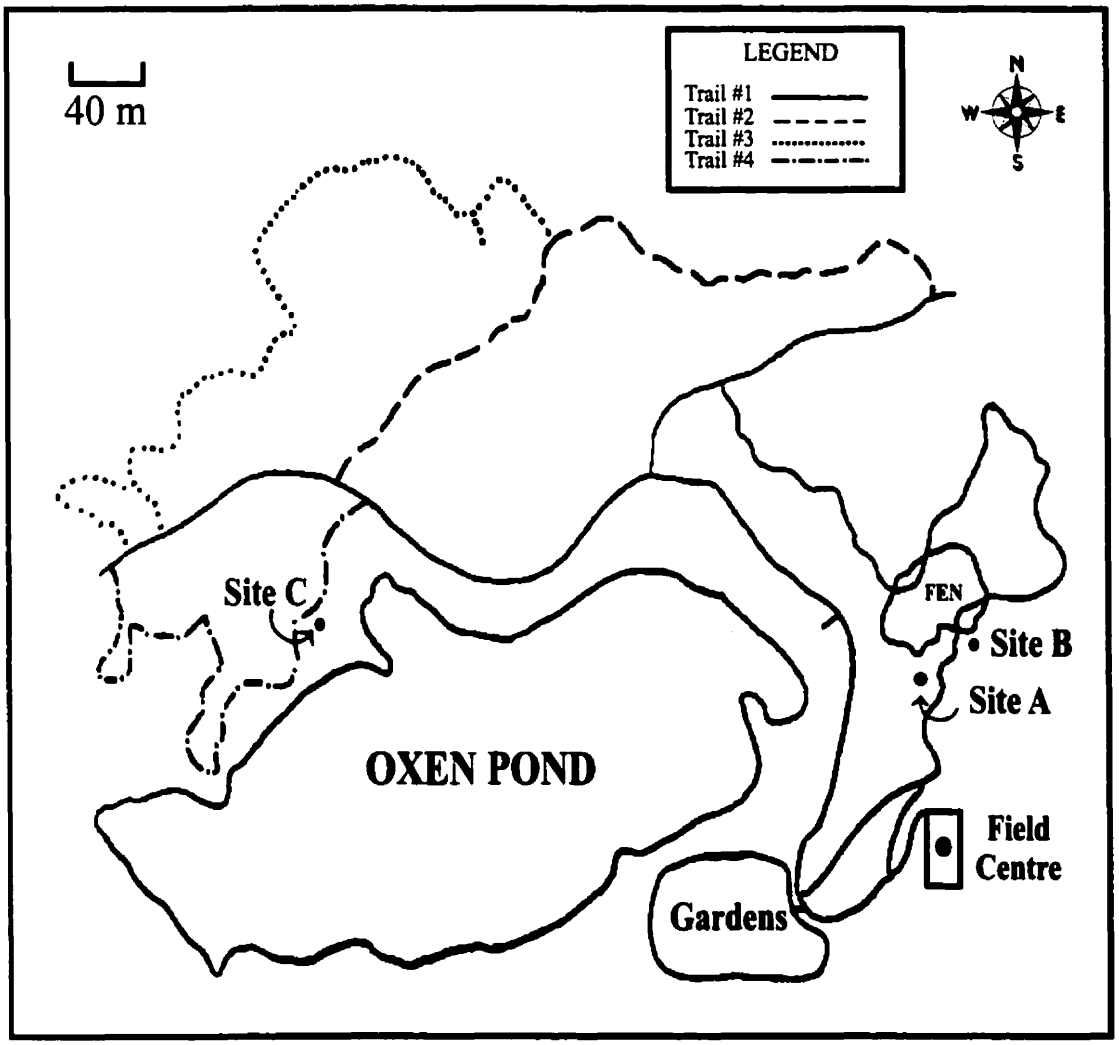


Figure 2.4 Location map of the three transplant sites along the trails of the MUN Botanical Garden (modified from MUN Botanical Garden pamphlet, used with permission).

2.1.3 Experimental Design

In the design of the primary experiment (Experiment I) some thought was given to the possibility of lichens dying through the course of the study. Three collection sites and three transplant sites were chosen so that in the event of the failure of one or more samples/sites there would still be a chance to complete the experiment. Lichens were collected from three separate sites along the Bonavista Peninsula expecting that at least one of the sites would have lichens with an isotopic composition higher than that of the Botanical Garden and close to 15‰. One-third of the lichen-covered branches from each Bonavista site (X, 15, 17) were then transplanted to each of the botanical garden sites (A, B, C).

The primary transplant experiment commenced in June 1997. On a monthly basis for a period of one year, a composite sample of approximately three lichen thalli from each site was collected and brought to the laboratory for analysis.

Based on preliminary analytical results a second transplant experiment (Experiment II) was started in June 1998 after the first experiment ended. This experiment lasted for three months with sample collection every week.

2.1.4 Control Measures

In order to determine if the sulphur isotopic compositions in Experiment I and II changed because of differences in the atmosphere surrounding the lichens, it was necessary to perform two control experiments.

The first control experiment was performed to test for natural variation in the sulphur isotopic composition and concentration of *A. sarmentosa* originally growing in the MUN Botanical Garden. Native lichens were sampled periodically for one year to determine the sulphur isotopic composition and concentration.

A second control experiment was performed to ensure that the act of transplantation (i.e., moving lichens from one location to another) did not influence the isotopic compositions of Experiments I and II. Five branches containing *A. sarmentosa* originally growing in the MUN Botanical Garden were moved from one site and transplanted to another site in the Botanical Garden. This transplanted lichen was then sampled every two weeks for the period of three months.

2.2 Lichen Identification

The transplant study was designed to utilize pendulous epiphtyic lichens mainly due to their great abundance across Newfoundland. Also, the majority of the lichens utilized in previous Newfoundland studies were of this type, thus there was a significant amount of data already collected that could aid in analysis.

In the field, the pendulous lichens from each of the three sites along the Bonavista Peninsula looked very similar upon initial observation. The lichens collected from sites X and 15 were identified as *Alectoria sarmentosa* while those from site 17 were identified as *Bryoria capillaris*.

The *A. sarmentosa* samples had a definite pendant yellow-green thallus lacking a central cord. The chemistry of the site X lichens yielded a KC+ red reaction with the

medulla. The samples from site 15, on the other hand, displayed a KC- reaction in the medulla. Evidently, the *A. sarmentosa* from site 15 were a relatively uncommon chemical race lacking alectoronic acid (Brodo *et al.*, in preparation; Brodo & Hawksworth, 1977; Richardson, 1992).

The *Bryoria capillaris* samples had a greenish-gray pendant thallus. The two distinguishing characteristics that led to accurate identification included the presence of distinct pseudocyphellae and a K+ bright yellow reaction with the thallus (Brodo *et al.*, in preparation; Brodo & Hawksworth, 1977; Richardson, 1992).

Even though the epiphytic pendulous lichens used in this study belonged to two different genera, it was expected that they would react similarly due to their similar morphology and overall ecology. Any difference in results between the two genera would be interesting for interpretation purposes.

2.3 Stable Isotopic Analysis

Preparation of lichen samples for stable isotopic analysis is quite extensive (Figure 2.5). Each step in the procedure will be described briefly below.

2.3.1 Sample Collection

At specified time intervals, samples of *A. sarmentosa* and *B. capillaris* were collected from the group of transplanted lichens placed in the MUN Botanical Garden. To prevent contamination of the sample, non-powdered latex gloves were worn when removing the lichen from the branch. To ensure homogeneity in the sample, composite

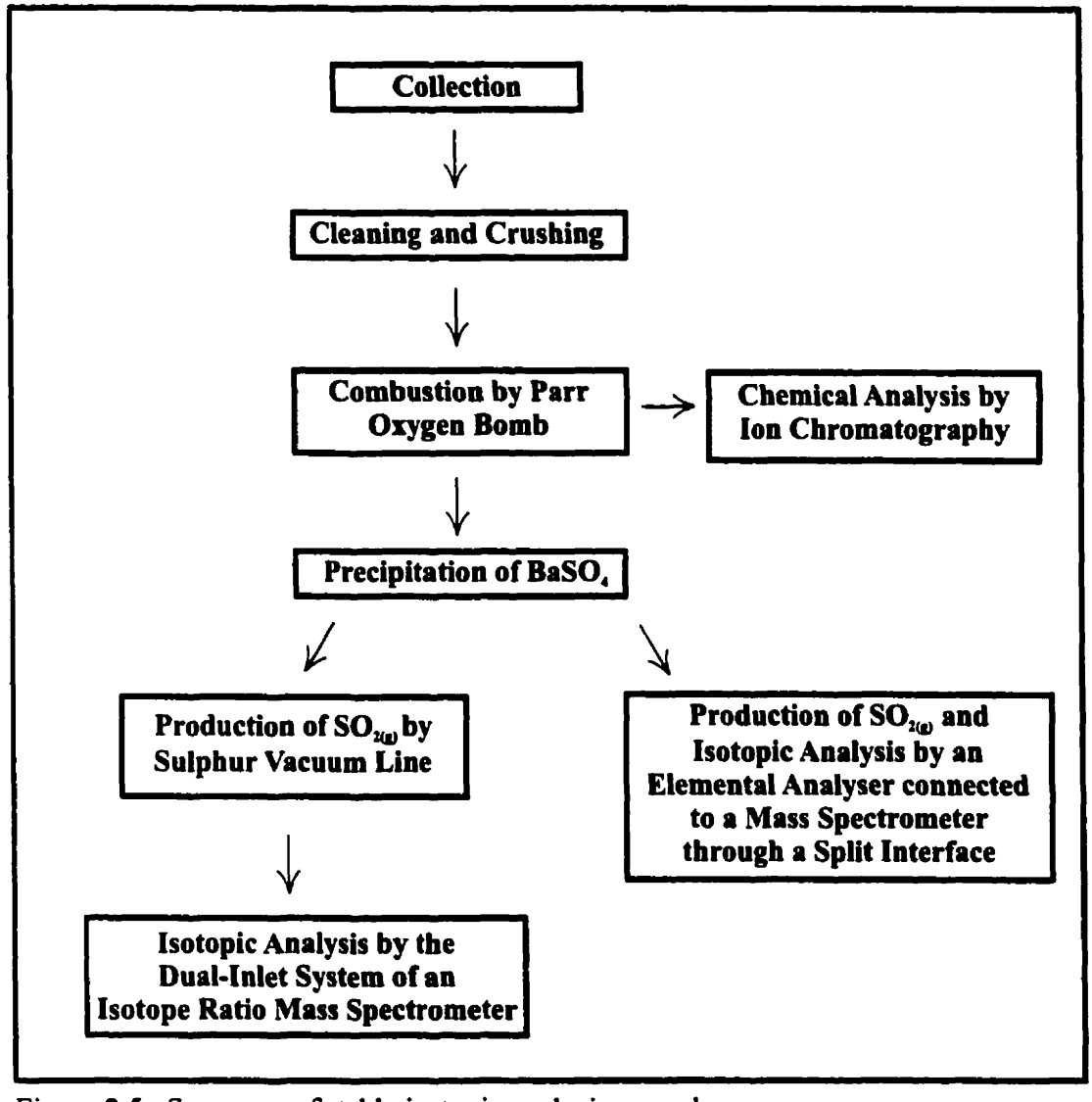


Figure 2.5 Summary of stable isotopic analysis procedure.

samples consisting of thalli from approximately three different plant-bodies were collected. The specimen was placed in a plastic Ziploc™ storage bag when processed immediately. To store a sample for later use, paper-sampling bags are preferable to prevent mold growth.

After the collection of *A. sarmentosa* and *B. capillaris* in the field, the specimens were transported to the laboratory, spread out on a clean surface, covered by large lint-free Kimwipe™ towels and allowed to air dry for approximately one week or until the lichens were brittle to touch.

2.3.2 Sample Cleaning and Crushing

When the samples were completely dry, all detritus such as twigs, insects and other lichen species was removed using clean stainless steel forceps and a large magnifying lamp. The lichen samples were crushed into a fine powder using a tungsten carbide puck mill. The powder was transferred into 20 ml vials, labeled and stored at room temperature. The puck mill was thoroughly cleaned with quartz sand and denatured alcohol before use and between samples to prevent cross-contamination.

2.3.3 Sample Combustion by Parr Oxygen Bomb

The Parr Oxygen Bomb is a stainless steel container able to withstand high pressure and temperature. The powdered lichen sample is combusted by the ignition of nickel alloy fuse wire under 30 atmospheres of oxygen. As in all combustion reactions,

many products result including CO_2, H_2O and SO_2. However, for the purpose of this experiment only the resultant sulphur compounds were utilized for further analysis.

Before the ignition of the fuse wire, a mixture of deionized water and 50% hydrogen peroxide was added to the bottom of the Parr bomb container to ensure that all oxidized sulphur was in the form of soluble sulphate after combustion. A series of washings with deionized water resulted in a solution containing all oxidized sulphur from the lichen sample. A more detailed description of the Parr Oxygen Bomb procedure can be found in the Appendix I.

Due to the fact that lichens contain a relatively small amount of sulphur, it was necessary to combust 3-4 g of powdered lichen sample to obtain enough sulphur for analysis. Thus, 3-4 separate Parr bomb combustions were performed on each lichen sample.

2.3.4 Aliquot Retrieval for Chemical Analysis

The total washings from 3-4 combustions were collected in one beaker and filtered through 0.45 μm cellulose nitrate membrane filters.

After filtration, the solution was brought to a known volume (i.e., 200 ml or 500 ml) in a volumetric flask with deionized water. The solution was mixed well and a small amount (approximately 15 ml) was transferred into a vial and refrigerated until analysis by ion chromatography (see Section 2.4). The remaining solution was utilized in the next step to obtain the $BaSO_4$ precipitate.

2.3.5 Precipitation of $BaSO_4$

The filtered solution was placed in a beaker and acidified to a pH of 4 by adding 2-3 drops of 8N HNO_3 (nitric acid) to ensure optimal conditions for $BaSO_4$ precipitation. The solution was placed on a hot plate and allowed to come to a boil. When the solution came to a boil, 10 ml of 10% 0.5M $BaCl_2$ (barium chloride) was added producing a white $BaSO_{4(s)}$ precipitate.

After 2-3 hours, the solution was filtered through Fisher Quant ashless filter paper to collect the precipitate. Two hundred fifty ml of warm water was poured through the filter to remove any Cl^- (chloride ions) that may have been present. The filtrate was discarded and the filter paper with the precipitate was dried in an oven.

The dry filter paper and precipitate was placed in a VYCOR™ crucible and the filter paper was slowly removed by heating over a Bunsen burner, leaving pure $BaSO_4$ powder.

2.3.6 Production of $SO_{2(g)}$ for Isotopic Analysis

The barium sulphate ($BaSO_{4(s)}$) obtained from the Parr bomb procedure was further processed for isotopic analysis. $SO_{2(g)}$ is the form of sulphur needed for isotopic analysis in the mass spectrometer. Thus, it was necessary to extract the sulphur from the barium sulphate powder and convert it to sulphur dioxide gas. This extraction was achieved by two methods due to a change in the lab equipment available for use. These were: (1) Conventional Sulphur Vacuum Line and (2) Continuous Flow-IRMS. When this experiment began in 1997, we used the conventional off-line method to convert the

barium sulphate powder into gaseous sulphur dioxide. The sulphur dioxide sample then was introduced into the mass spectrometer through the dual inlet system. In February 1998, the Isotope Ratio Mass Spectrometry lab at Memorial University acquired an elemental analyser and ConFlo II interface that upgraded the lab to the on-line continuous flow method. This apparatus allows $BaSO_{4(s)}$ to be combusted in an elemental analyzer and the $SO_{2(g)}$ is separated from the other combustion gases by gas chromatography. The $SO_{2(g)}$ enters the ion source of the mass spectrometer through a split interface (Giesemann *et al.*, 1994).

The major advantage of the on-line method is a reduction in the time- and chemical-consuming preparation of sulphur from organic samples associated with the off-line method. It was found by Giesemann *et al.* (1994) that the on-line method required a much smaller amount of sulphur and the $\delta^{34}S$ values obtained by the on-line method were identical within the standard deviation range to the off-line method.

Both the on-line and off-line methods were utilized during this experiment therefore each method will be explained separately.

2.3.6.1 Sulphur Vacuum Line

Thermal decomposition of $BaSO_4$ has been used for several years as a reliable method to prepare $SO_{2(g)}$ for sulphur isotopic analysis (Yanagisawa & Sakai, 1983). A mixture of barium sulphate ($BaSO_{4(s)}$), vanadium pentoxide (V_2O_5) and silica (SiO_2) is combusted at ~ 950°C and the gaseous products from the combustion reaction moved through a network of glass tubing. By using cryogenic separation, $SO_{2(g)}$ is purified and

collected for analysis. The procedure as outlined in Yanagisawa & Sakai (1983) was followed to collect $SO_{2(g)}$ for isotopic analysis.

2.3.6.2 Continuous Flow-IRMS

For on-line analysis, a Carlo Erba NA 1500 elemental analyzer was connected to a Finnigan MAT 252 mass spectrometer through a split interface (Figure 2.6). The $BaSO_{4(s)}$ samples were wrapped together with 0.1mg of V_2O_5 in tin capsules and flash combusted at 1050°C with a pulse of oxygen in the elemental analyzer. All gases produced during the combustion were carried in a stream of helium (80 ml/min flow rate) through a column packed with tungstic oxide, elemental copper and quartz wool to trap any excess O_2 and oxidize any traces of SO_3 produced from the combustion to SO_4. A desiccant trap filled with anhydrous $Mg(ClO_4)_2$ removed all water vapour. At this point, the remaining gas passed through a Teflon GC column (heated to 90°C) where $SO_{2(g)}$ was separated from $N_{2(g)}$ and $CO_{2(g)}$. The $SO_{2(g)}$ leaving the elemental analyzer was routed through the split interface to the ion source of the mass spectrometer.

2.3.7 Mass Spectrometry

The sulphur isotopic analysis was performed on a Finnigan MAT 252 isotope ratio mass spectrometer. Depending on the method used to obtain the $SO_{2(g)}$ (off-line vs. on-line), either the dual-inlet or interface system was used to introduce the gas to the mass spectrometer. The SO_2 samples obtained from the sulphur vacuum line entered the mass spectrometer by the dual inlet system. This system consisted of a symmetrical dual

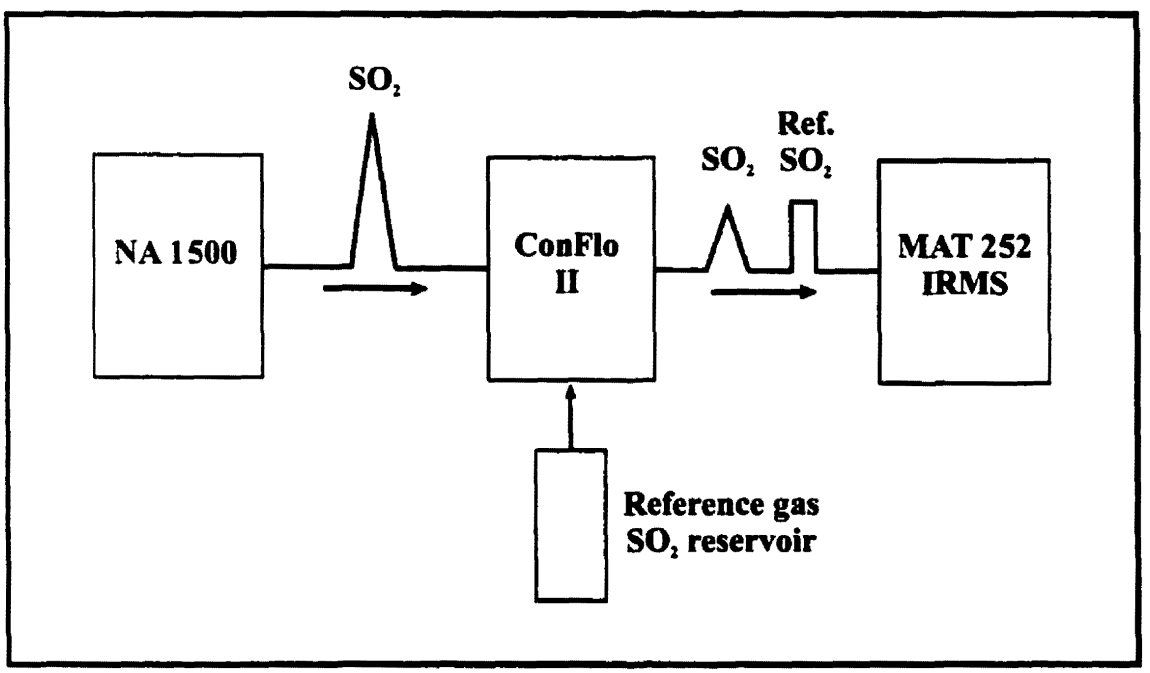


Figure 2.6 Schematic of on-line analysis using a Carlo Erba NA 1500 elemental analyzer connected to a Finnigan MAT 252 mass spectrometer through a ConFlo II split interface (modified from Finnigan MAT Application Flash Report).

set-up enabling alternating measurements of sample and standard gases. The internal standard was MUN pyrite.

After the sample was introduced into the mass spectrometer via dual inlet, it encountered an electron impact source. Samples were ionized by collision with a stream of electrons produced by thermionic emission of a tungsten filament (Potts, 1992). The beam of electrons from the filament was directed between two parallel plates and collimated using a weak magnetic field. An electric field drew the positive ions out of the electron beam and accelerated them towards the analyzer.

The mass analyzer split the ion beams emerging from the source according to their mass/charge ratios (Potts, 1992). The ions were injected into a magnetic field where the heavier and lighter ions were separated and followed different trajectories (Figure 2.7).

After passing through the magnetic field, the separated ions were collected in ion detectors called Faraday cups. The two Faraday cups used for sulphur analysis collected ions with mass 66 and 64. The ion detectors were grounded through a high capacity resistor. As the current went to ground, the potential drop in the resistor acted as a measure of the ion current. Ion beams resulting from the various masses of interest were collected simultaneously and their respective ratios were measured. The ion current of the masses 66 and 64 in the sample gas was compared to the corresponding ion currents of an internal reference gas. Each sample was analyzed for ten sampling intervals during which time both the standard and the sample were measured. The ISODAT software calculated the isotopic ratios as well as raw δ values and corrected δ values to the

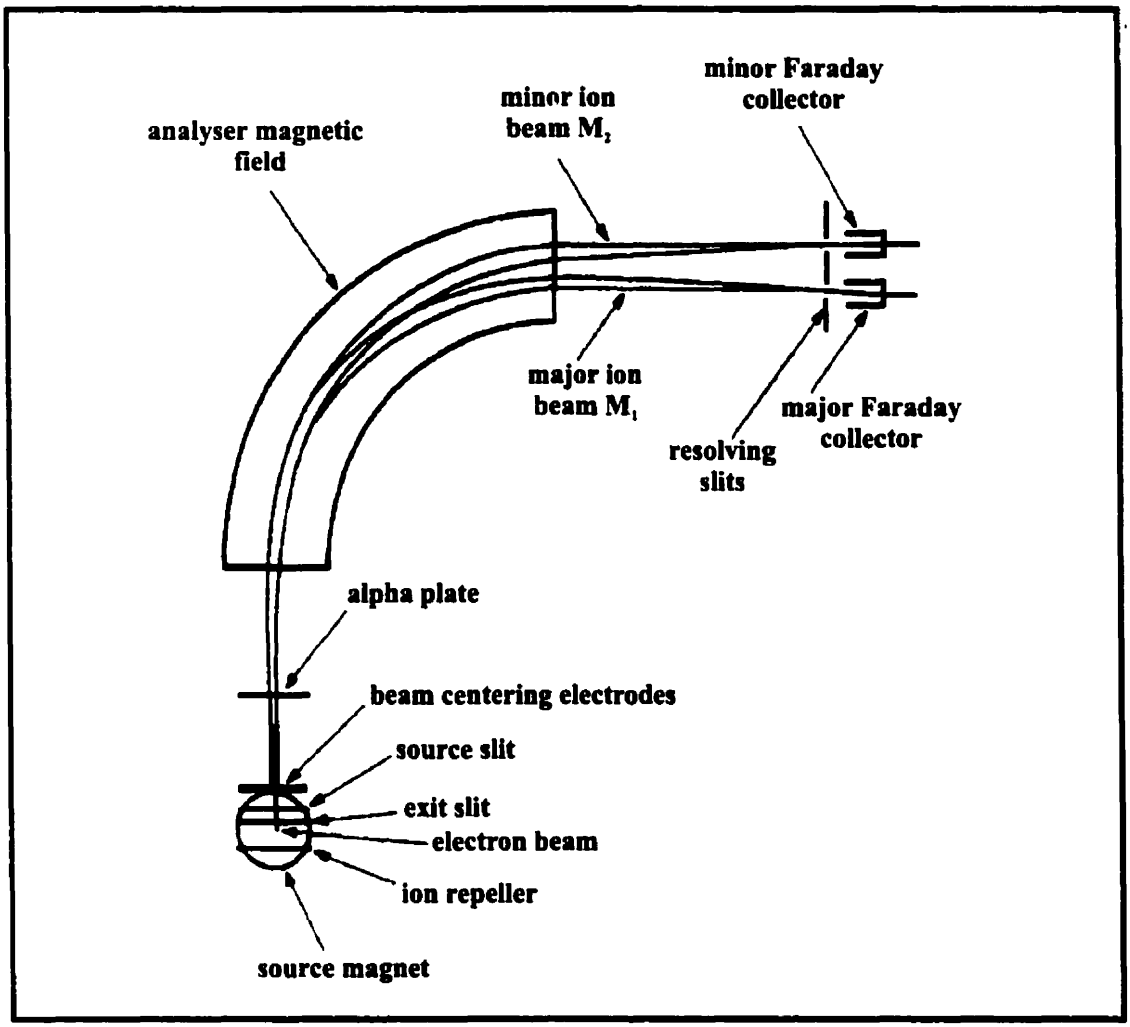


Figure 2.7 A gas source mass spectrometer designed for dual collection of a major and minor ion beam (from Potts, 1992).

international CDT standard. Along with the lichen samples, the international NIST standard, NBS-127, was analyzed to ensure accuracy throughout the analysis.

The SO_2 samples produced by the on-line method entered the mass spectrometer through a ConFlo II split interface. This system consisted of a capillary tube that directs helium carrier gas and SO_2 from the EA into the mass spectrometer (Figure 2.8). Once inside the mass spectrometer, the sample $SO_{2(g)}$ was ionized and followed the same path as described above with the dual inlet system.

The reference gas originated from a separate reservoir attached to one of the inlet valves used in the dual inlet method. It was injected for a specified time interval when the sample gas was not being measured. The software then measured and integrated the areas underneath the peaks to determine the isotope ratio. Numerous standards (NBS-127; BaSO4 #10) were analysed with each set of samples. These were used to calibrate the raw data and calculate a corrected $\delta^{34}S$ value for each sample.

2.4 Chemical Analysis by Ion Chromatography

The sulphur concentration of each lichen sample was also determined. The small aliquots of Parr bomb aqueous solutions were analyzed for SO_4^{2-} using 1.8mM sodium carbonate (Na_2CO_3) + 1.7mM sodium bicarbonate ($NaHCO_3$) solution as eluent flowing at a rate of 1.5 ml/min through an AS4A resin-packed column on a Dionex DX-100 ion chromatograph. The ion chromatograph was calibrated using National Institute of Standards and Technology (NIST) stock standard solutions. To ensure accuracy throughout the run, United States Geological Survey (USGS) reference waters, M108,

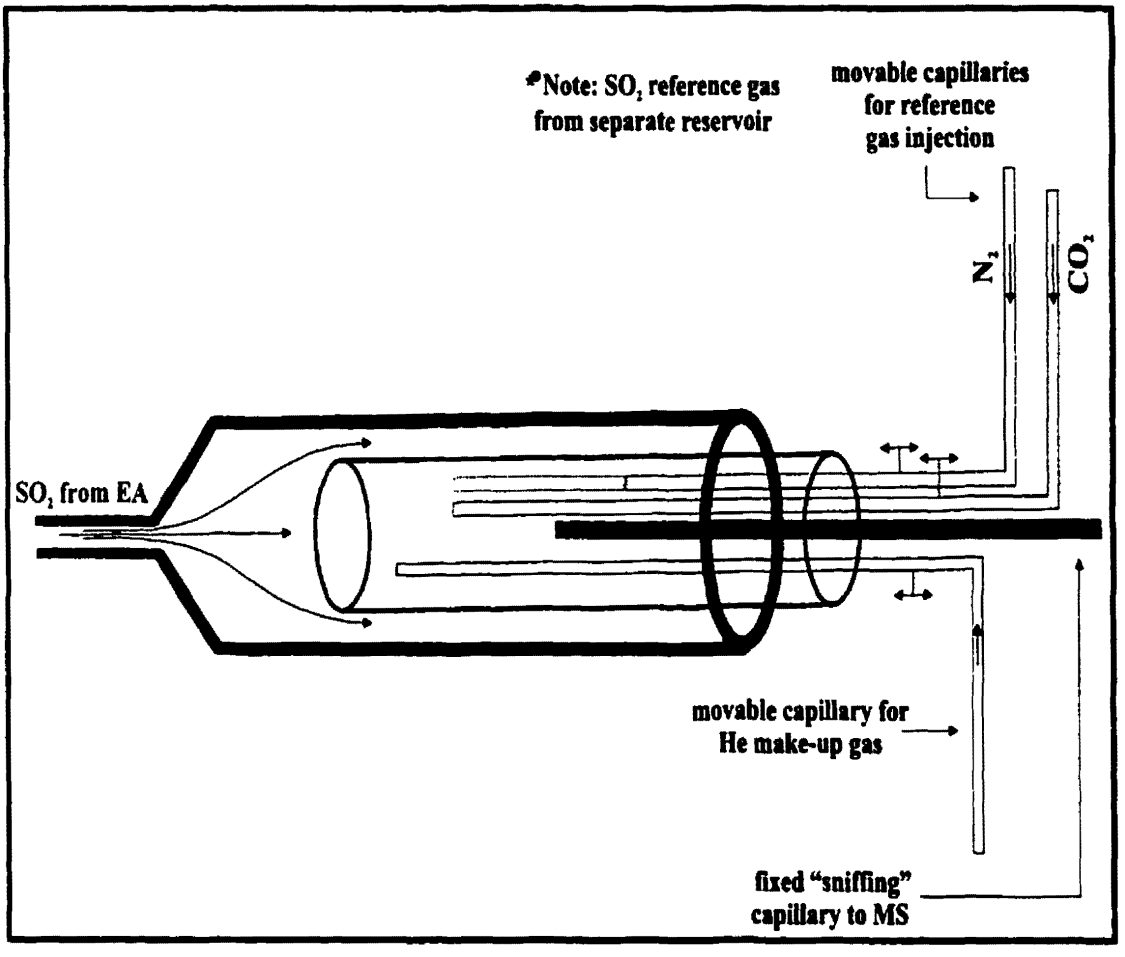


Figure 2.8 Schematic of ConFlo II split interface (from Finnigan MAT 252 Operator Course Notes, Nov 17-21, 1997).

M110, and M112, were used. The concentration of sulphate was measured in parts per million (ppm). By utilizing the sample calculation detailed in Appendix II, the concentration of sulphur in the lichen sample was determined.

2.5 Error Associated with Analytical Procedures

The $\delta^{34}S$ values obtained by using the conventional off-line method have a maximum error of ● 0.4‰ (standard deviation (1σ)). Five samples of $BaSO_{4(S)}$ obtained from the same lichen samples were analysed consecutively by Blake (1998) and it was found that the sample preparation led to an error of approximately 0.4‰. The majority of this error was due to a slight difference in the crushing method utilized. Blake (1998) used liquid nitrogen to crush the lichen samples as opposed to the puck mill utilized in this study. It is believed that crushing of lichen samples utilizing the puck mill results in a more homogeneous sample as a fine powder. Thus, the error associated with the $\delta^{34}S$ values obtained by the conventional method is actually better than ● 0.4‰.

The $\delta^{34}S$ values obtained using the on-line method have an associated error of ± 0.31‰. Thirty $BaSO_{4(S)}$ samples obtained from the same lichens were run consecutively using the on-line method and the measured value was +5.9 ● 0.31‰.

Finally, the error associated with the ion chromatograph was also calculated. A total of four samples of the M108 standard and five samples of the M112 standard was analysed for SO_4^{2-}. The measured value of M108 was 182 ± 2ppm while the accepted value is 185.1 ● 3.2ppm. The measured value of M112 was 25.0 ± 0.3ppm while the

accepted value is 30.4 ● 0.4ppm. There is an error of approximately 5% associated with each sulphur concentration.

2.6 Meteorological Considerations

As was stated in Chapter One, the weather patterns of a specific location can significantly affect the amount of sulphur taken up by the lichen thallus. For this reason weather information was collected throughout the time span of this study. The St. John's Airport was the nearest location to the MUN Botanical Garden that produced weather information daily. To determine if the St. John's Airport weather data was similar to that of the MUN Botanical Garden, a comparison of temperature, relative humidity and wind speed was performed.

A total of 34 weather-testing events were taken over 14 days. The relative humidity at the MUN Botanical Garden was measured using a sling psychrometer. Triplicate readings of the wet and dry bulbs were averaged and the relative humidity was then determined using these values on a psychrometric chart.

The temperature at the MUN Botanical Garden was recorded as the average of the three dry bulb readings from the sling psychrometer.

Wind speed was measured using a wind anemometer.

Data on the weather at the St. John's Airport were accessible through the Environment Canada web page (http://www.ns.ec.gc.ca/weather/). At specific times daily, it was possible to compare measurements of temperature, relative humidity and wind speed at the MUN Botanical Garden and the St. John's Airport.

CHAPTER 3

RESULTS

3.1 Control Measures

In the first control experiment, it was found that the sulphur isotopic compositions and concentrations in the native lichens of the Botanical Garden changed over the course of the year (Table 3.1). The mean of the $\delta^{34}S$ values of lichens originally growing in the MUN Botanical Garden was +5.8 ± 0.8‰ while the mean of the concentrations was 500 ± 74 ppm. There was no obvious trend but clearly natural variation occurred which exceeded the analytical error of the experiment. This experiment also provided important baseline information about the lichens originally growing in the MUN Botanical Garden.

In the second control experiment, it was found that the isotopic compositions did not change significantly after the actual transplant (Table 3.2). The delta value of the lichen prior to this transplant was 6.9‰. Two weeks after the transplant the delta value was 7.0‰. For 12 weeks following the transplant procedure the isotope signature remained within the range of ± 0.4‰ which can be attributed to error associated with the analytical technique. Thus, it was concluded that the act of transplantation did not contribute to any observed change in isotopic composition of Experiments I and II.

Table 3.1 $\delta^{34}S$ and [S] values of local Botanical Garden lichens over a one year period (Control Experiment I). (n = 4)

MONTH	$\delta^{34}S$ (‰)	[S] (ppm)
Jun-97	5.1	594
Nov-97	5.8	457
Jan-98	5.2	521
May-98	6.9	427

Table 3.2 $\delta^{34}S$ and [S] values of local lichens moved from one site to another site in the Botanical Garden (Control Experiment II). (n = 7)

WEEK	$\delta^{34}S$ (‰)	[S] (ppm)
0	6.9	427
2	7.0	439
4	7.3	448
6	6.7	424
8	6.6	485
10	6.9	443
12	7.5	470

3.2 Experiment I

Lichens from three sites along the Bonavista peninsula (site X, 15, 17) were transplanted into three sites at the MUN Botanical Garden (site A, B, C) in June 1997 (Figures 2.2 and 2.4; Section 2.1.3). Sampling took place at the MUN Botanical Garden on a monthly basis.

A designated label identified each sample. The first letter (A, B or C) signified the Botanical Garden site from which the sample was collected. The second unit (X, 15 or 17) signified the site in Bonavista where the sample originated. The third digit (0,1,2,...) signified the month when the sample was collected with July being month 1. Therefore, the sample labeled A/X/3 was originally taken from site X on the Bonavista Peninsula and then placed in site A at the MUN Botanical Garden. The sample was collected from the Botanical Garden in the third month after the experiment commenced (i.e., September 1997).

It is important to note that certain data points are missing from the data set because the supply of transplanted lichens was becoming depleted. It was necessary to conserve the lichen by sampling some sites less often to ensure that the experiment lasted for a period of a year.

Throughout the one-year experiment a total of 82 samples was collected from all sites at the Botanical Garden and analysed for sulphur isotopic composition and sulphur concentration. Complete tables of all analytical results for Experiment I can be found in Appendix III.

Figure 3.1 graphically illustrates the $\delta^{34}S$ values and sulphur concentrations obtained for all lichen samples from Experiment I. The $\delta^{34}S$ values of site X lichens (*A. sarmentosa*) in site A decreased over a year from 9.0‰ to 6.7‰ (Figure 3.1a). Site X lichens in site B decreased from 9.0‰ to 7.3‰ (Figure 3.1a). Site X lichens in site C decreased from 9.0‰ to 6.9‰ (Figure 3.1a).

Site 15 lichens (*A. sarmentosa*) in site A decreased over a year from 13.3‰ to 8.6‰ (Figure 3.1b). Site 15 lichens in site B decreased from 13.3‰ to 8.7‰ (Figure 3.1b). Site 15 lichens in site C decreased from 13.3‰ to 8.0‰ (Figure 3.1b).

Site 17 lichens (*B. capillaris*) in site A decreased over a year from 12.0‰ to 9.4‰ (Figure 3.1c). Site 17 lichens in site B decreased from 12.0‰ to 9.1‰ (Figure 3.1c). Site 17 lichens in site C decreased from 12.0‰ to 8.5‰ (Figure 3.1c).

In all cases the isotopic compositions decreased over the one-year study period.

The sulphur concentrations of site X lichens (*A. sarmentosa*) in site A increased over a year from 242 ppm to 399 ppm (Figure 3.1d). Site X lichens in site B increased from 242 ppm to 412 ppm (Figure 3.1d). Site X lichens in site C increased from 242 ppm to 413 ppm (Figure 3.1d).

Site 15 lichens (*A. sarmentosa*) in site A increased over a year from 234 ppm to 457 ppm (Figure 3.1e). Site 15 lichens in site B increased from 234 ppm to 330 ppm (Figure 3.1e). Site 15 lichens in site C increased from 234 ppm to 452 ppm (Figure 3.1e).

Site 17 lichens (*B. capillaris*) in site A increased over a year from 494 ppm to 844 ppm (Figure 3.1f). Site 17 lichens in site B increased from 494 ppm to 928 ppm (Figure 3.1f). Site 17 lichens in site C increased from 494 ppm to 945 ppm (Figure 3.1f).

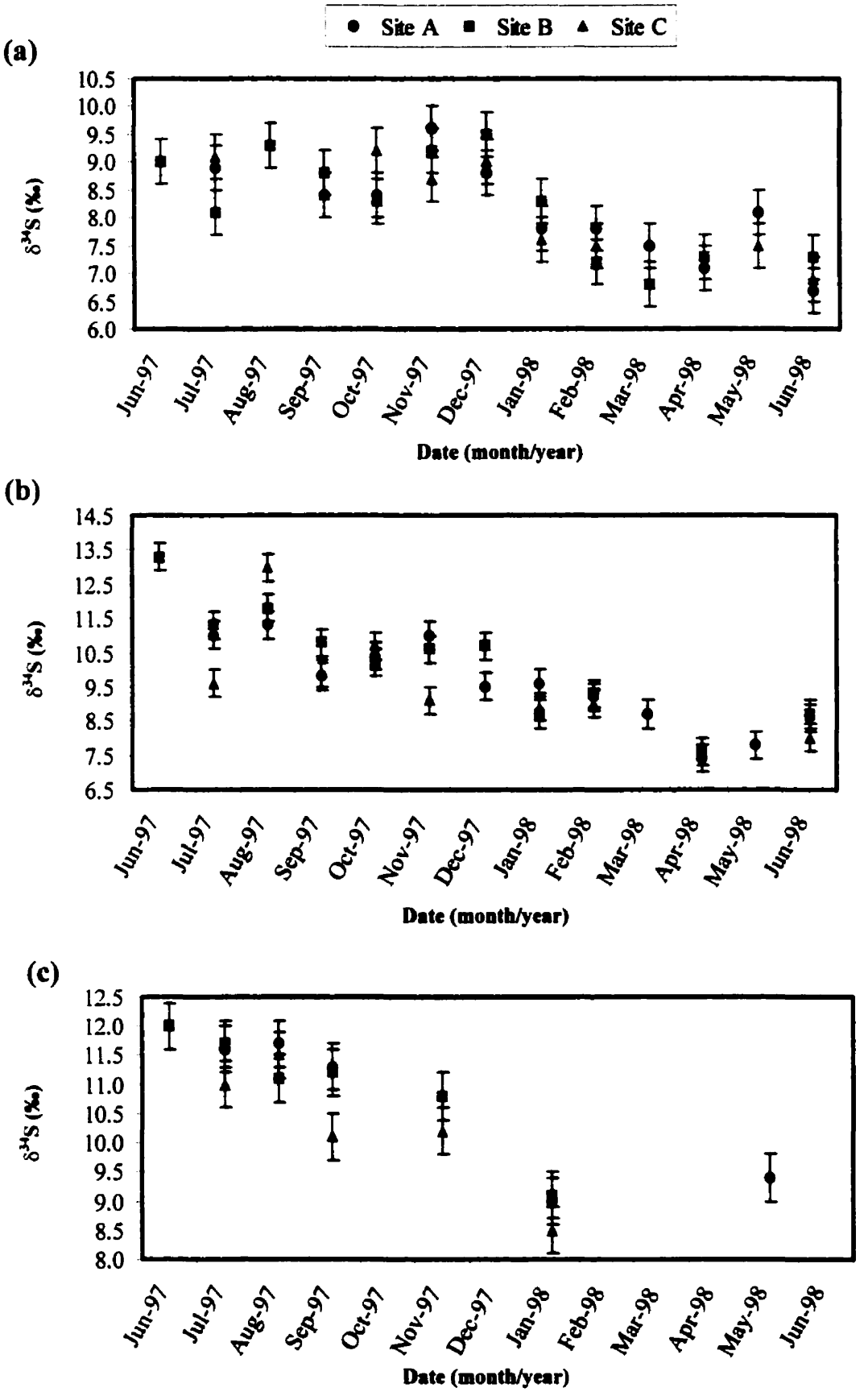


Figure 3.1 (a, b, c) $\delta^{34}S$ values for lichens moved from Bonavista sites (a) X (b) 15 (c)17 to the Botanical Garden over a one year period (Experiment I).

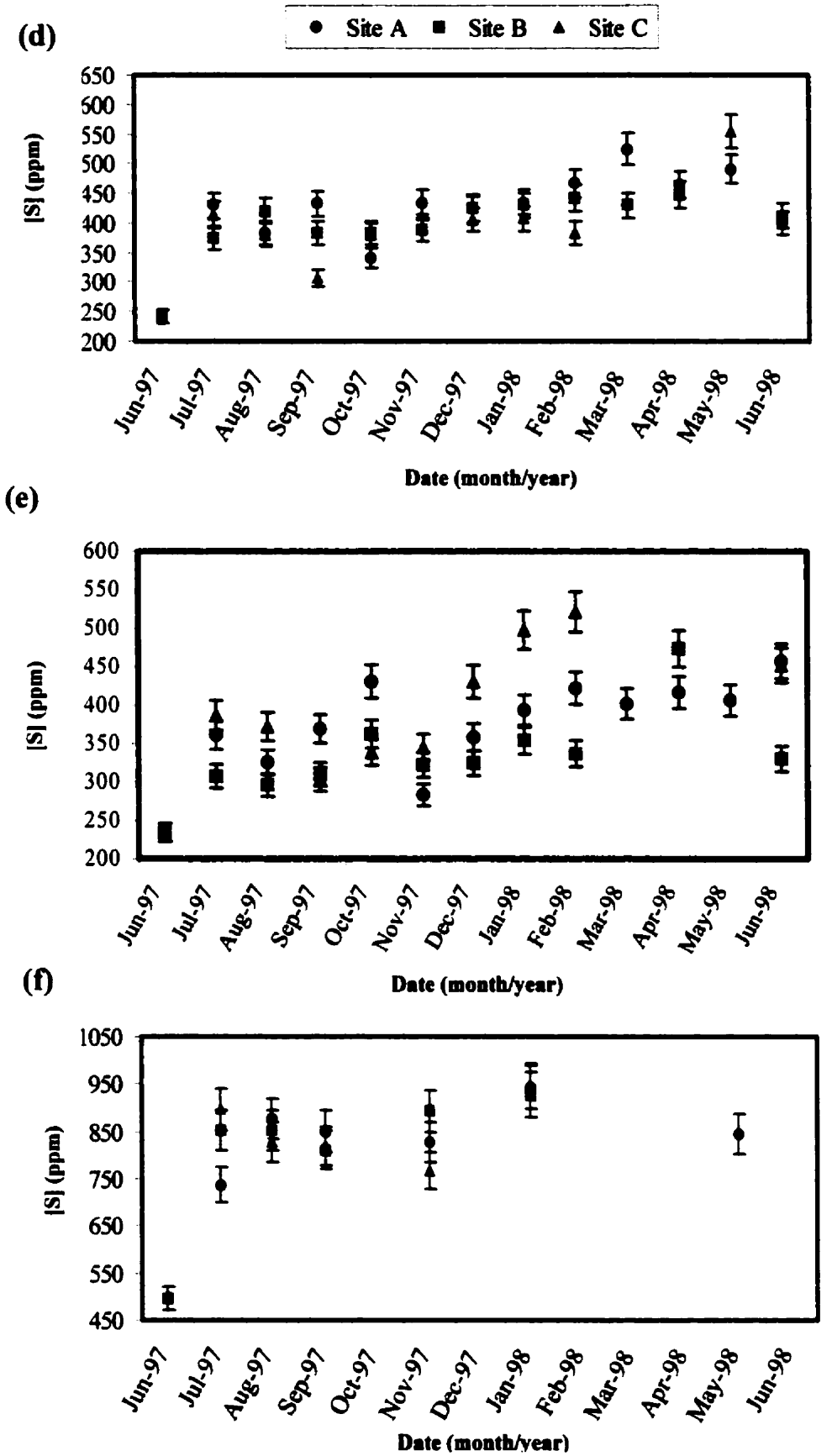


Figure 3.1 (d, e, f) [S] values for lichens moved from Bonavista sites (d) X (e) 15 (f) 17 to the Botanical Garden over a one year period (Experiment I).

In all cases the concentrations increased over the one-year study period.

It is evident from Figure 3.1 that the sulphur isotopic compositions and sulphur concentrations of each of the Bonavista lichens (X, 15, 17) reacted very similarly in the three Botanical Garden sites (A, B, C). Thus, statistical analysis as described in the following section was used to determine if the three sites in the Botanical Garden were similar enough to average.

3.2.1 Statistical Analysis of Experiment I Data

Statistical analysis was performed using SPSS Graduate Pack 9.0 for Windows. The level of significance (α) is 0.05. Two separate two-way analysis of variance statistical tests were applied to the sulphur isotopic compositions and sulphur concentrations collected from Experiment I.

The two-way analysis of variance of the sulphur isotopic compositions indicated that there was no interaction among the means from the Bonavista sites and the means from the Botanical Garden sites with a p-value of 0.924 (> 0.05). From this result, it was then possible to inspect the results of the Botanical Garden sites and Bonavista sites separately. As was expected, the means of the Bonavista sites were significantly different from each other with a p-value of 0.000 (< 0.05). This result was due to the difference among the starting isotopic compositions of the lichens from the Bonavista sites. Also from the two-way analysis of variance test, it was determined that the means of the Botanical Garden data were similar, with a p-value of 0.846 (> 0.05). There was no significant difference in transplant response between the three Botanical Garden sites

(A, B or C). Therefore, it is acceptable to average the isotopic data according to collection site in the Botanical Garden. The complete results for the two-way analysis of variance using the Experiment I isotopic data can be seen in Table 3.3.

The two-way analysis of variance of the sulphur concentration data for Experiment I displayed similar results as the statistical analysis of the isotopic data. Again, the statistical analysis demonstrated that there was no interaction between the means from the Bonavista sites and the means from the Botanical Garden sites with a p-value of 0.737 (> 0.05). From this, the Bonavista means were found to be significantly different with a p-value of 0.000 (< 0.05) while the Botanical Garden means were found to be very similar with a p-value of 0.753 (> 0.05). From this analysis, it was again acceptable to average the concentration data collected from the three Botanical Garden sites. The complete results for the two-way analysis of variance using the Experiment I concentration data can be seen in Table 3.4.

It is important to note that the above p-values of both analysis of variance tests are accurate because the sample size was large (82), and the residuals were homogeneous and normally distributed.

Table 3.3 Two-way analysis of variance results of sulphur isotopic data for Experiment I.

Source	TYPE III SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE	OBSERVED POWER
Corrected Model	95.721	8	11.965	7.198	0.000	1.000
Intercept	7526.649	1	7526.649	4527.645	0.000	1.000
Source	93.657	2	46.829	28.170	0.000	1.000
Location	0.557	2	0.278	0.167	0.846	0.075
Source * Location	1.491	4	0.373	0.224	0.924	0.096
Error	131.328	79	1.662			
Total	8163.350	88				
Corrected Total	227.049	87				

Table 3.4 Two-way analysis of variance results of sulphur concentration data for Experiment I.

Source	TYPE III SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE	OBSERVED POWER
Corrected Model	2605632.8	8	325704.10	38.183	0.000	1.000
Intercept	22135217	1	22135217	2594.957	0.000	1.000
Source	2568484.7	2	1284242.4	150.554	0.000	1.000
Location	4866.306	2	2433.153	0.285	0.753	0.094
Source * Location	17014.870	4	4253.717	0.499	0.737	0.163
Error	682407.33	80	8530.092			
Total	23161154	89				
Corrected Total	3288040.1	88				

3.2.2 Sulphur Isotopic Composition

The average isotopic compositions over the period of one year for each original Bonavista site can be seen in Table 3.5. Graphically, it is evident that the three groups of lichens display decreasing isotopic compositions over the period of one year. Site X lichens (*A. sarmentosa*) had a starting isotopic composition of 9.0‰ and decreased to 7.0‰ after 12 months (Figure 3.2). Site 15 lichens (*A. sarmentosa*) began at 13.3‰ and decreased to 8.4‰ (Figure 3.3). Site 17 lichens (*B. capillaris*) began at 12.0‰ and decreased to 9.4‰ (Figure 3.4).

The error bars surrounding each data point in Figures 3.2, 3.3 and 3.4 represent the ± 0.4‰ error associated with analytical methods. It is important to note that in all three cases the starting isotopic composition is significantly different from the final isotopic composition. However, based on the results of the control (section 3.1), the transplanted lichens were given insufficient time to reach the isotopic signature of the local lichens growing in the Botanical Garden (+5.8 ± 0.8‰).

Table 3.5 Average $\delta^{34}S$ values for lichens moved from Bonavista sites 15, X and 17 to the Botanical Garden over a one year period (Experiment I). (n = 33)

MONTH	$\delta^{34}S$ (‰)		
	Site 15	Site X	Site 17
JUN-97	13.3	9.0	12.0
JUL-97	10.6	8.7	11.4
AUG-97	12.0	9.3	11.4
SEP-97	10.2	8.5	10.9
OCT-97	10.4	8.6	
NOV-97	10.2	9.2	10.5
DEC-97	10.3	9.1	
JAN-98	9.1	7.9	8.9
FEB-98	9.2	7.5	
MAR-98	8.7	7.2	
APR-98	7.5	7.2	
MAY-98	7.8	7.8	9.4
JUN-98	8.4	7.0	

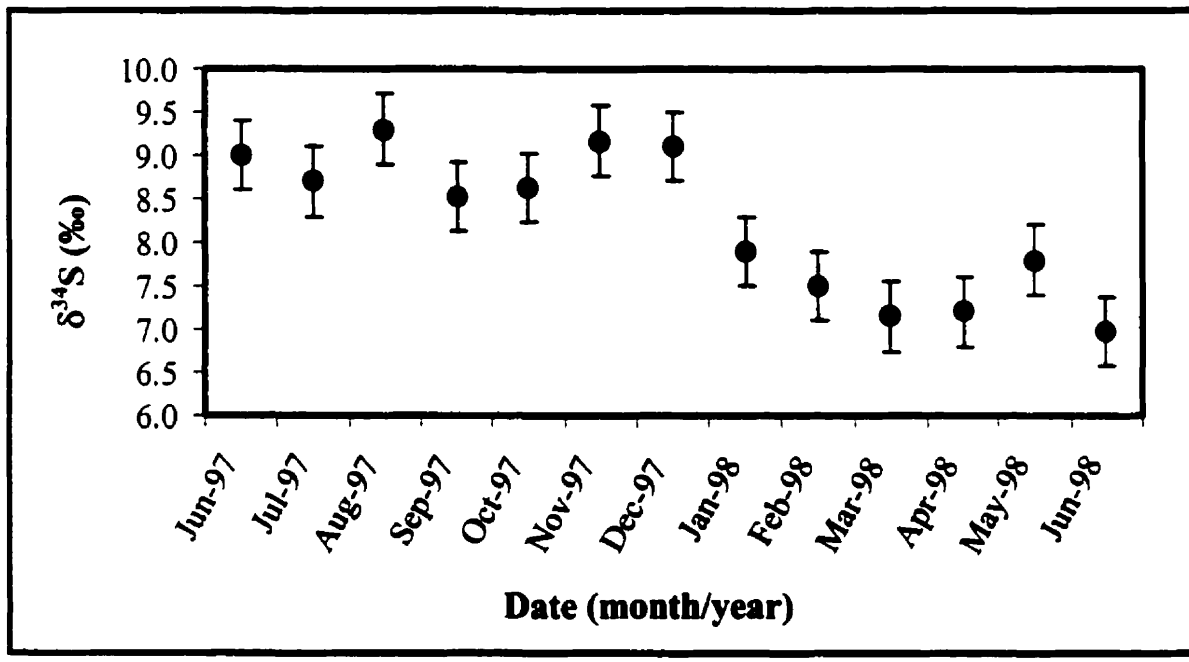


Figure 3.2 Average $\delta^{34}S$ values for lichens moved from Bonavista site X to the Botanical Garden over a one year period (Experiment I).

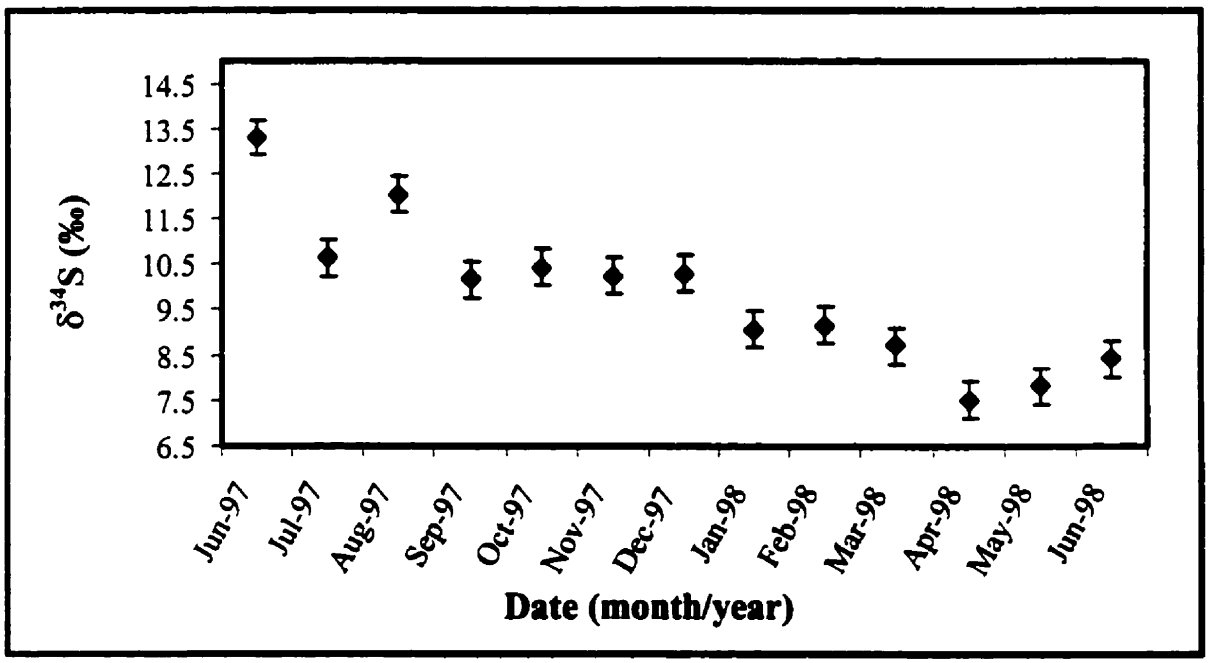


Figure 3.3 Average $\delta^{34}S$ values for lichens moved from Bonavista site 15 to the Botanical Garden over a one year period (Experiment I).

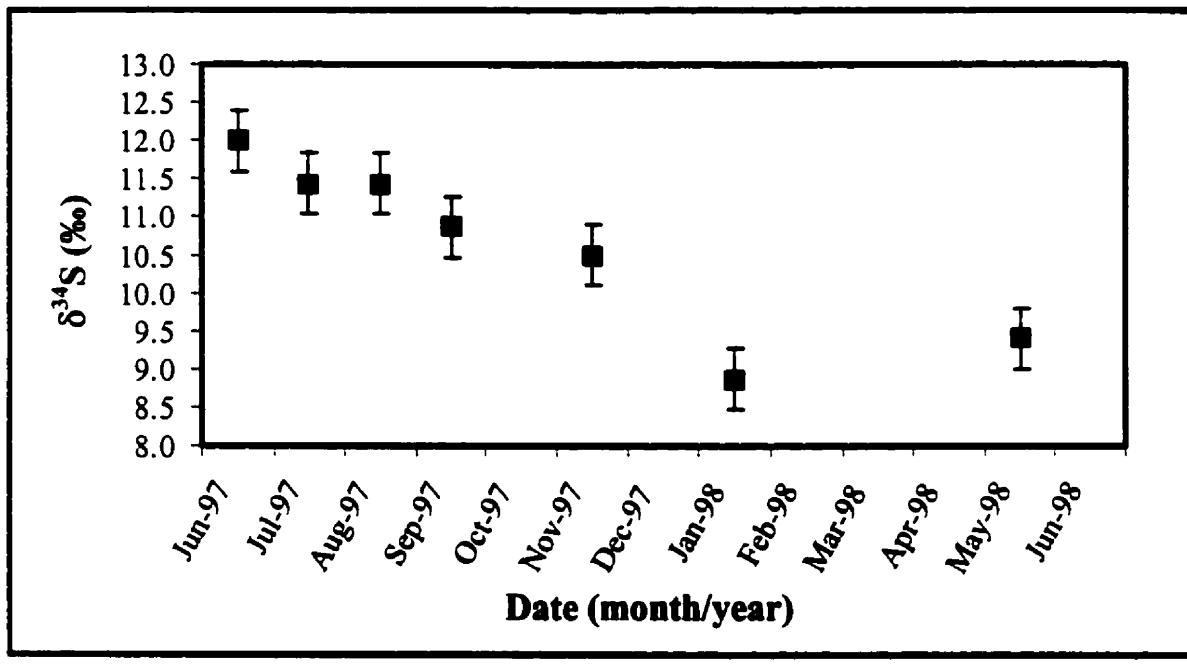


Figure 3.4 Average $\delta^{34}S$ values for lichens moved from Bonavista site 17 to the Botanical Garden over a one year period (Experiment I).

3.2.3 Sulphur Concentration

As was stated earlier, the statistical analysis demonstrated that it was acceptable to average the sulphur concentration values from the three Botanical Garden sites (Table 3.6). Each group of lichens from Bonavista increased in sulphur concentration over the period of one year. Site X lichens (*A. sarmentosa*) began with a starting concentration of 242 ppm and ended with a concentration of 408 ppm (Figure 3.5). Site 15 lichens (*A. sarmentosa*) began at 234 ppm and increased to 413 ppm (Figure 3.6). Site 17 lichens (*B. capillaris*) began at 494 ppm and increased to 844 ppm (Figure 3.7).

Recall in section 2.5 that there is a 5% error due to analytical methods associated with each concentration shown by the error bars in Figures 3.5, 3.6 and 3.7. It is evident that in all cases, the starting concentrations are significantly different from the final concentrations. However, based on the results of the control experiment (section 3.1), the transplanted lichens were given insufficient time to reach the sulphur concentration of the local lichens growing in the Botanical Garden (500 ± 74 ppm).

Table 3.6 Average [S] values for lichens moved from Bonavista sites 15, X and 17 to the Botanical Garden over a one year period (Experiment I). (n = 33)

MONTH	[S] (ppm)		
	Site 15	Site X	Site 17
JUN-97	234	242	494
JUL-97	352	406	829
AUG-97	331	395	852
SEP-97	327	374	827
OCT-97	377	368	
NOV-97	317	404	829
DEC-97	371	418	
JAN-98	415	424	939
FEB-98	427	430	
MAR-98	402	477	
APR-98	445	456	
MAY-98	406	522	844
JUN-98	413	408	

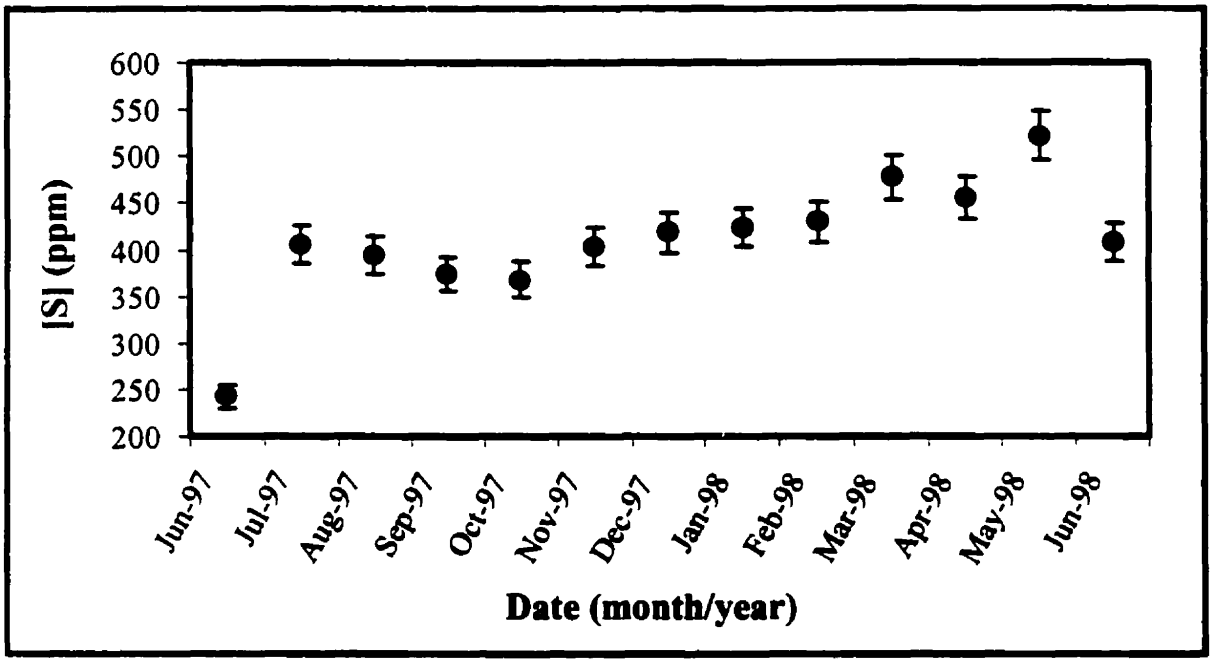


Figure 3.5 Average [S] values for lichens moved from Bonavista site X to the Botanical Garden over a one year period (Experiment I).

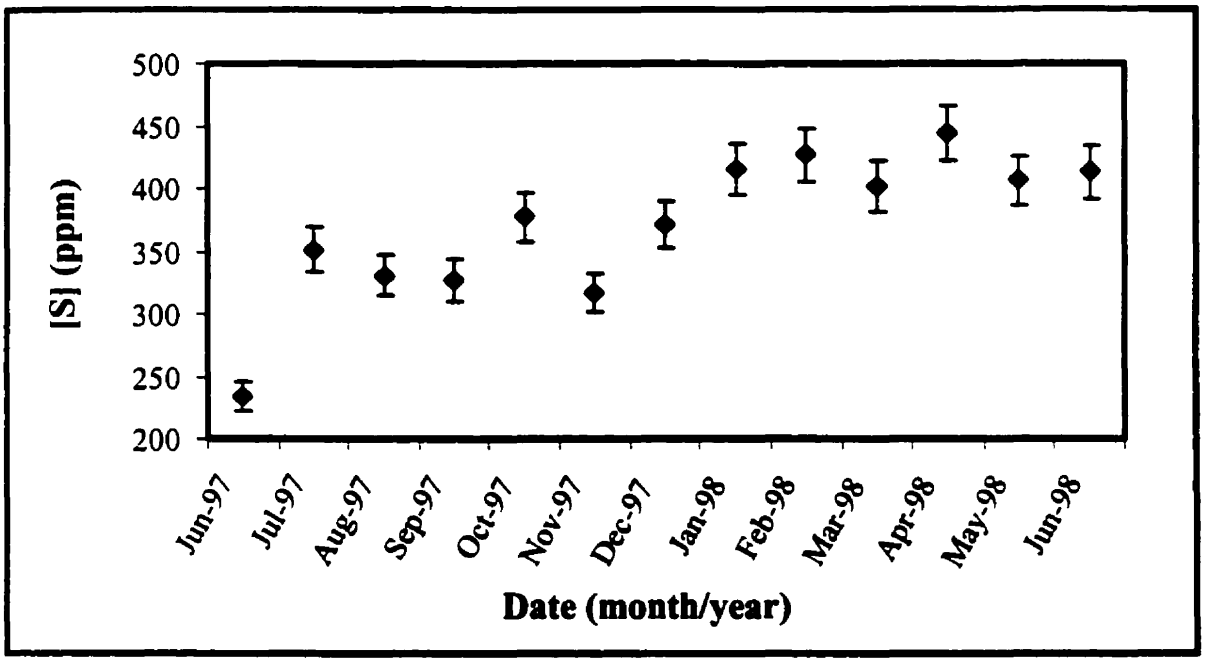


Figure 3.6 Average [S] values for lichens moved from Bonavista site 15 to the Botanical Garden over a one year period (Experiment I).

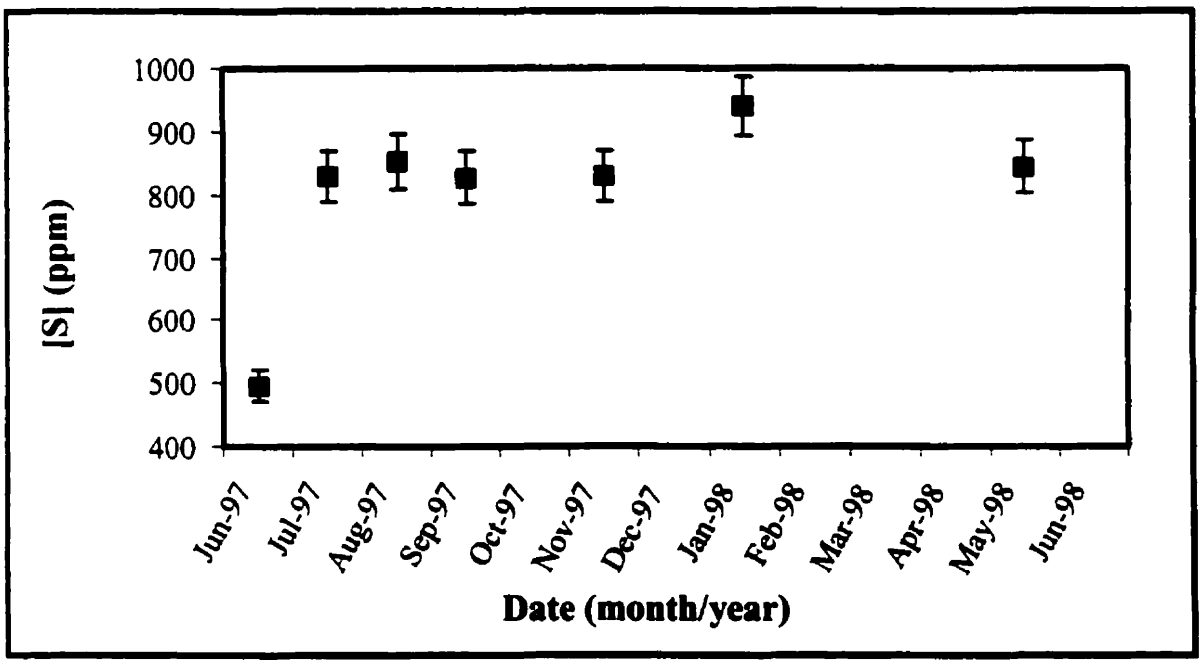


Figure 3.7 Average [S] values for lichens moved from Bonavista site 17 to the Botanical Garden over a one year period (Experiment I).

3.3 Experiment II

Experiment II began in June 1998 and ran for a period of three months until September 1998. Samples of *A. sarmentosa* and *B. capillaris* were collected from the same three sites along the Bonavista Peninsula (site X, 15, 17) that were utilized for Experiment I. These lichens were transplanted into two sites at the MUN Botanical Garden (site A, B). Sampling at the MUN Botanical Garden took place weekly. This experiment was performed to complement and reinforce the results obtained from Experiment I.

Each sample collected throughout Experiment II was identified similarly to the samples of Experiment I. Thus, the sample labeled B/15/W4 was originally taken from site 15 in Bonavista and then placed in site B at the MUN Botanical Garden. This sample was collected from the Botanical Garden during the fourth week of the experiment.

As was the case in Experiment I, it is important to note that certain data points are missing from this data set because the supply of transplanted lichens was becoming depleted.

A total of 67 samples was collected throughout the three-month period and analysed for both sulphur isotopic composition and sulphur concentration. Complete tables of all analytical results for Experiment II can be found in Appendix IV.

Figure 3.8 graphically illustrates the $\delta^{34}S$ values and sulphur concentrations obtained for all lichen samples from Experiment II. The $\delta^{34}S$ values of site X lichens (*A. sarmentosa*) in site A changed over a 12 week period from 7.8‰ to 9.1‰ (Figure 3.8a). Site X lichens in site B changed from 7.8‰ to 9.7‰ (Figure 3.8a).

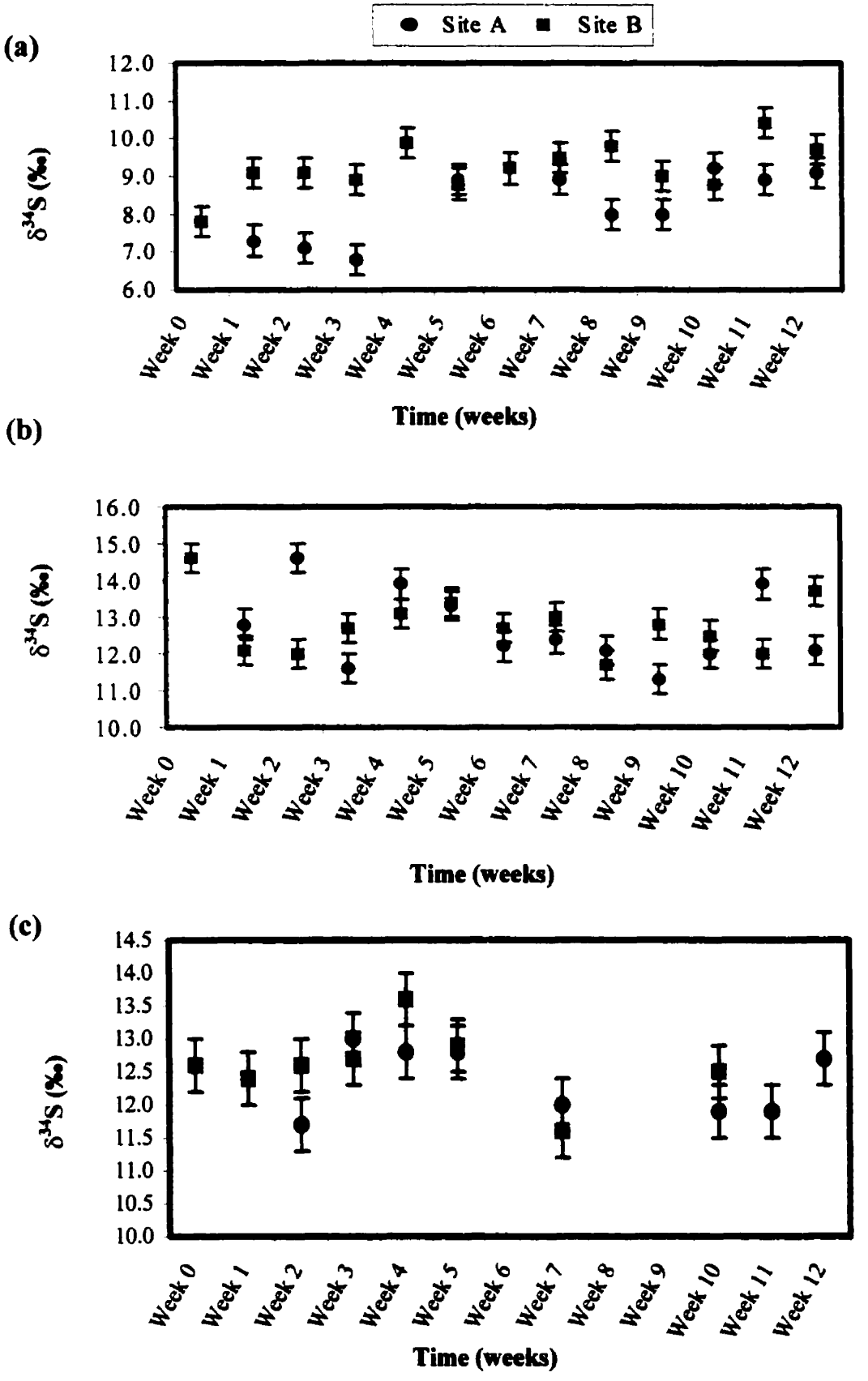


Figure 3.8 (a, b, c) $\delta^{34}S$ values for lichens moved from Bonavista sites (a) X (b) 15 (c)17 to the Botanical Garden over a twelve week period (Experiment II).

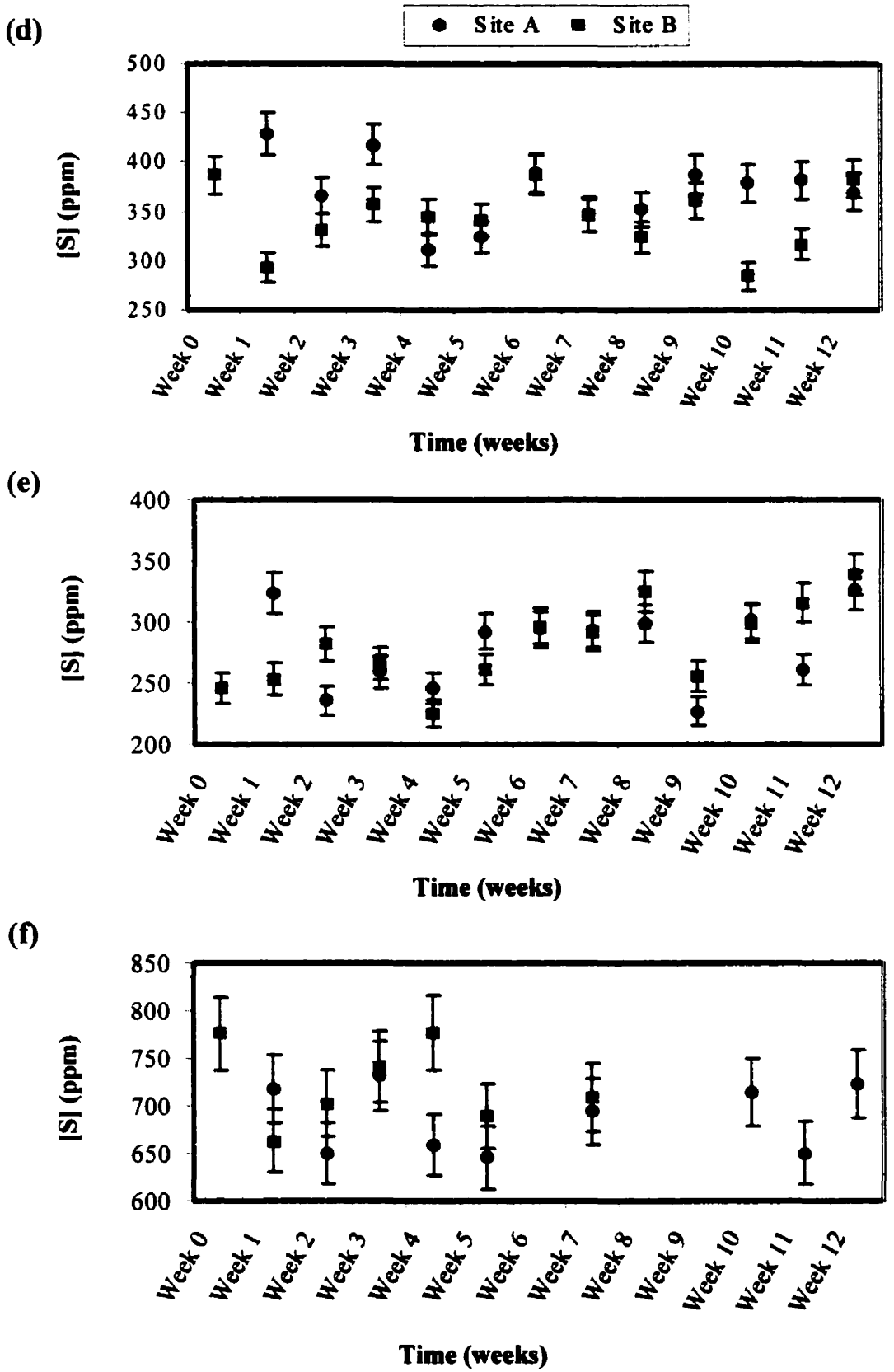


Figure 3.8 (d, e, f) [S] values for lichens moved from Bonavista sites (d) X (e) 15 (f) 17 to the Botanical Garden over a twelve week period (Experiment II).

Site 15 lichens (*A. sarmentosa*) in site A changed over a 12 week period from 14.6‰ to 12.1‰ (Figure 3.8b). Site 15 lichens in site B changed from 14.6‰ to 13.7‰ (Figure3.8b).

Site 17 lichens (*B. capillaris*) in site A changed over a 12 week period from 12.6‰ to 12.7‰ (Figure 3.8c). Site 17 lichens in site B changed from 12.6‰ to 12.5‰ (Figure 3.8c).

The sulphur concentrations of site X lichens (*A. sarmentosa*) in site A changed over a 12 week period from 387 ppm to 370 ppm (Figure 3.8d). Site X lichens in site B changed from 387 ppm to 383 ppm (Figure 3.8d).

Site 15 lichens (*A. sarmentosa*) in site A changed over a 12 week period from 246 ppm to 326 ppm (Figure 3.8e). Site 15 lichens in site B changed from 246 ppm to 339 ppm (Figure 3.8e).

Site 17 lichens (*B. capillaris*) in site A changed over a 12 week period from 776 ppm to 724 ppm (Figure 3.8f). Site 17 lichens in site B changed from 776 ppm to 709 ppm (Figure 3.8f). The large difference in the initial sulphur concentration values of the site 17 lichens for Experiment I (494 ppm) and Experiment II (776 ppm) will be explained in the discussion.

As was the case in Experiment I, statistical analysis was once again performed on both the isotopic compositions and concentrations separately to determine if the data from the two sites in the Botanical Garden (A, B) were similar enough to average.

3.3.1 Statistical Analysis of Experiment II Data

Two separate two-way analysis of variance statistical tests were performed on the sulphur isotopic compositions and sulphur concentrations collected from Experiment II.

The two-way analysis of variance of the sulphur isotopic compositions indicated that there was no interaction among the means of the three Bonavista sites and the two Botanical Garden sites with a p-value of 0.151 (> 0.05). From this result, it was then possible to inspect the results of the Botanical Garden sites and Bonavista sites separately. Again, it was confirmed that the means of the Bonavista sites were significantly different with a p-value of 0.000 (< 0.05). Also, it was determined that means of the Botanical Garden data were very similar, demonstrating a p-value of 0.088 (> 0.05). There was no significant difference between the two Botanical Garden sites (A, B). As was the case in Experiment I, it was acceptable to average the isotopic data for the Botanical Garden. The complete results for the two-way analysis of variance using the isotopic data from Experiment II can be seen in Table 3.7.

The two-way analysis of variance test of the concentration data from Experiment II displayed different results from the previous two-way ANOVA tests. In this case, there was a slight interaction between the means of the concentration data from the Bonavista sites and the Botanical Gardens sites indicated by a p-value of 0.047 (< 0.05). With this significant interaction term it was not accurate to accept the p-values for the Bonavista sites and Botanical Gardens sites separately. To determine if any particular group of data was contributing to the interaction, three separate one-way analysis of variance tests were performed on each Bonavista location separately. The p-value of the

Table 3.7 Two-way analysis of variance results of sulphur isotopic data for Experiment II.

Source	TYPE III SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE	OBSERVED POWER
Corrected Model	250.191	5	50.038	75.072	0.000	1.000
Intercept	8741.470	1	8741.470	13114.686	0.000	1.000
Source	245.602	2	122.801	184.237	0.000	1.000
Location	2.001	1	2.001	3.002	0.088	0.400
Source * Location	2.592	2	1.296	1.944	0.151	0.389
Error	42.659	64	0.667			
Total	9138.730	70				
Corrected Total	292.850	69				

Table 3.8 Two-way analysis of variance results of sulphur concentration data for Experiment II.

Source	TYPE III SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE	OBSERVED POWER
Corrected Model	2025791.0	5	405158.20	311.439	0.000	1.000
Intercept	13164525	1	13164525	10119.384	0.000	1.000
Source	1993731.9	2	996865.95	766.277	0.000	1.000
Location	1.234E-02	1	1.234E-02	0.000	0.998	0.050
Source * Location	8342.506	2	4171.253	3.206	0.047	0.593
Error	81958.060	63	1300.922			
Total	13940698	69				
Corrected Total	2107749.1	68				

one-way ANOVA for the site 15 concentration data for both Botanical Garden sites was 0.776 (> 0.05). Thus, for this group of data it was evident that there was no significant difference between sites A and B at the Botanical Garden. Similar results were obtained for the site 17 concentration data with a p-value of 0.248 (< 0.05). The problem arose with the site X concentration data at both Botanical Garden sites. The p-value obtained from the one-way ANOVA was 0.032 (> 0.05) indicating that the means of the concentration data at sites A and B for the site X lichens were significantly different. It was decided to perform a two-way ANOVA on the site 15 and 17 concentration data from both sites at the Botanical Garden. The site X concentration data were omitted from this test. The results indicated that there was no interaction among the means of the Bonavista and Botanical Garden sites with a p-value of 0.357 (> 0.05). With this result it was possible to inspect the Bonavista and Botanical Garden sites separately. As was expected, the means of the concentration data for the two Bonavista sites 15 and 17 were significantly different with a p-value of 0.000 (< 0.05). The results for the two Botanical Gardens sites were found to be similar with a p-value of 0.219 (> 0.05). Again, there was no significant difference between sites A and B at the Botanical Garden. Thus, it is acceptable to average the concentrations from both Botanical Garden sites for sites 15 and 17 lichens only. The complete results for the two-way and one-way analysis of variance tests using the concentration data can be seen in Tables 3.8, 3.9 and 3.10.

Table 3.9 One-way analysis of variance results of sulphur concentration data for Bonavista sites (a) X (b) 15 (c) 17 lichens.

(a)

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE
Between Groups	92.346	1	92.346	0.083	0.776
Within Groups	26708.308	24	1112.846		
Total	26800.654	25			

(b)

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE
Between Groups	2768.256	1	2768.256	1.443	0.248
Within Groups	28776.214	15	1918.414		
Total	31544.471	16			

(c)

	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE
Between Groups	5700.962	1	5700.962	5.168	0.032
Within Groups	26473.538	24	1103.064		
Total	32174.500	25			

Table 3.10 Two-way analysis of variance results of sulphur concentration data for Experiment II (excluding site X values).

Source	TYPE III SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F STATISTIC	P-VALUE	OBSERVED POWER
Corrected Model	1886359.2	3	628786.42	441.973	0.000	1.000
Intercept	9854112.9	1	9854112.9	6926.443	0.000	1.000
Source	1867392.4	1	1867392.4	1312.588	0.000	1.000
Location	2223.222	1	2223.222	1.563	0.219	0.230
Source * Location	1237.793	1	1237.793	0.870	0.357	0.149
Error	55484.522	39	1422.680			
Total	10585561	43				
Corrected Total	1941843.8	42				

3.3.2 Sulphur Isotopic Composition

From the above analysis of variance results, it was acceptable to average the isotopic compositions obtained from the two Botanical Garden sites (Table 3.11). When transplanted lichens are sampled weekly as opposed to monthly, the isotopic results are much more variable. The starting composition for site X was 7.8‰ and increased slightly over 12 weeks to 9.4‰ (Figure 3.9). The starting composition for site 15 was 14.6‰ and decreased slightly to 12.9‰ (Figure 3.10). Finally, the starting isotopic composition for site 17 was 12.6‰ and ended after 12 weeks at a similar value of 12.7‰ (Figure 3.11).

In this experiment, there were no consistent trends over time as was the case in Experiment I. Site 15 lichens were the only group to show a decrease perhaps due to the higher initial isotopic composition. It is evident that weekly sampling reveals many increases and decreases within the data set. These results are important because they give an indication of how much variability among the isotopic compositions actually occurs from week to week.

3.3.3 Sulphur Concentration

As was stated in section 3.3.1, it was acceptable to average the concentrations from both the Botanical Garden sites for only the sites 15 and 17 lichens from the Bonavista Peninsula. The average concentrations can be seen in Table 3.12. The concentrations of the sites 15 and 17 lichens were variable over 12 weeks. The site 15

Table 3.11 Average $\delta^{34}S$ values for lichens moved from Bonavista sites 15, X and 17 to the Botanical Garden over a twelve week period (Experiment II). (n = 36)

WEEK	$\delta^{34}S$ (‰)		
	Site 15	Site X	Site 17
0	14.6	7.8	12.6
1	12.5	8.2	12.4
2	13.3	8.1	12.2
3	12.2	7.9	12.9
4	13.5	9.9	13.2
5	13.4	8.9	12.9
6	12.5	9.2	
7	12.7	9.2	11.8
8	11.9	8.9	
9	12.1	8.5	
10	12.3	9.0	12.2
11	13.0	9.7	11.9
12	12.9	9.4	12.7

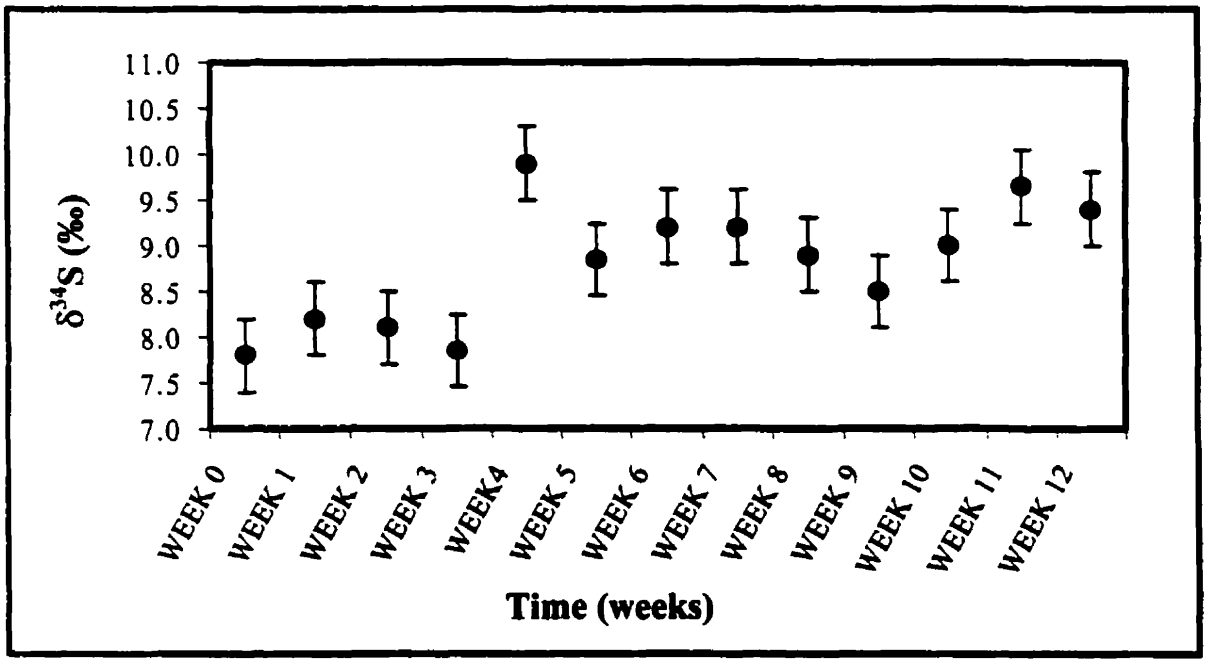


Figure 3.9 Average $\delta^{34}S$ values for lichens moved from Bonavista site X to the Botanical Garden over a twelve week period (Experiment II).

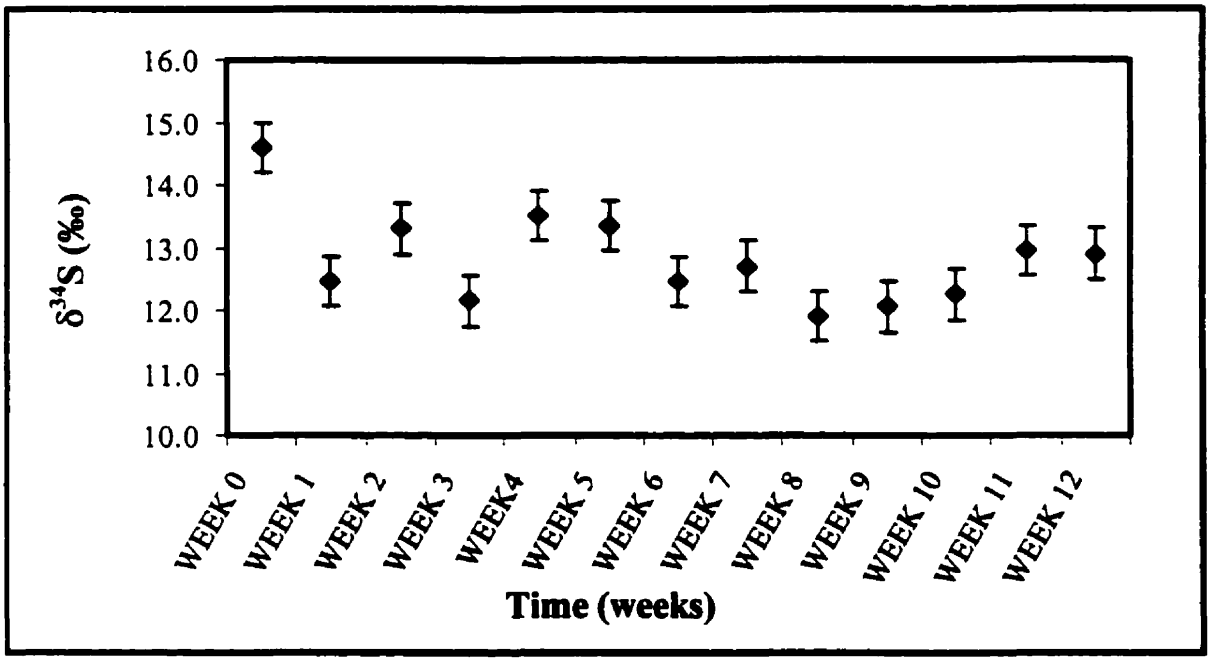


Figure 3.10 Average $\delta^{34}S$ values for lichens moved from Bonavista site 15 to the Botanical Garden over a twelve week period (Experiment II).

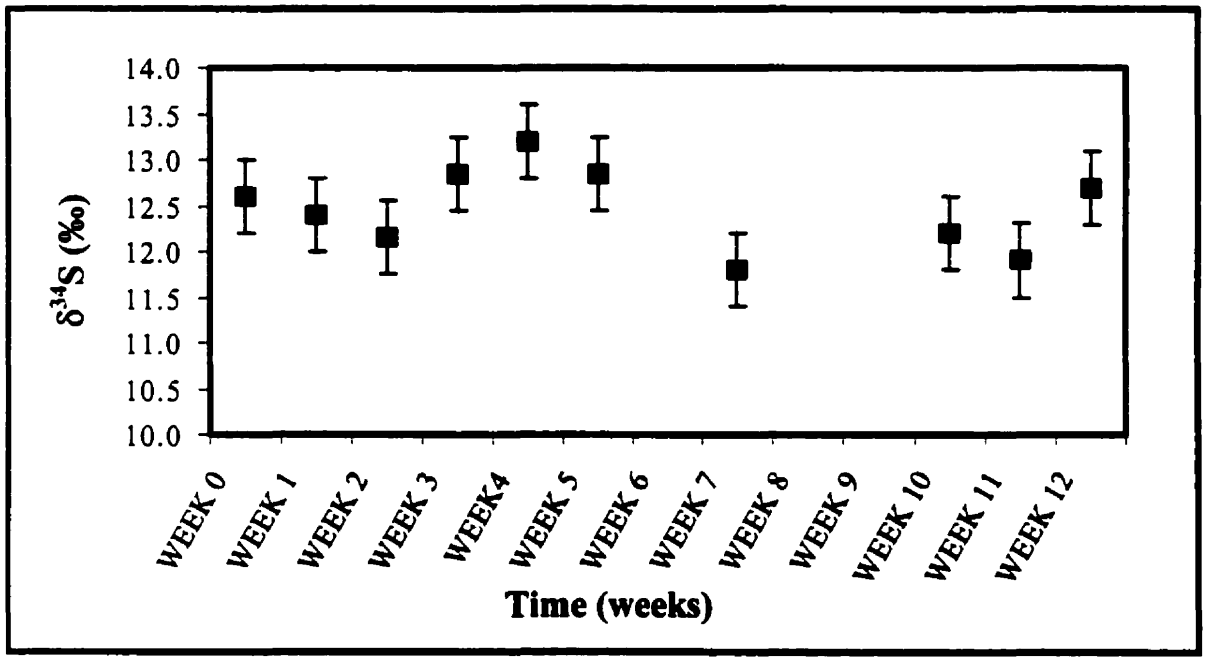


Figure 3.11 Average $\delta^{34}S$ values for lichens moved from Bonavista site 17 to the Botanical Garden over a twelve week period (Experiment II).

Table 3.12 Average [S] values for lichens moved from Bonavista sites 15 and 17 (excluding site X values) to the Botanical Garden over a twelve week period (Experiment II). (n = 23)

WEEK	[S] (ppm)	
	Site 15	Site 17
0	246	776
1	289	691
2	259	676
3	263	737
4	235	718
5	277	667
6	295	
7	292	702
8	312	
9	241	
10	300	715
11	288	651
12	332	724

lichens (*A. sarmentosa*) began with a starting concentration of 246 ppm and increased slightly over 12 weeks to a final concentration of 332 ppm (Figure 3.12). The site 17 lichens (*B. capillaris*) did not change significantly over the 12 week period. The initial concentration was 776 ppm and the final concentration was 724 ppm (Figure 3.13).

Recall in section 2.5 that there is a 5% error due to analytical methods associated with each concentration shown by the error bars in Figures 3.12 and 3.13. It is evident that there is only a slight increase in concentration for site 15 lichens over a 12 week period. The site 17 lichens did not demonstrate a significant increase, essentially the concentrations remained very similar.

3.4 Meteorological Considerations

The sites at the MUN Botanical Garden experienced unique microclimates that varied from the St. John's Airport with respect to temperature, relative humidity and wind speed (Appendix V).

In the forested sites at the Botanical Garden, it was found that the temperatures were similar to those recorded from the St. John's Airport with means of 3.9°C and 3.6°C respectively. The maximum variation in temperature between the Botanical Garden and the St. John's Airport was 2.2°C in one case while all other temperature variations fell below this value.

The mean relative humidity at the Botanical Garden (88.3%) was found to be significantly higher than that of the St. John's Airport (77.6%). The site at the Botanical Garden where the weather measurements were taken was in a densely forested area very

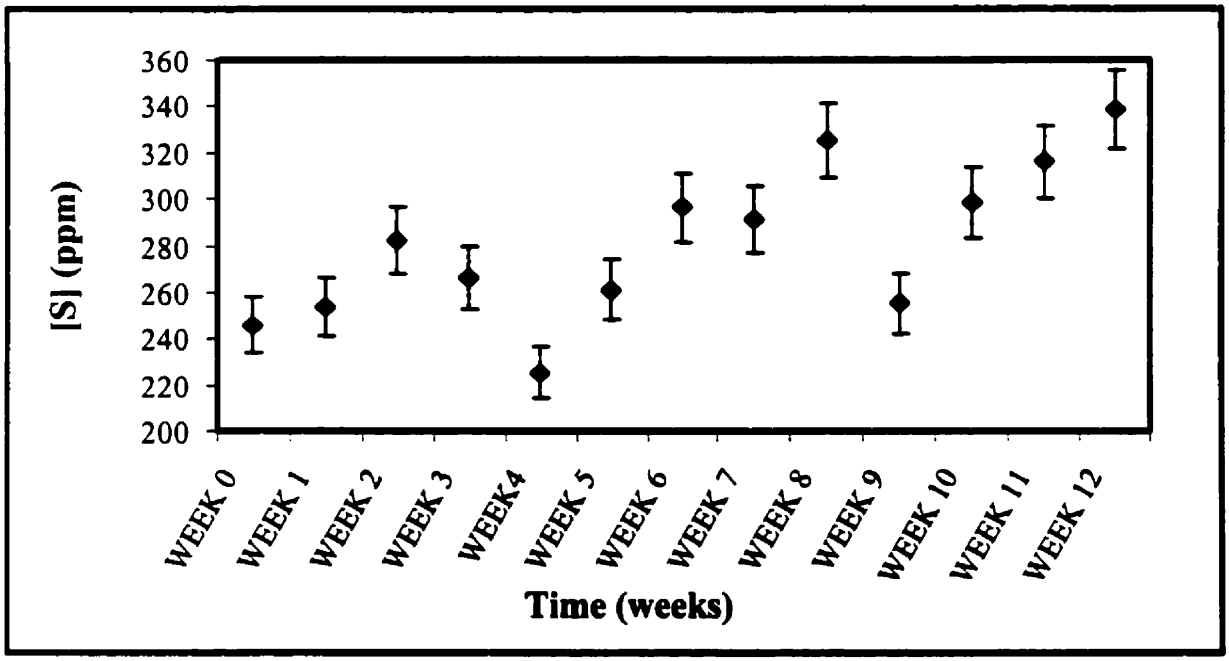


Figure 3.12 Average [S] values for lichens moved from Bonavista site 15 to the Botanical Garden over a twelve week period (Experiment II).

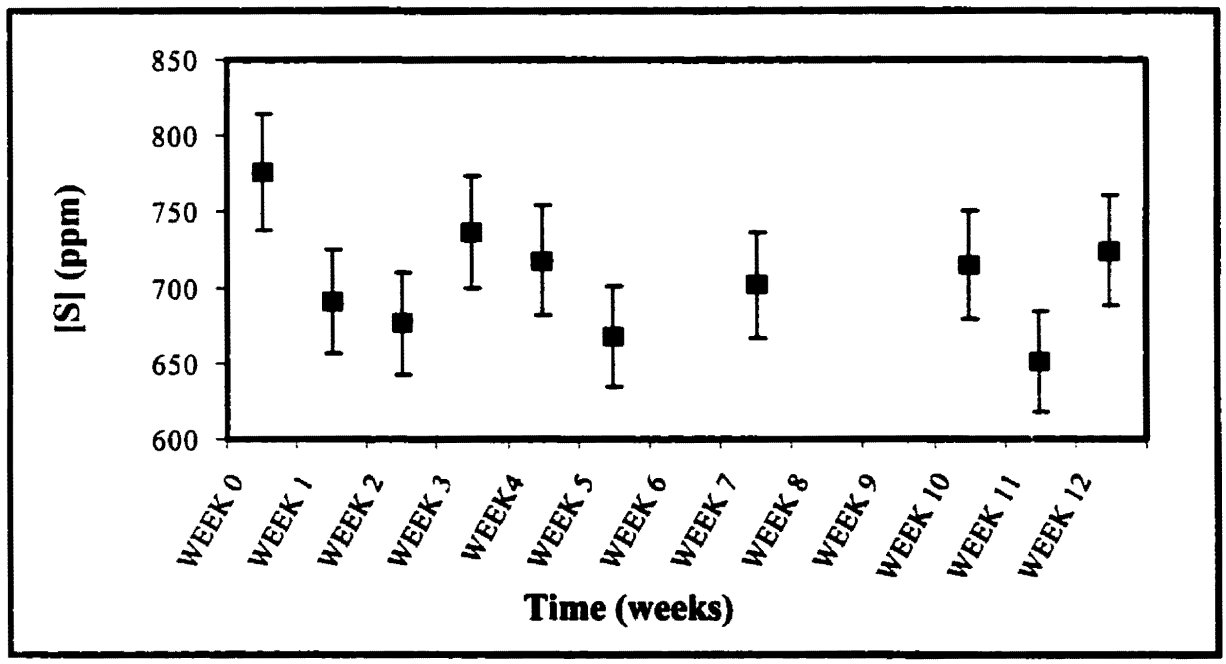


Figure 3.13 Average [S] values for lichens moved from Bonavista site 17 to the Botanical Garden over a twelve week period (Experiment II).

near a pond. Evidently, there was significantly more water in the atmosphere because of the nearby pond and the transpiration of the numerous trees.

Finally, the wind speed in the site at the Botanical Garden was consistently measured at 0 m/s for 27 out of the 34 weather-recording events. The other 7 remaining events did not reach wind speeds greater than 1.5 m/s. These results vary greatly from those of the St. John's Airport where the wind speeds ranged from 2.5 m/s to 13.3 m/s. Obviously, the densely forested site was blocking the wind that was being detected in an open area at the St. John's Airport.

Overall, this relatively small comparison study of the MUN Botanical Garden and St. John's Airport weather recordings indicated that the climate of the MUN Botanical Garden sites differed from that of the St. John's Airport.

CHAPTER 4

DISCUSSION

4.1 Transplant Sites

The island of Newfoundland was an ideal location for this study. The sites chosen for the transplant experiments were the Bonavista Peninsula and the city of St. John's due to the difference in sulphur isotopic compositions of lichens at each location. From a previous study by Wadleigh & Blake (in press) lichens along the Bonavista Peninsula were found to have a high sulphur isotopic composition (~ +15‰) while the lichens in the city of St. John's had a lower sulphur isotopic composition (~ +7‰). To support the choice of locations, rain studies by Evans (1996) and Jamieson (1996) were also used. Evans (1996) found that the $\delta^{34}S$ values of six rain events in the town of Bonavista ranged from 6.2‰ to 18.5‰ with a weighted average of 14.3‰. Jamieson (1996) analysed rain samples in the city of St. John's with $\delta^{34}S$ values ranging from 2.2‰ to 18.6‰ with an average of 7.1‰.

4.1.1 Bonavista Peninsula

The initial sulphur isotopic compositions from the three sites in Bonavista were slightly different than expected, however, it is possible to explain the differences by considering specific sites characteristics. The sulphur isotopic compositions of the lichens from sites 15 and 17 were relatively high (+13.3‰ and +12.0‰ respectively) and close to the expected value of 15‰ obtained from Wadleigh & Blake (in press). The

sulphur isotopic composition of site X lichens, on the other hand, was a little lower at 9.0‰.

Site 17 was located in the town of Bonavista along the coastline of the Atlantic Ocean (Figure 2.2). The lichens in this particular site were evidently accumulating sulphur from the seaspray that exhibits a fairly constant isotopic composition of +21‰ as determined by Rees *et al.* (1978). The rest of the sulphur in these lichens probably originated from the day-to-day anthropogenic activities around the town of Bonavista leaving a mixed isotopic composition of 12.0‰.

Site 15 also exhibited a high sulphur isotopic composition of 13.3‰. This site was located along the Bonavista Peninsula about 1 km away from the main highway on the edge of a forest clearing (Figure 2.2). These lichens were clearly subjected to the atmosphere that contained sulphur originating from seaspray. The anthropogenic sulphur accumulated in the lichen possibly originated from the exhaust of numerous vehicles travelling along the Bonavista highway causing a mixed composition of 13.3‰, however, the amount of anthropogenic sulphur was limited due to the distance between the highway and the actual site of collection.

The final site along the Bonavista Peninsula, site X, exhibited the lowest isotopic composition (+9.0‰) of the three sites. This particular site was located close (~ 50 m) to the Bonavista highway (Figure 2.2). It is evident that the sources of sulphur in these lichens originated from both seaspray and the anthropogenic vehicle exhaust emissions produced along the Bonavista highway.

A transplant study by Tuba and Csintalan (1993) measured the accumulation of metals in the lichen, *Cladonia convoluta*, at specific distances away from a main highway. All metals studied were found in highest concentrations in samples closest to the road (1 m) and their concentrations gradually decreased with distance from the road. However, samples furthest from the road (90 m) still contained higher amounts of all metals than the control sites. This study by Tuba and Csintalan (1993) tested metal concentrations thus it cannot be directly compared to this study that analysed sulphur, however, it does verify that pollution from road traffic can have an impact on vegetation greater than 90 m away from the highway. As was stated previously, it is possible that anthropogenically-derived sulphur from road traffic isotopically influenced the lichens in site X located only 50 m from the Bonavista highway.

4.1.2 MUN Botanical Garden, St. John's

The lichens originally growing in the MUN Botanical Garden have an average isotopic composition of +5.8‰ that varied by as much as 1.8‰ over the course of one year (Table 3.1). The isotopic compositions did not change in any particular pattern over the study period. This increase and decrease of isotopic compositions can likely be attributed to natural variation influenced by a number of events some of which can be explained and some of which are beyond the scope of this study.

It is possible that numerous physiological features of lichens such as thallus morphology, thallus exposure and species variation may lead to slight natural variation in

isotopic compositions, however, the majority of physiological features are not investigated in this study.

The natural variation in isotopic compositions of local lichens may be influenced by variations in source strengths of sulphur contributions to the atmosphere that will be explained in more detail. At any given time, sulphur in the atmosphere may have originated from a variety of sources. The low sulphur isotopic composition obtained for the local MUN Botanical Garden lichens (+5.8‰) suggests that the majority of the sulphur in these lichens is from an anthropogenic source.

There are a number of sources contributing anthropogenic sulphur to the lichens in the Botanical Garden. The largest point source is the stack located at the Utilities Annex on the Memorial University of Newfoundland campus <1 km southeast of the MUN Botanical Garden. $SO_{2(g)}$ is emitted from the stack through burning of Bunker C fuel and may be transported under favourable wind conditions into the atmosphere surrounding the MUN Botanical Garden.

A study by Ennis (1999) analysed the sulphur isotopic composition of the Bunker C fuel consumed in the Utilities Annex. A series of 17 samples of fuel yielded isotopic compositions ranging from –2.98‰ to 0.17‰ with an average of –1.51‰. It is evident that the Utilities Annex is contributing to atmospheric sulphur, however, the fuel isotopic compositions are lower than the rain and lichen isotopic compositions (~7‰) obtained by Wadleigh & Blake (in press) and Jamieson (1996). Part of the difference in isotopic compositions may be due to the fractionation of the fuel sample during the combustion process, during subsequent atmospheric oxidation or simply different fuel shipments.

The difference in isotopic compositions is also an indication that the Utilities Annex is not the sole contributor of anthropogenic sulphur to the Garden.

Two years prior to the time period of this study, a new four-lane highway was constructed around the outer property line of the Botanical Garden. Over a period of 5 months (April 1995- September 1995), heavy equipment (i.e., dump trucks) commuted into the MUN Botanical Garden on a regular basis to dump organic material from the highway construction site in a designated pit (pers. comm. Charlie Horwood). These large vehicles primarily utilized diesel fuel that has a high sulphur content. Thus, the exhaust from the heavy equipment and the day-to-day construction of the highway may have contributed significantly to the anthropogenic signal detected by the lichens originally growing in the MUN Botanical Garden.

Another source of anthropogenic sulphur may be the vehicle emissions originating from Mt. Scio Rd. that runs alongside the MUN Botanical Garden property. Also, the everyday industrial activities throughout the city of St. John's contributed to the amount of sulphur in the atmosphere surrounding the MUN Botanical Garden. Finally, it is important to note that seaspray sulphur originating from the nearby Atlantic Ocean can also greatly influence the atmosphere at any given time.

Due to the numerous contributions of sulphur sources to the atmosphere, natural variation in isotopic compositions should be expected and taken into consideration in subsequent interpretations.

4.2 Effect of Transplantation Procedure

The results of this transplant study suggest that the actual transplant procedure did not affect the isotopic compositions of the transplanted lichens (Table 3.2). It was expected that the transplant procedure would not affect the isotopic compositions because the epiphytic pendulous lichens used in this study do not receive nutrients from the substrate on which they grow. Thus, detaching the branch from the tree and attaching it to another tree should not have affected the lichen. Also, extreme caution was taken when detaching, transporting and reattaching the branches to ensure contamination was not introduced during this process.

A study by Loppi *et al.* (1998) transplanted *Hypogymnia physodes* in their original site and analysed for elemental concentrations after one year. The mean trace element concentrations in control lichens analysed at the beginning and end of the experiment did not differ significantly ($p < 0.05$). Thus, the influence, if any, of the transplant process was negligible and the element concentrations were not affected by the transplant procedure.

Another study by Bennett *et al.* (1996) also tested the effects of the act of transplantation on the element concentrations of the lichen, *Hypogymnia physodes*, at a control site. In this case, local lichens were not transplanted but rather elemental concentrations of local lichens were simply compared to transplanted lichens. It was found that variation in concentration was greater in local lichens sampled three times throughout 1992 than the transplanted lichens sampled from 1993-1995. Statistical analysis (one-way analysis of variance) of the data indicated that once again the influence

of the transplant procedure was negligible. Other studies (Garty *et al.*, 1996; Evans & Hutchinson, 1996) have found similar results to confirm that the act of transplantation does not significantly alter the chemistry or physiology of the various lichen species examined.

4.3 Transplant Experiments

4.3.1 Experiment I

As detailed in section 2.1.3, the primary experiment involved transplanting lichens from the Bonavista Peninsula into the MUN Botanical Garden. Samples were collected monthly and analysed for both sulphur isotopic composition and sulphur concentration.

4.3.1.1 Sulphur Isotopic Composition

As expected, the delta values of each group of Bonavista lichens decreased significantly over the period of one year. Figures 3.2, 3.3 and 3.4 all begin with high isotopic compositions and decrease to significantly lower isotopic compositions, however, there is not a consistent amount of decrease each month.

While each group of lichens from the Bonavista Peninsula reacted independently, there are similar trends between the three groups of lichens. These will be discussed later. The site 15 isotopic data displays the clearest trend of the three sites. *A. sarmentosa* from site 15 had the highest initial starting isotopic composition (+13.3‰) providing an ideal situation to see the most pronounced changes in isotopic compositions

over the period of one year. At the end of this experiment it was found that site 15 lichens exhibited the largest difference between the initial and final isotopic compositions of all three groups of lichens. The site 15 lichens are therefore considered to represent the most successful of the three experiments and will be discussed in most detail.

The site 15 lichens decreased by 4.9‰ over the course of one year. However, throughout this time period the isotopic compositions rose and fell from month to month. Much of this increase and decrease in isotopic compositions over the one year period can be attributed to natural variation as was the case with the local lichens growing in the MUN Botanical Garden.

A study by Jamieson (1996) demonstrated that individual rain events are quite variable in SO_4 concentration, $\delta^{34}S$ value and the amount of sea salt they contain. Thus, it would not take much of a difference in the atmosphere to alter the isotopic composition of the local and transplanted lichens by 1-2‰. At any given time, the majority of sulphur in the atmosphere may be anthropogenically-derived leading to a decrease in sulphur isotopic compositions. Each decrease in isotopic compositions cannot be attributed to a sole pollution event, but rather a combination of numerous activities such as emissions from the stack of the Utilities Annex, vehicle exhaust, highway construction and general industrial activity throughout St. John's. On the other hand, it is also possible that the majority of sulphur in the atmosphere at a certain point in time may have originated from the seaspray of the nearby Atlantic Ocean leading to an increase in sulphur isotopic compositions.

Even though there is natural variation among the isotopic compositions, site 15 lichens demonstrated a net decrease of 3.1‰ over the first three months. This net change is significant and should be attributed to more than natural variation. This large initial decrease can be explained by the fact that the transplanted lichens were immersed into an atmosphere with a significantly different source of sulphur than the atmosphere from where they originated. Evidently, the transplanted lichens were greatly influenced by the anthropogenically-derived sulphur in the atmosphere surrounding the Botanical Garden over the first three months.

The lichens from sites X and 17 reacted similarly to the site 15 lichens, however the changes were not as pronounced. As was the case with the site 15 lichens, there are increases and decreases in the isotopic compositions throughout the one-year period. Again, these changes can be attributed primarily to natural variation.

Interestingly, the isotopic compositions of sites X, 15 and 17 lichens decreased significantly between December 1997 and January 1998 (Figure 3.2, 3.3 and 3.4). In both site X and 15 lichens there was a decrease of 1.2‰. The site 17 lichen supply was becoming depleted so a sample was not taken during December 1997. However, the difference between the November 1997 and January 1998 samples was 1.6‰. A smaller decrease of 0.6‰ was also seen in the local lichens between November 1997 and January 1998 (Table 3.1). This parallel between transplanted and local lichens indicate that a seasonal change occurred. Personal communication with Mr. John Dunne of the Utilities Annex of Memorial University of Newfoundland determined that the fuel consumption increased during the winter months over the time period of this study (Table 4.1). A

Table 4.1 Monthly fuel consumption by the Utilities Annex over a one year period.

MONTH	FUEL CONSUMED (gls)
JUN-97	123,933
JUL-97	97,480
AUG-97	101,770
SEP-97	112,290
OCT-97	168,803
NOV-97	189,732
DEC-97	216,570
JAN-98	291,266
FEB-98	214,817
MAR-98	211,596
APR-98	185,915
MAY-98	147,988
JUN-98	120,663

decrease in temperature during the fall and winter led to increased heating demands. This increase of anthropogenic sulphur to the atmosphere from the Utilities Annex, along with increased fuel consumption by other sources throughout St. John's, contributed to the observed decreasing isotopic compositions in local and transplanted lichens during December 1997.

It is important to note that site 17 lichens belong to the genus *Bryoria*, unlike sites 15 and X that belong to the genus *Alectoria*. Even though the site 17 lichens belong to a different genus, there was no difference in the movement of isotopic compositions over the one year period when compared to *Alectoria*.

As was stated in section 4.1.2, it is possible that numerous physiological features of lichens may lead to a slight natural variation in isotopic compositions. The majority of physiological features are beyond the scope of this study, however, it was decided that desiccation/rehydration cycles of the lichens would be examined using available meteorological data. Reports indicate that lichens accumulate sulphur efficiently when they are moist (Palomäki *et al.*, 1992). Thus, total precipitation data recorded at the St. John's Airport for the time period of this study was compared to the isotopic compositions obtained from Experiment I to identify any apparent relationships or patterns with periods of extreme wetness or dryness. Figure 4.1 indicates that no relationship between isotopic compositions and total precipitation is evident. This can be explained by the fact that the sites at the MUN Botanical Garden experience a unique microclimate that differs from that of the St. John's Airport (section 3.4). The transplanted lichens were placed in sites that were not subjected to extreme dry periods.

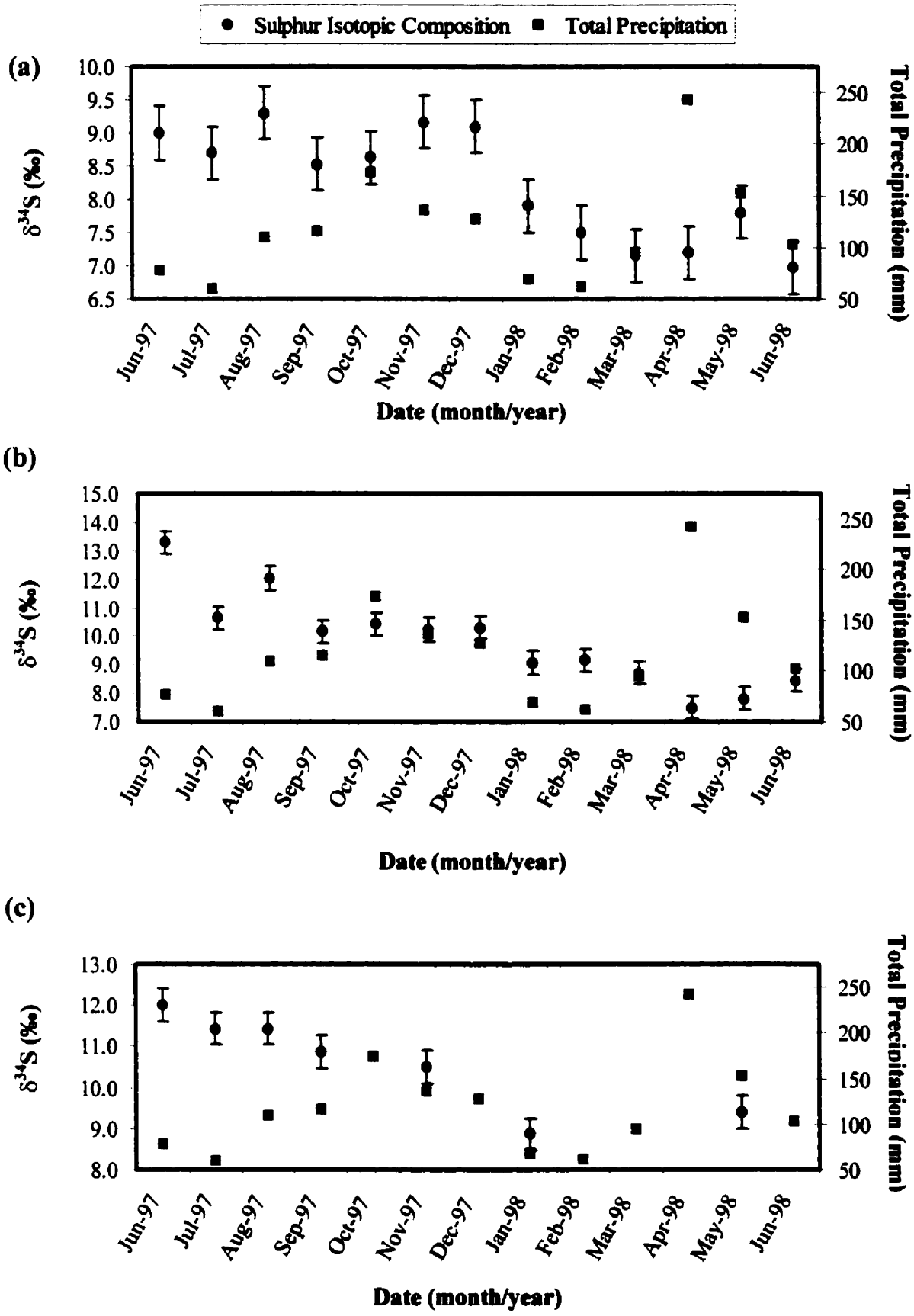


Figure 4.1 Plot of total monthly precipitation and average $\delta^{34}S$ values for lichens moved from Bonavista sites (a) X (b) 15 (c) 17 to the Botanical Garden over a one year period (Experiment I).

Even when the total precipitation was low, the lichens remained moist. All three sites were located very close to a pond that constantly contributed to the moisture content in the atmosphere. Also, the three sites were located in the interior of a densely forested area which has been found to be more favourable for retaining thallus water probably due to the less evaporative conditions within forests (Renhorn *et al.*, 1997). The transplanted lichens did not encounter extended periods of dryness in this particular study so wet/dry cycles cannot be used to explain the changes in isotopic compositions.

While there was a significant decreasing trend in each case over the period of one year, the results of this transplant experiment did not establish a definitive length of time for pendulous epiphytic lichens to assume the sulphur isotopic signature of the surrounding atmosphere. As was discussed, the local lichens growing in the Botanical Garden exhibited an average $\delta^{34}S$ value of +5.8 ● 0.8‰. The isotopic compositions at the end of the one year study period for sites X, 15 and 17 were 7.0‰, 8.4‰ and 9.4‰, respectively. In each case the final isotopic compositions after the one-year period did not fall within the ambient range. It is evident that the transplanted lichens needed more than one year to exhibit the same isotopic compositions as those of the lichens originally growing in the Botanical Garden (+5.8 ● 0.8‰). It is difficult to estimate the additional amount of time that would be needed to completely allow the transplanted lichens to assume the isotopic signature of the Botanical Garden atmosphere because of the amount of natural variability. However, if the isotopic compositions continue to decrease in a linear fashion as was the case with the site 15 lichens ($r^2 = 0.8279$), a minimum additional six months would be needed to allow the transplanted lichens to assume the isotopic

signature of the local lichens in the Botanical Garden. It is expected that when this is accomplished the isotopic compositions would level-off and remain fairly constant over time. Only slight changes would occur due to natural variation from numerous events occurring around the Botanical Garden which alter the isotopic signature of the atmosphere. A long-term transplant study for a period of at least 2 years would likely clarify this.

There is a gap in the literature concerning the use of sulphur stable isotopes to determine the amount of time for lichens to accumulate sulphur. Thus, comparisons with other studies are not possible, however, there are studies that estimated the accumulation time of sulphur by measuring sulphur concentration. These comparisons will be made in the following section.

4.3.1.2 Sulphur Concentration

The results from the concentration data for Experiment I are detailed in section 3.2.3. The sulphur concentrations of each group of Bonavista lichens increased significantly over the period of one year. The graphs in Figures 3.5, 3.6 and 3.7 all began with low concentrations and increased to significantly higher concentrations, however, they exhibited a great amount of natural variability.

In all three cases, the greatest amount of increase in sulphur concentration occurred within the first month of the experiment. Site X lichens increased by 164 ppm. Site 15 lichens increased by118 ppm. Site 17 lichens increased by 335 ppm. This increase in concentration immediately after the transplant procedure can be explained by

the fact that the lichens were immersed in an atmosphere with a much higher total sulphur concentration. Evidently, the lichens were encountering significantly more sulphur, thus they were forced to process this sulphur at a faster rate and in greater quantities. The lichens utilized the first month to acclimatize to the new and different surrounding atmosphere. After this initial intense acclimatization period, the lichens apparently accumulated sulphur at a much slower rate that varied somewhat from month to month.

The transplant study by Cañas *et al.* (1997) showed contrasting results. The lichens, *Parmotrema austrosinense* and *P. conferendum*, transplanted in a non-polluted site and an urban site demonstrated a slight decrease in sulphur concentration during the first month of exposure. Other studies that tested sulphur concentration did not do so on a monthly basis and therefore could not be compared.

When the sulphur concentrations of all three groups of lichens are compared, it is difficult to find similarities other than the initial significant increase in each case. Evidently, there is a great amount of natural variability associated with the sulphur concentrations. This variability is expected because the sulphur concentration of the lichens reflects the sulphur concentration of the atmosphere. Thus, if anthropogenic activities contribute sulphur to the atmosphere, it will be reflected in the lichen sulphur concentrations. For example, if the stack of the Utilities Annex contributes sulphur dioxide emissions to the atmosphere and these emissions are moved toward the Botanical Garden, the lichens will directly reflect this increase in the sulphur concentrations. Numerous anthropogenic activities contribute daily to the amount of atmospheric sulphur

such as vehicle exhaust, fire burning, highway construction and industrial activity thus altering sulphur concentrations.

Total precipitation data from the St. John's Airport were compared to the sulphur concentrations from Experiment I. Figure 4.2 demonstrates that there is no evident relationship between total precipitation and concentration. The changes in sulphur concentration cannot be attributed to wet/dry cycles because, as was stated in section 4.3.1.1, the lichens in this particular study were not subjected to periods of extreme dryness.

The transplanted lichens X and 15 had insufficient time to reach the level of sulphur concentration of the lichens originally growing in the Botanical Garden (500 ± 74 ppm). The final concentrations for sites X and 15 lichens were 408 ppm and 413 ppm, respectively. Both groups of lichens were close to the local level. Again, it is difficult to estimate the additional amount of time that would be needed to completely allow the transplanted lichens to assume the sulphur concentration of the Botanical Garden atmosphere because of the amount of natural variation. However, if the sulphur concentrations continue to increase in a linear fashion as was the case with the site 15 lichens ($r^2 = 0.6769$), a minimum additional three months would be needed to allow the transplanted lichens to achieve the same concentration as the local lichens in the Botanical Garden.

In comparison to previous transplant studies, the exposure time of 12-15 months falls within the range of commonly-used exposure periods. The transplant studies

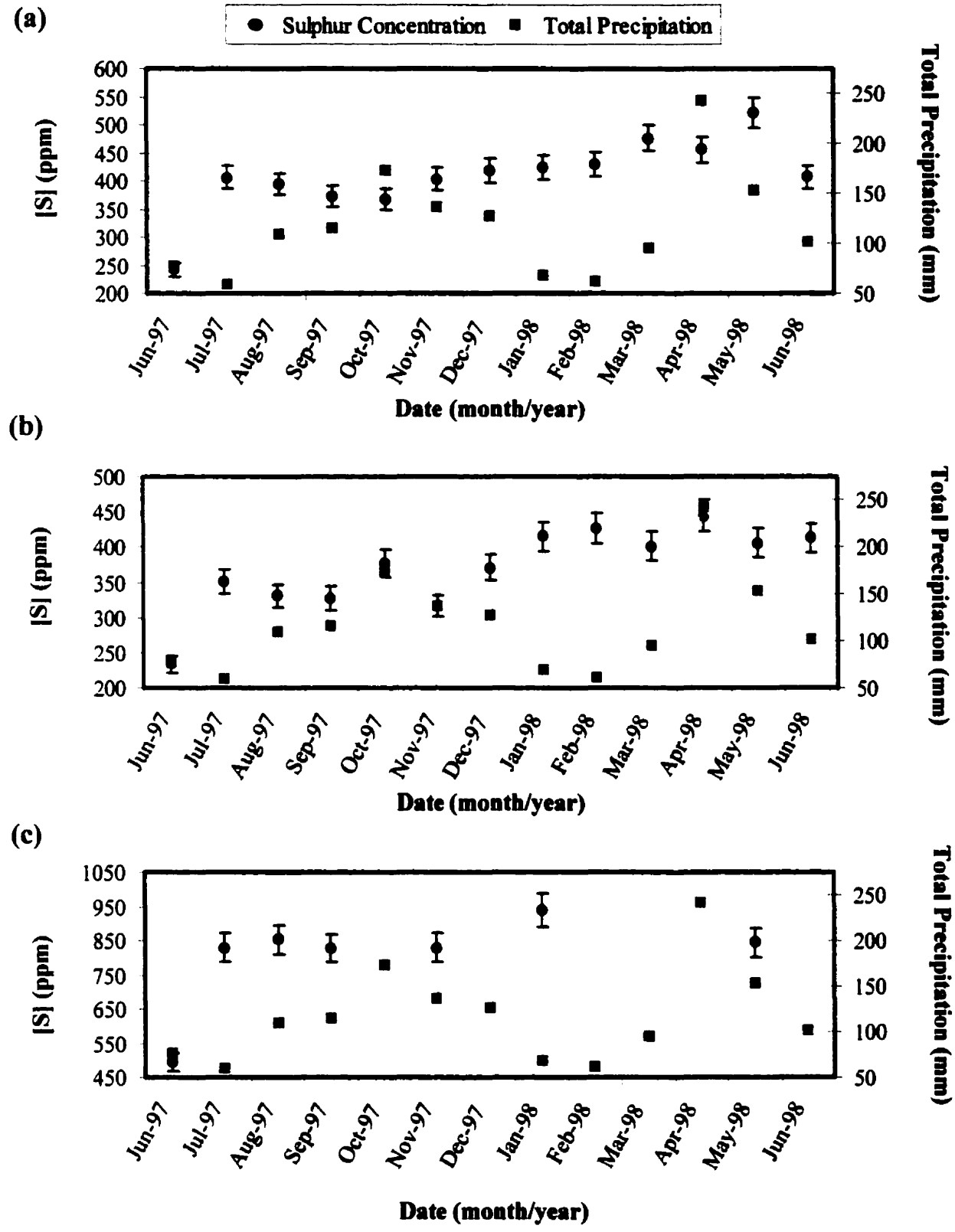


Figure 4.2 Plot of total monthly precipitation and [S] values for lichens moved from Bonavista sites (a) X (b) 15 (c) 17 to the Botanical Garden over a one year period (Experiment I).

discussed in Chapter One utilized exposure times ranging from 2 months to 3 years to measure the accumulation of a variety of elements.

It was determined by Gailey & Lloyd (1986) that metal accumulation by the lichen, *Hypogymnia physodes,* occurred after as little as 2 months. A period of 2 months was determined to be an appropriate length of exposure because it met the following criteria: detectable accumulated concentrations, reliable values with high replicability and within the limits of practical consideration (Gailey & Lloyd, 1986). These findings were reinforced by Loppi *et al.* (1998).

On the opposite end of the spectrum, a transplant study by Bennett *et al.* (1996) utilized an exposure period of three years to allow the accumulation of 20 chemical elements. The exposure period of this study was almost three times longer than the majority of transplant studies with exposure periods from 30-214 days, thus comparison was difficult. It was found that increase in element concentrations fell in the same general range as those studies with shorter exposure periods.

In the middle of the range, a transplant study by Sloof (1995) measuring cobalt, scandium and zinc determined that an exposure period of at least 12 months was required to distinguish the concentration of the exposed lichen from the initial concentration of the lichen used in this study.

It is evident from the literature that questions still remain concerning the accumulation of a variety of elements in transplanted lichens. The accumulation rate of sulphur in particular remains unclear. The study by Palomäki *et al.* (1992) found that after a period of 5-6 months the transplanted lichens had the same sulphur concentration

as the original lichens growing at the same sites. On the other hand, the study by Garty *et al.* (1997) found that 10 months was sufficient time to observe a significant increase in sulphur concentration of transplanted lichens. There are numerous transplant exposure periods reported in the literature, thus it is possible that a wide range of factors affect the accumulation rate of sulphur such as distance from a pollution point source, degree of thallus wetness and difference in lichen species.

The site 17 lichen, *Bryoria capillaris*, demonstrated interesting sulphur concentration results. It is important to note that *B. capillaris* initially had higher sulphur concentrations than both groups of *A. sarmentosa*. The starting concentration of the site 17 lichens was 494 ppm, only 6 ppm away from the average concentration of the lichens originally growing in the Botanical Garden. Over the year, the concentrations increased to a very high level of 844 ppm.

Evidently, *B. capillaris* has the ability to accumulate and process greater amounts of sulphur than *A. sarmentosa*. However, this excess accumulation of sulphur by *B. capillaris* led to physiological damage. Upon initial collection of *B. capillaris*, the thalli of the lichen samples were greenish-gray in colour and had a fine texture. After *B. capillaris* was transplanted to the MUN Botanical Garden, the thalli changed from greenish-gray to a dull brown. The thalli became so brittle that the samples crumbled when they were touched. Thus, the data set for *B. capillaris* is incomplete for both Experiment I and II because this lichen was more apt to fragment and blow away.

This finding of physiological damage to *B. capillaris* is reinforced in a study by Holopainen (1984). The lichens, *Hypogymnia physodes* and *Bryoria capillaris*, were

transplanted to the proximity of a paper mill/fertilizer complex in central Finland. In the highly polluted SO_2 sites, acute injury, including cell organelle degeneration and plasmolysis, was observed. Further away from the point source in a lower level of SO_2 pollution, chronic injury including changes in chloroplast and vacuolar shapes as well as swelling of the algal mitochondria was observed. It was estimated in the Holopainen (1994) study that, in less polluted areas, months or years might be necessary before macroscopic changes would be evident.

The above estimation by Holopainen (1984) proved to be accurate with respect to this transplant study because visible macroscopic changes in the colour and texture of the transplanted *B. capillaris* were evident 3-4 months after the transplant procedure. The degeneration in appearance continued over the period of the one-year study, however, microscopic examination of the lichen at the end of the study period indicated that it did not die.

In contrast, the transplanted *A. sarmentosa* samples (sites X and 15) were bright yellow-green in colour with stiff basal attachment and flaccid hollow branches that remained unchanged macroscopically throughout the study period. The stability of appearance in *A. sarmentosa* can be attributed to the initial lower concentrations of sulphur than that of *B. capillaris*.

4.3.1.3 Sulphur Isotopic Composition and Sulphur Concentration

Figures 4.3, 4.4 and 4.5 graphically illustrate that the sulphur isotopic compositions decrease while the sulphur concentrations increase over the period of one

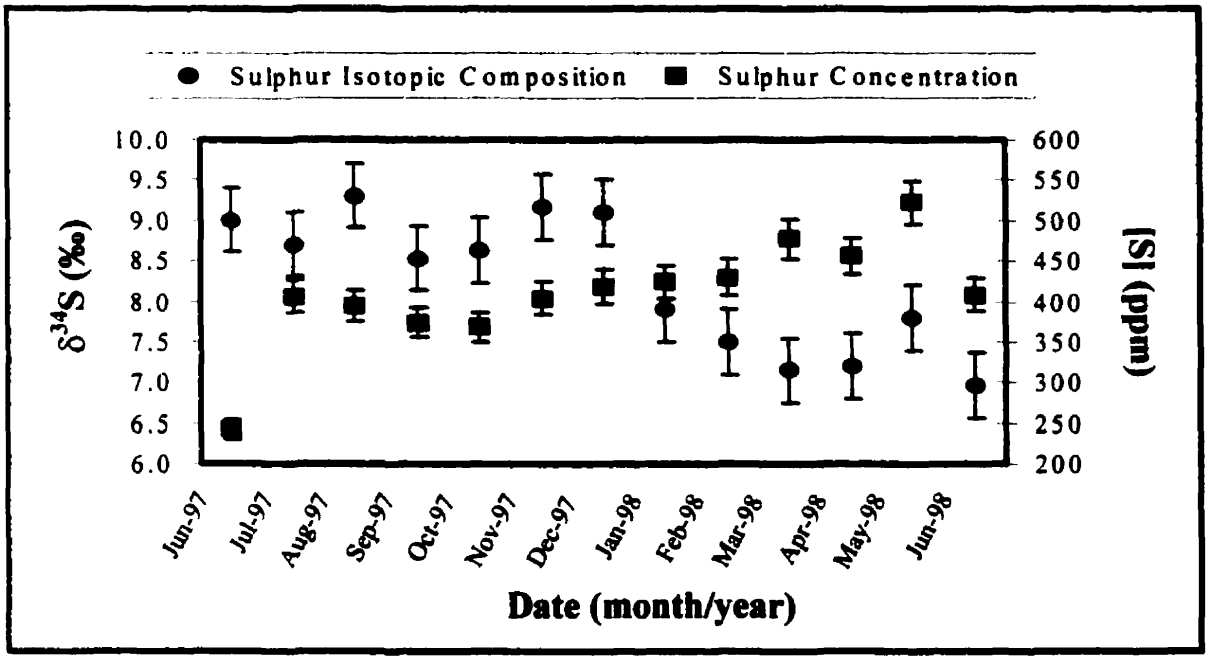


Figure 4.3 Plot of average $\delta^{34}S$ values and [S] values for lichens moved from Bonavista site X to the Botanical Garden over a one year period (Experiment I).

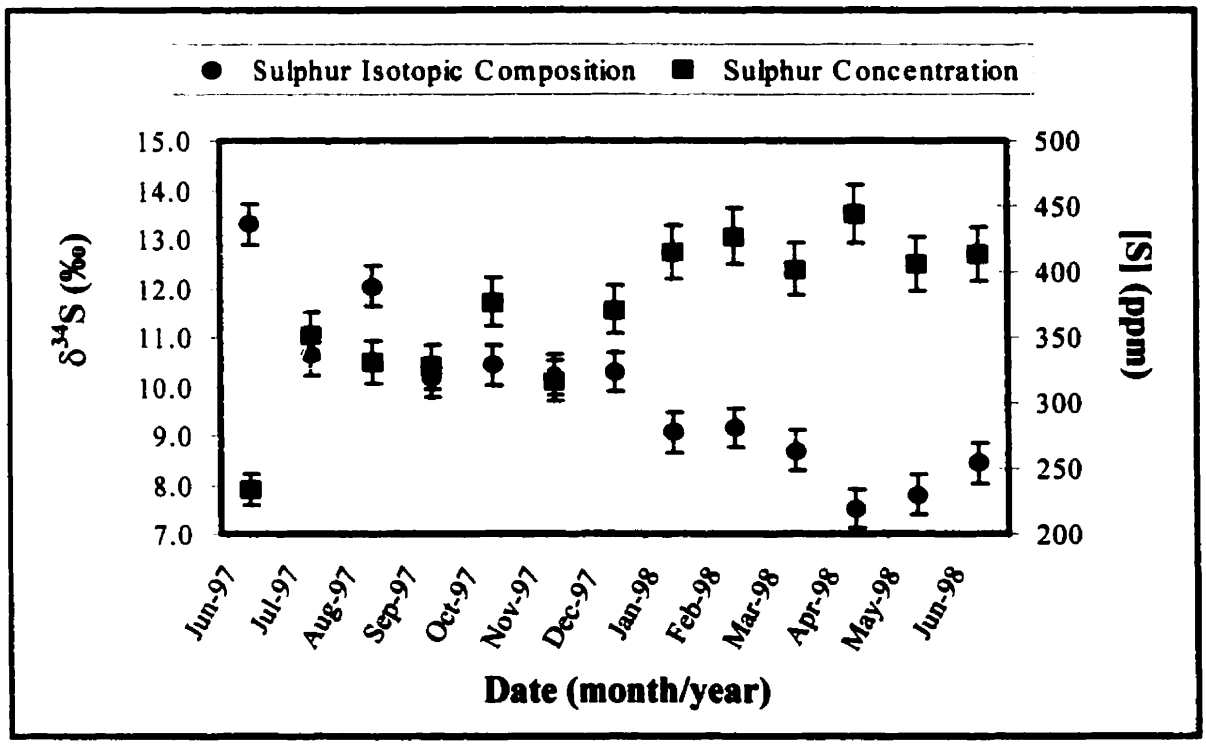


Figure 4.4 Plot of average $\delta^{34}S$ values and [S] values for lichens moved from Bonavista site 15 to the Botanical Garden over a one year period (Experiment I).

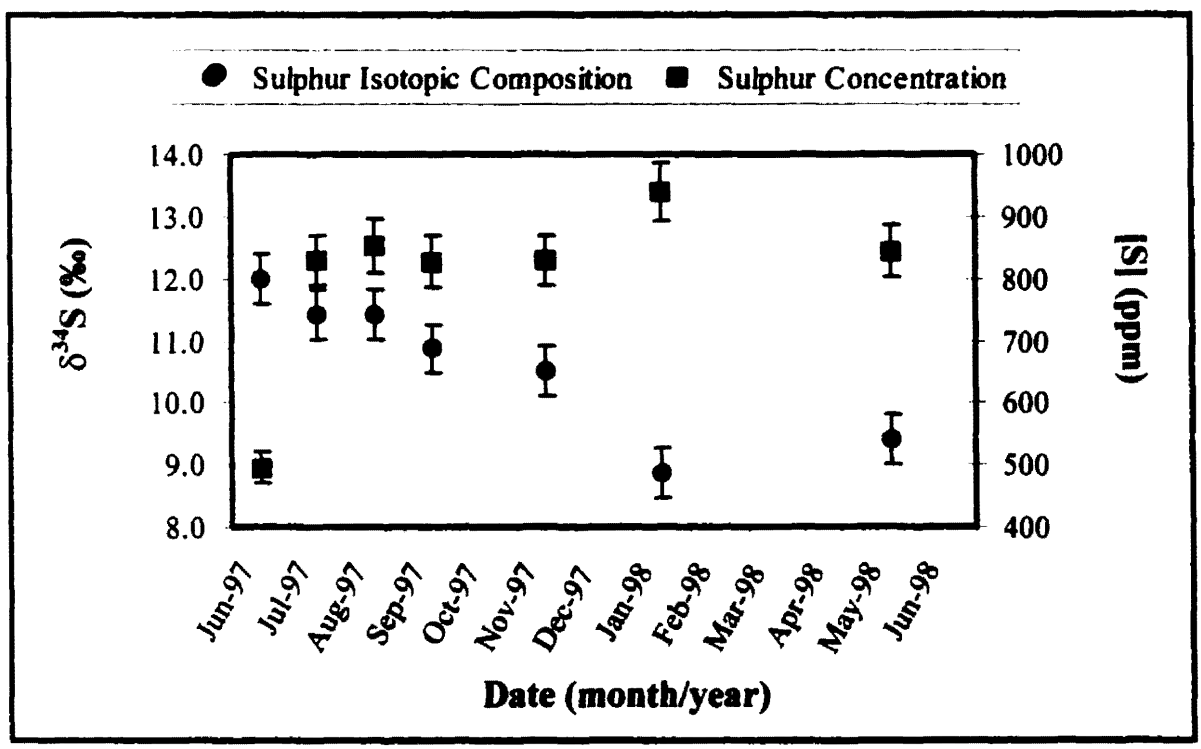


Figure 4.5 Plot of average $\delta^{34}S$ values and [S] values for lichens moved from Bonavista site 17 to the Botanical Garden over a one year period (Experiment I).

year in all three groups of lichens. The anthropogenic sulphur surrounding the Botanical Garden was being accumulated by the pendulous epiphytic lichens as reflected by the decreasing isotopic compositions and increasing concentrations. Figure 4.6 demonstrates the relationship between the decreasing sulphur isotopic compositions and increasing sulphur concentrations for the site 15 lichens. The r^2 for the regression of $\delta^{34}S$ on [S] is 0.8047. If the trendline is extended it will intercept at the isotopic composition (+5.8 ● 0.8‰) and concentration (500 ± 74 ppm) of the local lichens in the MUN Botanical Garden.

The most noticeable observation when considering both the isotopic data and concentration data together is that during the first month of the experiment as the sulphur concentration increased greatly, the sulphur isotopic compositions decreased in each case. A significant adjustment occurred within the first month with respect to both sulphur isotopic composition and sulphur concentration. The transplanted lichens were immersed into an atmosphere influenced by a different source of sulphur in greater total amounts. Thus, it was expected that a change in isotopic compositions and concentrations would result almost immediately after the transplant procedure. After this initial introductory period, the isotopic compositions continue to decrease while the concentrations continue to increase with natural variation among the isotopic and concentration data.

4.3.2 Experiment II

A second transplant experiment was performed to complement the results obtained from Experiment I. Lichens were again moved from the Bonavista Peninsula

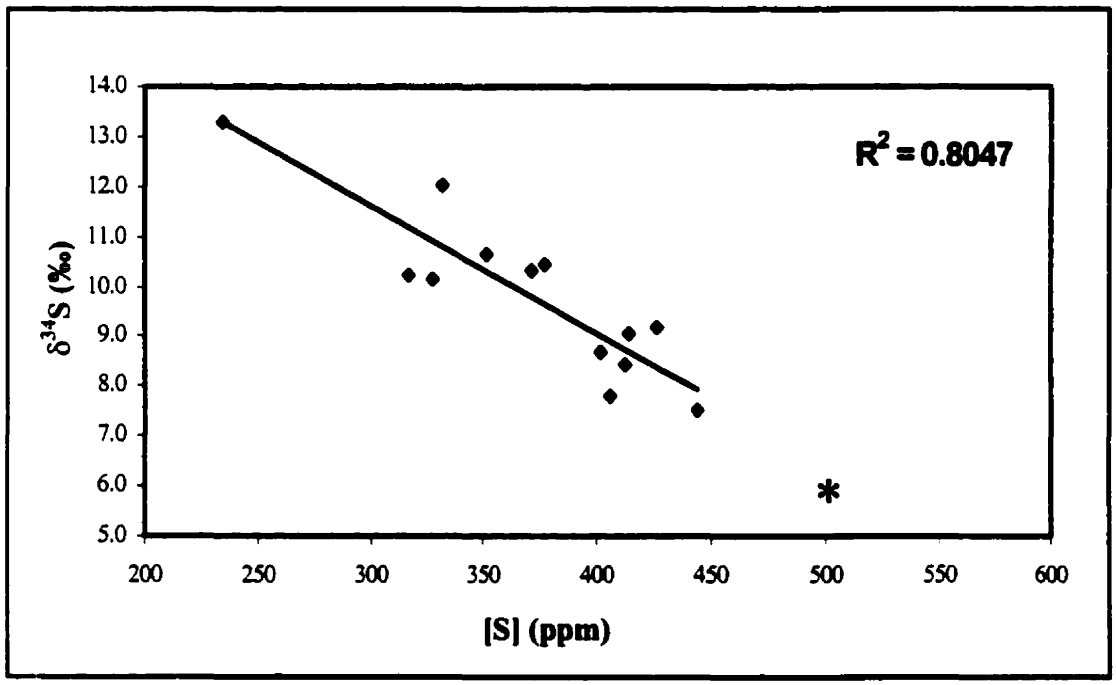


Figure 4.6 Average $\delta^{34}S$ values plotted against average [S] values for site 15 lichens. * represents the isotopic composition and concentration of local lichens in the Botanical Garden.

into the MUN Botanical Garden. Samples were collected weekly and analysed for both sulphur isotopic composition and sulphur concentration over a period of three months.

4.3.2.1 Sulphur Isotopic Composition

The most significant observation from section 3.3.2 is the great amount of variability in the isotopic data that occurs from week to week in all cases. The increases and decreases in the isotopic data on a weekly basis can be attributed to natural variation. At any given time, the atmosphere may be anthropogenically-influenced by a variety of sources including emissions from the Utilities Annex, vehicle exhaust, road construction and fire burning leading to a decrease in isotopic compositions. On the other hand, increases in isotopic compositions are most likely due to sulphur originating from the marine environment being carried into the atmosphere surrounding the Botanical Garden.

Site 15 lichens exhibited the highest initial starting isotopic composition (+14.6‰). There was an overall decrease of a total of 1.7‰ over the three months, however, the amount of variability is significant.

Sites X and 17 lichens did not decrease over the three-month period. Evidently, these lichens needed a longer time to assume the isotopic signature of the atmosphere surrounding the Botanical Garden. Interestingly, the greatest amount of natural variation among the isotopic compositions for site X, 15 and 17 lichens (2.1‰, 2.7‰ and 1.4‰ respectively) is close to the maximum amount of variation established for the local lichens (1.9‰).

4.3.2.2 Sulphur Concentration

The results from the concentration data for Experiment II are detailed in section 3.3.3. Again, the three-month period of this experiment did not show any significant patterns for the concentration data of the site 15 and 17 lichens. The concentrations for site X lichens were very variable thus they could not be averaged and were not included in the analysis (section 3.3.1).

Site 15 lichens exhibited a slight increase in concentrations over three months. Site 17 concentrations remained fairly constant, beginning and ending with similar values. However, the amount of variation in each case was great once again.

Evidently, anthropogenic or natural activities occurring on a weekly basis and contributing sulphur to the atmosphere are subsequently reflected in the lichen samples. It is important to note that the concentrations of this experiment did not increase greatly over the first month as was the case in Experiment I. Apparently, the initial shock after the transplant procedure was less extensive in the second experiment.

4.3.2.3 Sulphur Isotopic Composition and Sulphur Concentration

The three-month period did not provide enough time to see any significant changes in isotopic or concentration data (Figures 4.7 and 4.8). The most significant observation was that on a weekly basis there is a great amount of variability in both the isotopic compositions and concentrations. Due to this great amount of natural variability, there is no definite relationship between the sulphur isotopic compositions and sulphur concentrations (Figure 4.9) during this short experiment as was the case in Experiment I.

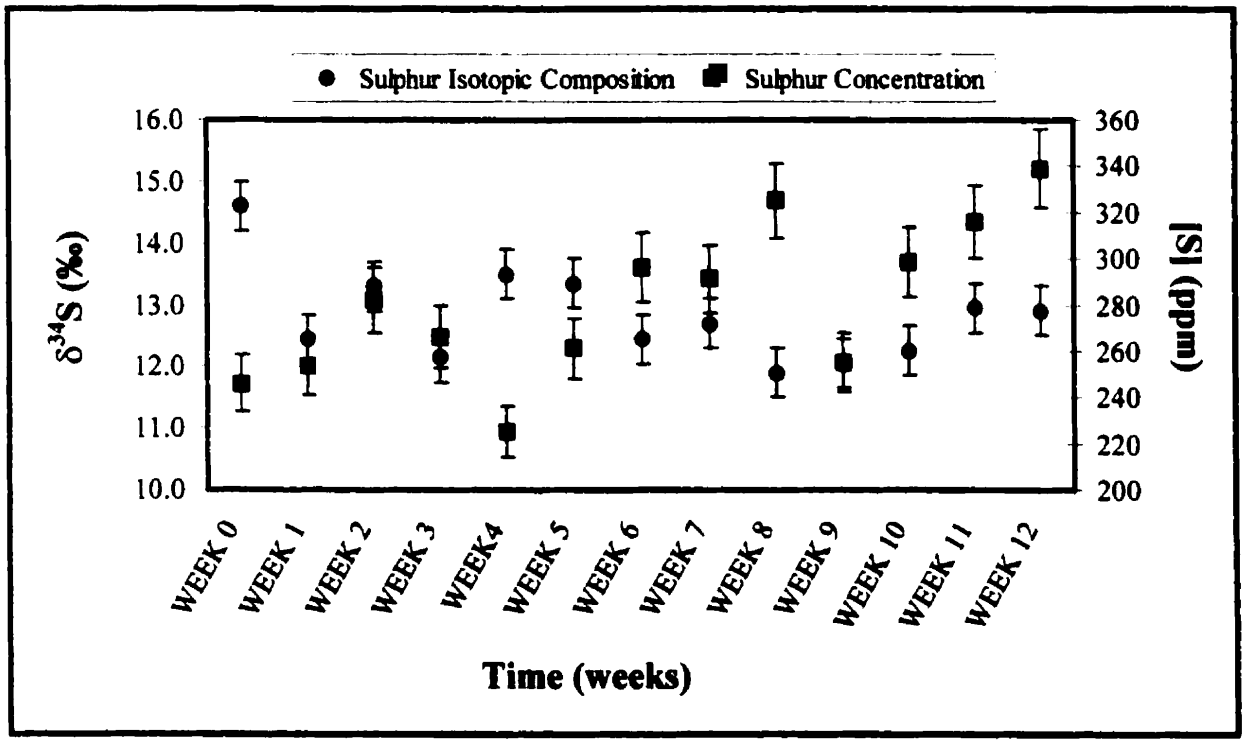


Figure 4.7 Plot of average $\delta^{34}S$ values and [S] values for lichens moved from Bonavista site 15 to the Botanical Garden over a twelve week period (Experiment II).

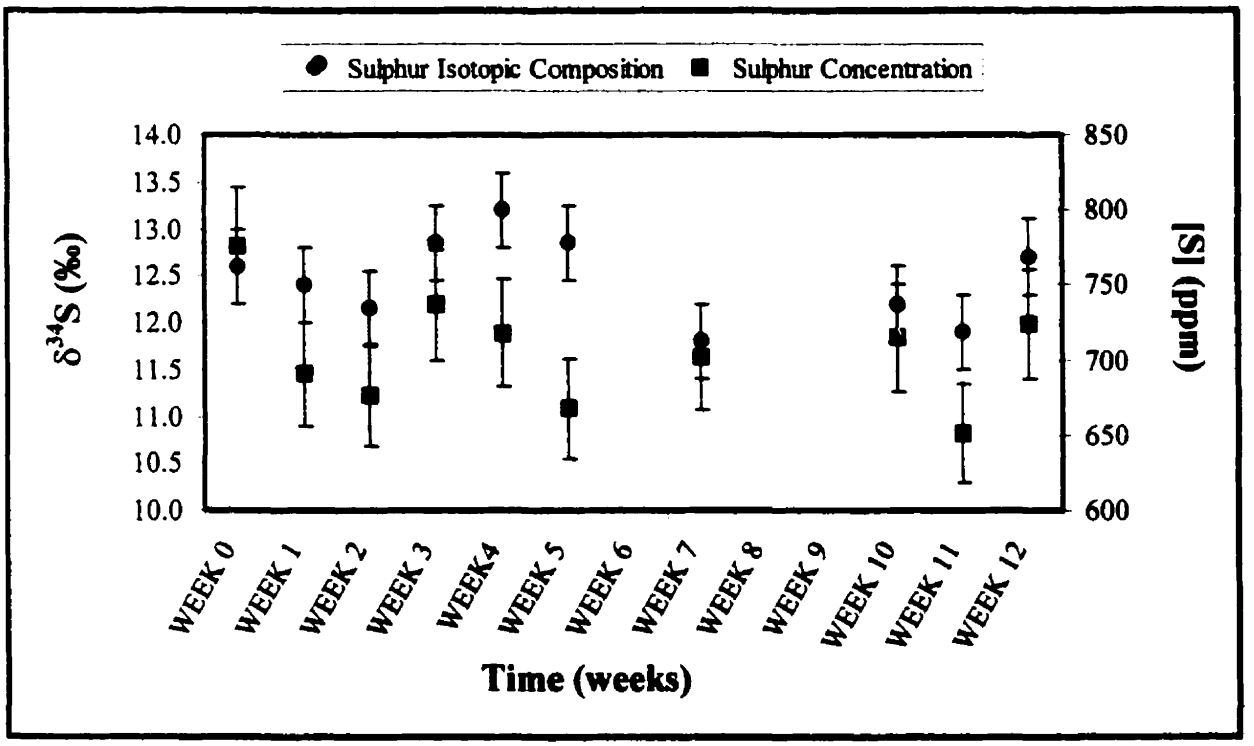
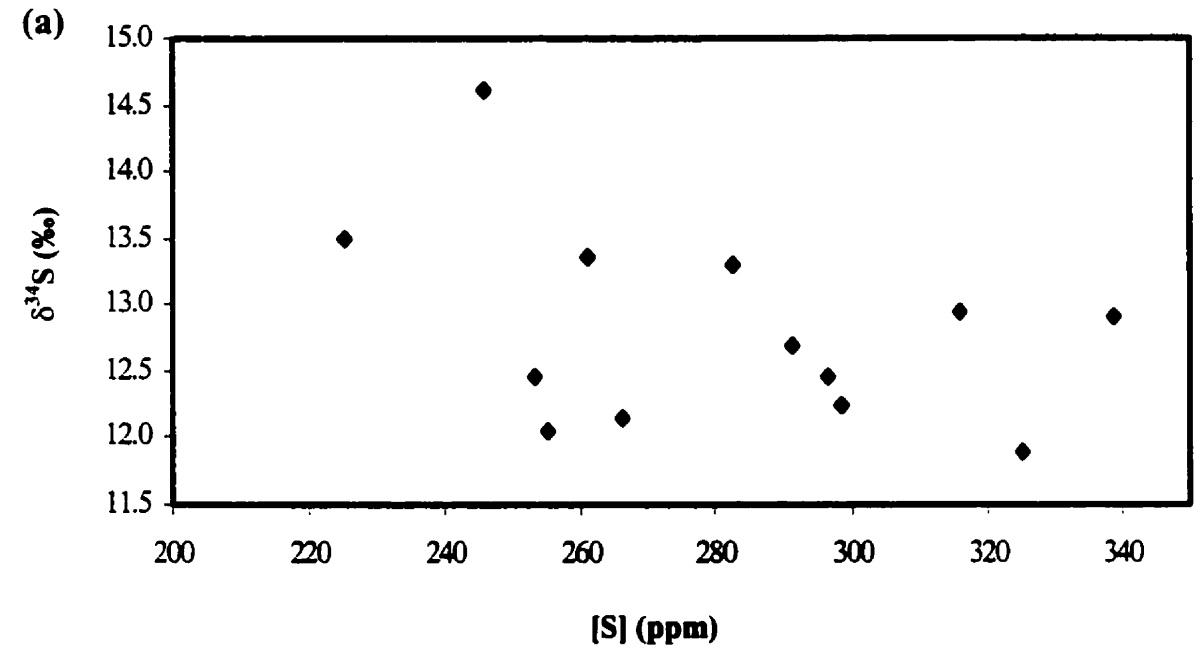


Figure 4.8 Plot of average $\delta^{34}S$ values and [S] values for lichens moved from Bonavista site 17 to the Botanical Garden over a twelve week period (Experiment II).



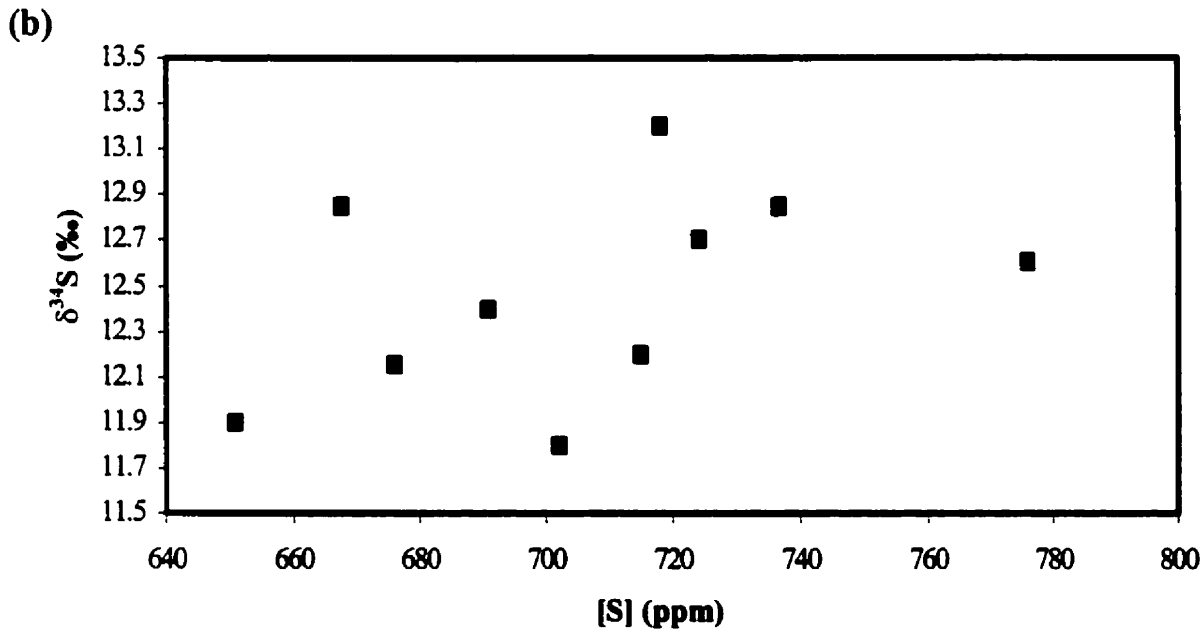


Figure 4.9 Average $\delta^{34}S$ values plotted against average [S] values for lichens from Bonavista sites (a) 15 (b) 17 over a twelve week period (Experiment II).

4.3.3 Experiment I and II

It is obvious that Experiment I demonstrated important findings. The one-year study provided a sufficient amount of time to see a definite change in both isotopic compositions and concentrations. The transplanted lichens began to react to the new environment within the first month and continued to change throughout the year. From this experiment it was determined that pendulous epiphytic lichens need more than one year to attain the same isotopic signature and sulphur concentration of the surrounding environment.

The second experiment was less significant however it showed that more than three months are needed to see a definite change in isotopic compositions and concentrations. Most importantly, it demonstrated the great amount of variability that takes place on a weekly basis. When this is taken into consideration, it is evident that a small amount of natural variation on a monthly basis can be expected.

Overall, the two experiments determined that the pendulous epiphytic lichens used in this study need over one year to attain the isotopic composition and sulphur concentration of the new surrounding atmosphere. These results are significant because they demonstrate that while lichens continuously accumulate sulphur from the atmosphere, more than one year is required for them to be representative of the atmosphere if its sulphur isotopic signature is suddenly altered.

CHAPTER 5

CONCLUSIONS

5.1 Overview

The main objective of this transplant experiment was to determine the length of time it takes pendulous epiphytic lichens to achieve the sulphur isotopic composition and sulphur concentration of the surrounding atmosphere. This study is the first attempt to monitor changes in sulphur isotopic composition of transplanted lichens. This information is essential to complement lichen studies, in general, and stable isotopic studies with lichens, specifically.

Two transplant experiments were performed. The primary transplant extended for a period of one year with sampling on a monthly basis. The secondary transplant ran for a three-month period with sampling on a weekly basis.

From Experiment I it was found that the isotopic compositions decreased while the concentrations increased over the one-year period for each group of lichens. These results confirmed that transplantation of pendulous epiphytic lichens into an area with a high content of anthropogenic sulphur in the atmosphere will indeed cause a decrease in isotopic compositions and a subsequent increase in sulphur concentrations. It was found consistently in each group that a significant decrease in isotopic compositions occurred during the month of December 1997. The concentration, on the other hand, increased greatly during the first month of the experiment. It is important to note that there is natural variability among the isotopic and concentration data, apparently unrelated to transplantation, as was demonstrated by Experiment II.

From the results of Experiment I, it was determined that one year was insufficient time for the transplanted pendulous epiphytic lichens to assume the same isotopic composition and concentration as the lichens originally growing in the Botanical Garden. It is estimated that a minimum additional six months would allow the transplanted lichens to reflect the sulphur isotopic composition and sulphur concentration of the new surrounding atmosphere.

The implications of this study for environmental monitoring and assessment are significant. It is possible to measure significant changes in sulphur isotopic compositions and sulphur concentrations over time without waiting extended periods for macroscopic indications of damage to the lichen. From this study, it was determined that lichens constantly react to various sulphur sources in the atmosphere. If the atmospheric sulphur is significantly altered isotopically, this will be seen in the isotopic signature of pendulous epiphytic lichens. Thus, isotopic and concentration monitoring of lichens around areas of proposed development provide a definitive measure of the impact of emissions on the surrounding vegetation over time.

5.2 Future Work

This study has laid the foundation for further research. A longer-term study of approximately 2-3 years would confirm the results obtained from this study and definitively determine the length of time transplanted pendulous epiphytic lichens need to reach the same sulphur isotopic composition and concentration of local lichens.

A reciprocal study where lichens from the MUN Botanical Garden are transplanted into the sites along the Bonavista Peninsula would provide valuable complementary results to this study.

It would also be interesting to perform a similar transplant study utilizing other abundant lichen species such as *Cladonia* to determine if various species react similarly.

Finally, the implications of this study for environmental monitoring and assessment are great. There are numerous industrial point-sources throughout Newfoundland and Labrador that emit pollutants into the surrounding atmosphere, thus calling for regular environmental monitoring. Studies involving transplanted and local lichens can effectively monitor improvement as well as deterioration in atmospheric quality surrounding industrial point-sources such as the Come By Chance oil refinery, Holyrood thermal generating station and proposed Voisey's Bay Nickel mine and smelter sites.

Overall, studies of atmospheric pollution utilizing lichens as effective bioindicators have provided significant findings in the past and should continue in the future.

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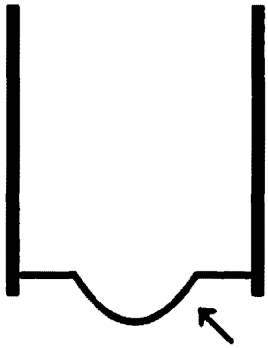
Appendix I

Parr Bomb Procedure

- Accurately weigh 0.75 – 1 g of crushed lichen and place into a clean combustion capsule.
- Attach nickel alloy fuse wire to the electrodes emerging from the bomb lid shown in the following diagram.



- Place 10 ml of deionized water and 3-5 drops of 50% hydrogen peroxide into the bottom of a clean Parr bomb container.
- Place combustion capsule with lichen into circular ring on the lid portion of the bomb.
- Adjust the fuse wire so it is positioned close to but not touching the lichen sample.
- Gently slip the lid into the bomb container (should rest ~1-2 mm above lip of container).
- Screw containment-ring onto the bomb container.
- Attach the connection fitting of the oxygen tank to the inlet valve on the Parr bomb with the holes aligned.
- Flush bomb with O_2 for three seconds.
- Fill bomb with 30 atmospheres of O_2.
- Attach the wires of the ignition unit to the bomb lid.
- Submerge bomb in cold water bath and check for leaks.

- Ignite bomb and allow to cool in the water bath for 15 minutes.
- Remove bomb from the water bath and slowly release the pressure.
- Remove the screw cap and separate the bomb lid from the container.
- Rinse all bomb components with deionized water into a 500 ml beaker.
- Remove any excess fuse wire and clean the Parr bomb thoroughly for the next combustion.
- Filter the Parr bomb washings through 0.45 μm cellulose membrane filters.
- $BaSO_{4(s)}$ is precipitated from the washing solution.

Note: Non-powdered latex gloves must be worn during this procedure to prevent contamination.

Appendix II

Sample Calculation of Total Amount of Sulphur in a Lichen Sample

Example using sample A/15/1:

- **0.0040751 kg** → total amount of crushed lichen combusted in Parr oxygen bomb
- **0.50 ml** → washing solution from Parr bomb procedure was diluted to this known volume for ion chromatography
- **8.802 ppm** → sulphate concentration obtained from ion chromatography
- **33.4%** → percentage of sulphur in a sulphate molecule

$$\frac{\textbf{8.802 mg}}{\textbf{1 L}} = \frac{\textbf{X mg}}{\textbf{0.50 L}}$$

= 4.401 mg of sulphate in 0.50 L of solution

$$\frac{\textbf{4.401 mg}}{\textbf{0.0040751 kg}} = \frac{\textbf{X mg}}{\textbf{1 kg}}$$

= 1079.97 ppm of sulphate in total lichen

1079.97 ppm x 0.334

= 361 ppm of sulphur in total lichen

Appendix III

Table III.1 $\delta^{34}S$ and [S] results for Experiment I.

MONTH	$\delta^{34}S$ (‰)				[S] (ppm)			
	Site A/15	Site B/15	Site C/15	Average	Site A/15	Site B/15	Site C/15	Average
JUN-97	13.3	13.3	13.3	13.3	234	234	234	234
JUL-97	11.0	11.3	9.6	10.6	361	307	387	352
AUG-97	11.3	11.8	13.0	12.0	325	296	372	331
SEP-97	9.8	10.8	9.9	10.2	369	310	303	327
OCT-97	10.4	10.2	10.7	10.4	431	362	338	377
NOV-97	11.0	10.6	9.1	10.2	283	322	345	317
DEC-97	9.5	10.7	10.7	10.3	358	325	430	371
JAN-98	9.6	8.7	8.9	9.1	394	354	497	415
FEB-98	9.2	9.3	9.0	9.2	422	337	521	427
MAR-98	8.7	-	-	8.7	402	-	-	402
APR-98	7.4	7.6	-	7.5	416	473	-	445
MAY-98	7.8	-	-	7.8	406	-	-	406
JUN-98	8.6	8.7	8.0	8.4	457	330	452	413
	Site A/X	Site B/X	SiteC/X	Average	Site A/X	Site B/X	SiteC/X	Average
JUN-97	9.0	9.0	9.0	9.0	242	242	242	242
JUL-97	8.9	8.1	9.1	8.7	430	373	415	406
AUG-97	9.3	9.3	9.3	9.3	383	420	381	395
SEP-97	8.4	8.8	8.4	8.5	433	383	307	374
OCT-97	8.4	8.3	9.2	8.6	340	383	381	368
NOV-97	9.6	9.2	8.7	9.2	434	387	389	404
DEC-97	8.8	9.5	9.0	9.1	425	425	405	418
JAN-98	7.8	8.3	7.6	7.9	434	429	407	424
FEB-98	7.8	7.2	7.5	7.5	466	441	382	430
MAR-98	7.5	6.8	-	7.2	525	429	-	477
APR-98	7.1	7.3	-	7.2	464	448	-	456
MAY-98	8.1	-	7.5	7.8	490	-	554	522
JUN-98	6.7	7.3	6.9	7.0	399	412	413	408

Table III.1 (continued)

MONTH	$\delta^{34}S$ (‰)				[S] (ppm)			
	Site A/17	Site B/17	SiteC/17	Average	Site A/17	Site B/17	SiteC/17	Average
JUN-97	12.0	12.0	12.0	12.0	494	494	494	494
JUL-97	11.6	11.7	11.0	11.4	737	853	897	829
AUG-97	11.7	11.1	11.5	11.4	877	853	827	852
SEP-97	11.3	11.2	10.1	10.9	850	811	820	827
OCT-97	-	-	-	-	-	-	-	-
NOV-97	-	10.8	10.2	10.5	828	893	767	829
DEC-97	-	-	-	-	-	-	-	-
JAN-98	9.0	9.1	8.5	8.9	944	928	945	939
FEB-98	-	-	-	-	-	-	-	-
MAR-98	-	-	-	-	-	-	-	-
APR-98	-	-	-	-	-	-	-	-
MAY-98	9.4	-	-	9.4	844	-	-	844
JUN-98	-	-	-	-	-	-	-	-

Appendix IV

Table IV.1 $\delta^{34}S$ and [S] results for Experiment II.

WEEK	$\delta^{34}S$ (‰)			[S] (ppm)		
	Site A/15	Site B/15	Average	Site A/15	Site B/15	Average
0	14.6	14.6	14.6	246	246	246
1	12.8	12.1	12.5	324	253	289
2	14.6	12.0	13.3	236	282	259
3	11.6	12.7	12.2	259	266	263
4	13.9	13.1	13.5	246	225	235
5	13.3	13.4	13.4	292	261	277
6	12.2	12.7	12.5	294	296	295
7	12.4	13.0	12.7	293	291	292
8	12.1	11.7	11.9	299	325	312
9	11.3	12.8	12.1	227	255	241
10	12.0	12.5	12.3	301	298	300
11	13.9	12.0	13.0	261	316	288
12	12.1	13.7	12.9	326	339	332
	Site A/X	Site B/X	Average	Site A/X	Site B/X	Average
0	7.8	7.8	7.8	387	387	387
1	7.3	9.1	8.2	429	293	361
2	7.1	9.1	8.1	366	331	349
3	6.8	8.9	7.9	417	357	387
4	9.9	9.9	9.9	311	345	328
5	8.9	8.8	8.9	324	341	332
6	9.2	9.2	9.2	389	387	388
7	8.9	9.5	9.2	347	346	347
8	8.0	9.8	8.9	352	324	338
9	8.0	9.0	8.5	387	361	374
10	9.2	8.8	9.0	379	284	332
11	8.9	10.4	9.7	382	316	349
12	9.1	9.7	9.4	370	383	376

Table IV.1 (continued)

WEEK	$\delta^{34}S$ (‰)			[S] (ppm)		
	Site A/17	Site B/17	Average	Site A/17	Site B/17	Average
0	12.6	12.6	12.6	776	776	776
1	12.4	12.4	12.4	718	663	691
2	11.7	12.6	12.2	650	702	676
3	13.0	12.7	12.9	732	741	737
4	12.8	13.6	13.2	659	777	718
5	12.8	12.9	12.9	646	689	667
6	-	-	-	-	-	-
7	12.0	11.6	11.8	694	709	702
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	11.9	12.5	12.2	715	-	715
11	11.9	-	11.9	651	-	651
12	12.7	-	12.7	724	-	724

Appendix V

Table V.1 Meteorological data.

		BOTANICAL GARDEN				ST. JOHN'S AIRPORT			
Date	**Time**	**Temp. (°C)**	**Relative Humidity (%)**	**Wind Speed (Parking Lot) (m/s)**	**Wind Speed (Site) (m/s)**	**Temp. (°C)**	**Relative Humidity (%)**	**Wind Speed (m/s)**	**Wind Direction (m/s)**
02/11/98	10:30am	12.0	100	0.0	0.0	12.0	100	2.5	WSW
02/11/98	3:30pm	10.0	100	0.0	0.0	10.0	93	3.1	W
03/11/98	9:30am	6.5	100	3.0	0.0	6.0	93	5.0	E
03/11/98	1:30pm	7.1	100	2.0	0.0	6.0	100	7.8	E
03/11/98	4:30pm	7.2	100	1.0	0.0	7.0	93	5.0	ESE
04/11/98	9:30am	6.5	92	1.0	0.0	8.0	81	6.1	SW
04/11/98	1:30pm	7.8	88	0.0	0.0	8.0	71	5.0	WSW
04/11/98	4:30pm	7.3	91	0.0	0.0	6.0	75	3.1	SW
05/11/98	9:30am	6.2	100	0.0	0.0	6.0	93	4.2	SSW
05/11/98	12:30pm	7.7	82	0.0	0.0	8.0	71	5.0	W
05/11/98	4:30pm	6.0	89	0.0	0.0	6.0	70	7.8	W
06/11/98	10:30am	5.3	94	0.0	0.0	5.0	81	7.8	WNW
09/11/98	9:30am	1.7	91	0.0	0.0	2.0	64	4.2	WSW
09/11/98	12:30pm	3.5	77	0.0	0.0	4.0	56	5.6	WSW
09/11/98	3:30pm	3.0	85	0.0	0.0	3.0	60	4.7	W
10/11/98	9:30pm	1.9	94	0.0	0.0	2.0	69	3.1	WNW

Table V.1 (continued)

		BOTANICAL GARDEN				ST. JOHN'S AIRPORT			
Date	Time	Temp. (°C)	Relative Humidity (%)	Wind Speed (Parking Lot) (m/s)	Wind Speed (Site) (m/s)	Temp. (°C)	Relative Humidity (%)	Wind Speed (m/s)	Wind Direction (m/s)
1011/98	1:30pm	2.6	85	0.0	0.0	2.0	69	4.7	NW
10/11/98	3:30pm	2.1	83	0.0	0.0	1.0	69	3.1	N
16/11/98	1:30pm	2.5	90	3.0	1.5	2.0	69	13.3	WSW
16/11/98	4:30pm	0.4	100	2.0	1.0	0.0	69	10.3	WSW
17/11/98	11:30am	4.1	80	1.5	1.5	4.0	65	12.2	W
17/11/98	3:30pm	3.9	79	1.0	1.0	3.0	70	8.3	W
18/11/98	8:30am	-0.2	100	0.0	0.0	-2.0	93	1.9	W
18/11/98	12:30pm	2.1	98	1.0	0.0	1.0	86	5.0	NNE
18/11/98	3:30pm	1.7	97	0.0	0.0	1.0	80	5.0	NNE
19/11/98	9:30am	0.0	-	2.0	0.0	-1.0	74	11.4	NW
19/11/98	12:30pm	0.1	-	1.5	0.0	0.0	69	10.3	WNW
20/11/98	10:30am	-2.3	-	0.0	0.0	-2.0	69	5.6	WNW
20/11/98	12:30pm	-0.6	-	0.0	0.0	-2.0	69	4.7	W
22/11/98	10:30am	2.1	98	1.0	0.0	2.0	87	5.0	NW
23/11/98	9:30am	2.7	91	2.0	1.5	3.0	75	8.6	WSW
23/11/98	11:30am	3.7	83	1.5	1.0	3.0	75	9.2	SSW
23/11/98	2:30pm	4.2	80	1.0	1.0	4.0	75	8.6	SW
23/11/98	3:30pm	4.2	83	0.0	0.0	4.0	75	6.7	SW