DIGESTION OF EPIPHYTIC LICHENS FOR ANALYSIS BY ICP-MS, AS APPLIED TO MONITORING ATMOSPHERIC HEAVY METALS

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Digestion Of Epiphytic Lichens For Analysis By ICP-MS, As Applied To Monitoring Atmospheric Heavy Metals

by

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To Marleen,

Abstract

Lichens are excellent biomonitors. This study was undertaken to develop and refine a partial digestion procedure for lichen, suitable for ICP-MS analysis of trace elements, for the purpose of environmental monitoring. The developed digestion method consisted of a series of alternating dry and wet ashings utilizing nitric acid and hydrogen peroxide. Acceptable ICP-MS data were obtained for the following suite of elements: Mg, P, Ca, Mn, Co, Zn, Sr, Ba, V, Cr, Fe, Cu, Rb, Cd, Sb, Cs, and Ce. The application of the procedure to lichens from different sites indicated that sites could be distinguished by their trace element concentrations; the elements with differences included elements of environmental interest such as V, Zn, and Cu. The concentrations determined by this research for Newfoundland lichens were generally much lower than those reported by other researchers. The digestion procedure was also applied to different lichen species (*Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*) collected at the same site. It was found that different species yielded different trace element information, thus direct comparisons cannot necessarily be made. Digestion residues were examined by SEM-EDX to determine general compositions. The majority of these residual particles had a high silicon content, with varying amounts of other elements, particularly aluminum and potassium; these minerals were silicates, likely to be quartz, feldspars, olivines, garnets, micas, and/or clay minerals. Differences in concentration (as determined by ICP-MS) were observed in lichen samples collected from the same site in

consecutive years. Some elements displayed differences between ICP-MS Runs, likely due to sample inhomogeneity. Other researchers have found that elemental concentrations can differ between species, that some elements (e.g. Cr, Fe, Ni, Cu, Zn) have higher concentrations in more polluted areas, and that the levels of some anthropogenic pollutants decrease with distance from the source; each of these points support the findings of this study.

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CHAPTER 1: INTRODUCTION

1.1 Objectives And Scope

As atmospheric pollution levels continue to rise, the need for environmental monitoring becomes increasingly more important. Lichens have been recognized as potential biomonitors since the 1800s (Nylander, 1866 and 1896, cited in Richardson, 1992). Lichens have been utilized to monitor various types of atmospheric pollutants, including: heavy metals (e.g. Pb, Zn, Hg), fluorides, organic compounds (e.g. PCBs, DDT), and acidic substances such as sulphates and nitrates (Richardson, 1992). In recent years, attention has been focused on the biomonitoring abilities of lichens, partially as a result of the need to monitor the effects of the Chernobyl accident in 1986 (Richardson, 1992). Epiphytic lichens are useful as atmospheric deposition monitors since they obtain most of their nutrients from dry and wet deposition (Bruteig, 1993). Epiphytes grow attached to other plants, using them for structural support. A comprehensive review of lichens and their applications to pollution monitoring can be found in Richardson (1992).

For successful monitoring, appropriate methods of obtaining reliable sample analyses must be established. A suitable analytical technique must be selected, and then appropriate sample preparation procedures must also be chosen. The primary objective of this research was to develop a lichen digestion procedure which yields solutions suitable for analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS was selected because it offers the low detection limits necessary for heavy metal monitoring, and this analytical instrument is now widely available. The research presented here evolved from a study which

was part of a Bachelor of Science Honours Thesis (Tucker, 1995).

The dissolution of samples must be given careful consideration when ICP-MS is the analytical instrument to be used. Ideally, the solutions to be analyzed should have a nitric acid concentration near 0.2 moles/litre, relatively low total dissolved solids, a minimum of organic material, and an absence of solids (Date and Gray, 1989). Chlorine-containing reagents should be avoided due to their interferences (i.e. combinations of molecular ions) (Friel et al., 1990). If hydrofluoric acid is used, the procedure must ensure that the final solution contains minimal HF because this acid can dissolve the sample introduction system (composed of quartz glass) typically used for the ICP-MS.

The secondary objective of this study was to apply the developed digestion method. This was done in two ways. First, lichens of the same species were analyzed from areas exposed to pollution and from relatively pristine areas to see if such sites can be distinguished from each other based on trace metal analysis. Second, different lichen species were analyzed to see if different species have the same heavy metal concentrations.

1.2 Background Information Concerning Lichens

1.2.1 General Information

Lichens are comprised of a fungal component (mycobiont) and a photosynthetic component (photobiont) which exist in a symbiotic relationship (Ahmadjian, 1993). The photobiont may be an alga, a cyanobacterium, or less commonly, both of these (Ahmadjian, 1993). The fungal component is dominant, at least in bulk, in most lichens (Lawrey, 1984). It has not yet been determined whether the relationship of the two "partners" is one of mutualism or of parasitism (Ahmadjian, 1993).

The fungus of the lichen produces a body, called a thallus, inside which the photobionts exist (Ahmadjian, 1993). The thallus is composed of fungal tissue in the form of a cortex and a medulla, as well as a photobiont layer where fungal hyphae (filaments) surround the cells of the alga or cyanobacterium (Ahmadjian, 1993). The cortex is the outermost layer of a lichen, providing protection (Richardson, 1992). The medulla is commonly composed of hyphae in a loosely packed arrangement (Dobson, 1979).

The most common lichen thallus morphologies are: crustose (having a crust-like appearance), foliose (having a leafy appearance), and fruticose (having a shrubby or pendant appearance) (Richardson, 1992). These forms are illustrated in Figure 1.1.

There are approximately 15,000 to 20,000 species of lichens which exist in diverse localities ranging from arctic to tropical, from mountain tops to low tide levels (Richardson, 1992; Nieboer and Richardson, 1981; Ahmadjian, 1993). Growth rates vary from less than one millimetre to up to a few centimetres per year, and may be dependent upon moisture, light intensity, temperature, and nutrients (Ahmadjian, 1993; Richardson, 1992). Lichens may have lifespans of hundreds or even thousands of years (Ahmadjian, 1993).

The suitability of lichens for monitoring pollution has been attributed to characteristics such as their ability to take up gases, minerals (in solution), and particulates, their long lifespan, and their ability to inhabit diverse ecosystems (Nash, 1996; Richardson, 1992; Galun, 1988). Lichens are non-selective in their capacity to remove material from the atmosphere, and so do not possess the ability to defend against pollution (Déruelle and Lallemant, 1983, cited in Carignan and Gariépy, 1995). As well, lichens do not possess a cuticle (a waxy protective layer) or stomata (pores that allow for gas exchange) as do vascular plants, therefore it is likely that uptake of matter occurs across the entire lichen

surface (Carignan and Gariépy, 1995). Lichens also lack roots, however, some possess rhizinae to attach themselves to their substrate (Richardson, 1992).

1.2.2 Metal Uptake

The uptake of metals in lichens occurs by three principle mechanisms: particulate entrapment, ion exchange, and intracellular uptake (Nash, 1996). Most of the elemental accumulation by lichens occurs extracellularly via particulate entrapment or ion exchange (Nieboer and Richardson, 1981).

1.2.2.1 Particulate Entrapment

The trapping of insoluble particulates is one method by which lichens can accumulate metals; these insoluble particles are commonly sulphates, sulphides, and oxides (Richardson, 1992). Industrial metal emissions are principally contained in insoluble particles which lichens can uptake in a similar manner to the way in which they uptake soil and rock particulates; these solid particulates are contained in the central medulla portion of the lichen thallus, having been trapped by the growing hyphae (Richardson, 1992). Over time, certain lichen metabolites may break down trapped particulates such that they become soluble and the elements are released to be utilized by the lichen and/or circulated back into the ecosystem (Tyler, 1989; Richardson, 1992).

1.2.2.2 Ion Exchange

For the uptake of metals through ion exchange, the lichen must be moist (Nieboer and Richardson, 1981). Ion exchange is a means of removing ions from a solution onto a solid

resin; upon the introduction of a solution containing different ions, some of the ions on the resin can be displaced by others which adsorb more strongly to the resin (Krauskopf, 1979; Manahan, 1994). Lichens function in a manner similar to ion exchange resins by absorbing metal ions from precipitation and releasing hydrogen ions or cations of low binding affinity as uptake continues (Richardson, 1992).

Metal ions with a greater binding affinity, or a lower binding affinity but higher concentration, can displace ions which are located at the ion exchange sites of lichens (Richardson, 1992). For some lichens, the specific binding affinity order can be determined (Richardson, 1992).

1.2.2.3 Intracellular Uptake

In general, the knowledge of intracellular uptake of metals does not seem to be as well established as the other uptake methods discussed above (Tyler, 1989). A slower uptake of metals into lichen cells may be associated with the more rapid uptake of metal ions onto the cell walls (Richardson, 1992). Buck and Brown (1979), demonstrated that for unstressed lichens, potassium ions are dominantly in intracellular locations, but calcium and magnesium ions are present intracellularly as well as at extracellular sites. The proportion of calcium and magnesium ions at each location is dependent upon the supply of the cations from the environment and the species being considered (Buck and Brown, 1979).

1.3 An Overview Of Atmospheric Pollution

1.3.1 General Information

The release of gaseous and particulate matter into the atmosphere is of concern because of its effects on global warming, human health, and the state of natural ecosystems (Hemond and Fechner, 1994). These releases can be of anthropogenic or natural origin (Ahrens, 1998). Anthropogenic pollutants can be emitted to the atmosphere from various types of sources such as: industrial, agricultural, forest fires, and other combustion processes (Hemond and Fechner, 1994; Manahan, 1994). Natural sources of gases and particulates to the atmosphere can include: volcanic eruptions, seaspray, soil/dust, release of pollen and spores by vegetation, decay of organic material, and forest fires (Hemond and Fechner, 1994).

The chief mechanisms for the removal of solid pollutants from the atmosphere are dry and wet deposition (Hemond and Fechner, 1994). Dry deposition encompasses the gravitational settling out of suspended particles and the impacting of particles at the earth's surface (Hemond and Fechner, 1994). Wet deposition includes the various forms of precipitation such as rain, snow, and sleet (Hemond and Fechner, 1994; Schlesinger, 1991).

Atmospheric pollutants can be in the form of gases or aerosols (Manahan, 1994). For this current research, inorganic aerosols are of primary interest.

1.3.2 Atmospheric Aerosols

Particles of solids or liquids in the atmosphere in the size range of a few molecules to about 20 μm are known as atmospheric aerosols (Berner and Berner, 1996). The most readily noticeable form of air pollution is particulate matter (Ahrens, 1998). These

particulates may be inorganic or organic, and can originate from anthropogenic and/or natural sources (Manahan, 1994; Government of Canada, 1991). Atmospheric particles serve certain ecological purposes (discussed further in the following section), however, when particle levels are high, they can be considered pollutants (Government of Canada, 1991). The removal of these pollutants from the atmosphere may occur through dry or wet deposition (Manahan, 1994).

1.3.2.1 Natural Atmospheric Aerosols

As mentioned above, natural sources contribute aerosols to the atmosphere (Berner and Berner, 1996). These solids and liquids can be biological or non-biological in origin (Manahan, 1994). Atmospheric particles can originate from a wide variety of natural processes, including: smoke from forest fires, emissions from volcanic eruptions, wind-blown dust, particles from seaspray, and reactions between atmospheric gases which produce particles (Schlesinger, 1991; Government of Canada, 1991). Natural liquid droplets in the atmosphere can consist of: fog, raindrops, and sulphuric acid mist (Manahan, 1994). Particles which are of direct biological origin include: pollen, fungal spores, and viruses (Manahan, 1994).

Particulates in the atmosphere have important ecological functions (Schlesinger, 1991; Israël and Israël, 1974). Perhaps the most important role of atmospheric particles is as condensation nuclei, thus contributing to the formation of clouds, fog, and rain (Manahan, 1994; Schlesinger, 1991). This role of atmospheric particles is clearly very important to the water cycle, and consequently also quite important to climatic conditions, radiation and thermal equalization, weathering processes, and the dissolved composition of rainfall (Israël

and Israël, 1974; Schlesinger, 1991). In addition, visibility, the intensity of the blue colour of the sky, and the electrical properties of the air are all dependent upon atmospheric particles (Israël and Israël, 1974).

1.3.2.2 Anthropogenic Atmospheric Aerosols

There are numerous anthropogenic sources of particulates to the atmosphere (Hemond and Fechner, 1994). Approximately 50 % of these particulates are from industrial activity (Government of Canada, 1991). Many of the human-generated sources of atmospheric particles involve combustion processes, such as: power plants using fossil fuels, internal combustion engines, incinerators, and household furnaces (Manahan, 1994). In addition, anthropogenic atmospheric particles can arise from: lubricating oils, incomplete combustion of hydrocarbons forming polycyclic aromatic hydrocarbons (PAHs), and dust from coal grinding (Manahan, 1994). Liquids such as water droplets and acidic mist can also exist in the atmosphere as a result of human activity (Manahan, 1994).

Atmospheric pollutants which are in the form of particulate matter can have harmful environmental and health effects (Moroz, 1996; Manahan, 1994). Particles in the air can have a significant effect in the reduction of visibility, they can damage materials, and they may also have unpleasant esthetic effects (Ahrens, 1998; Manahan, 1994). Particulate pollution may also influence weather phenomena (Israël and Israël, 1974; Manahan, 1994). Water droplets can be harmful under some circumstances: they are capable of functioning as carriers of other pollutants such as corrosive salts, and when the droplets are dense as in fog, the consequential reduction in visibility may have dire effects on the navigation of aircraft, vehicles, and ships (Manahan, 1994). Vegetation may be damaged by atmospheric

aerosol pollutants in various ways, such as the coating of fragile desert plants with dust dispersed by all-terrain vehicles (Manahan, 1994). Acidic deposition has been suggested to be the cause of substantial forest decline in Europe, and it can cause further deleterious effects through indirect means such as altering soil chemistry and acidification of natural waters (Government of Canada, 1991).

1.3.2.3 Composition Of Atmospheric Aerosols

Atmospheric particles can have varying compositions depending on their source (Manahan, 1994). Aerosols are composed mainly of carbonaceous matter, metal oxides and glasses, ionic species in solution, and ionic solids (Manahan, 1994). In general, the smaller aerosols tend to be acidic (originating from gaseous components), whereas the larger aerosols tend to be basic (originating from mechanically generated material such as by the grinding of limestone, $CaCO_3$) (Manahan, 1994).

Atmospheric particles of inorganic nature in a polluted region may encompass a range of compositions, including: nitrogen and sulphur compounds, oxides, salts, and metals (Manahan, 1994). Particulate forms of aluminum, calcium, carbon, iron, silicon, sodium, and potassium generally occur at levels above 1 $\mu g/m^3$ (Manahan, 1994). Copper, zinc, lead, and titanium occur below 1 $\mu g/m^3$ (Manahan, 1994). Other elements which occur in particulates at very low concentrations include: antimony, beryllium, bismuth, cadmium, cobalt, nickel, vanadium, chromium, cesium, lithium, manganese, rubidium, selenium, and strontium (Manahan, 1994). Table 1.1 groups constituents of inorganic particles into four categories based on their source. Table 1.2 presents some likely sources for specific elements found in inorganic atmospheric particles.

Table 1.1: Some constituents found in inorganic particles, as grouped into four categories (Manahan, 1994).

Elements With Natural Sources	Elements With Anthropogenic Sources	Species Formed By Atmospheric Reactions	Compounds In The Surrounding Atmosphere
Al, K, Na, Si, Fe, Cl, Ti, I	Pb, Mg, Zn, Fe, Ba, Ca, V, Mn, Ti, Cu, Be, Br	H_2O, NH_4^+, NO_3^-, SO_3^{2-}, SO_4^{2-}	H_2O, HBr, NH_3, HCl, SO_2

Table 1.2: Some likely sources for selected elements found in inorganic atmospheric particles (Manahan, 1994).

Element	Likely Sources
Al, Fe, Ca, Si	Soil erosion, rock dust, combustion of coal.
C	Incomplete combustion of carbonaceous fuel.
Na, Cl	Marine aerosols, chloride originating from organohalide polymer waste incineration.
Sb, Se	Combustion of oil, coal, or refuse. (Both Sb and Se are very volatile.)
V	Combustion of residual petroleum (V is especially high in Venezuelan crude residues).
Zn	Combustion.
Pb	Combustion of leaded fuels (not as great a concern today as in the past) and wastes which contain lead.

1.4 Sources Of Metals In The Environment

Metal input and transport in the environment is currently a prominent area of scientific interest, largely due to the significance of metals in biological life processes (Friedland, 1990; Kieffer, 1991). Metals are essential to plants and animals yet, at the same time, are of concern as environmental pollutants (Manahan, 1994). Although metals have

numerous natural sources, significant quantities can also originate from anthropogenic activities (Morgan and Stumm, 1991; Friedland, 1990). Table 1.2 includes sources for specific metals in atmospheric particles.

The physical and chemical weathering of glacial tills, bedrock, and ore bodies can release heavy metals which can remain in terrestrial systems (dust, soils, sediments), or can enter aquatic or atmospheric systems (Friedland, 1990). Volcanic emissions, forest fires, sea salt, and vegetative exudates are other natural sources which introduce metals to ecosystems (Friedland, 1990; Pacyna, 1986).

Anthropogenic sources of metals in the environment vary widely (Ross, 1994). Industrial sources of metals encompass releases from mining and smelting operations, the cement industry, electrolysis, wood preservation, and refineries (Ross, 1994; Ernst and Joosse, 1983, cited in Verkleij, 1993). Metals are released in association with energy production and supply through petroleum combustion, coal burning power stations, nuclear power plants, and high tension lines (Ernst and Joosse, 1983, cited in Verkleij, 1993). Another source of metals in the environment is from traffic, through such things as use of leaded gasoline and catalysts (Ernst and Joosse, 1983, cited in Verkleij, 1993). Current agricultural practices release metals through utilization of fertilizers, pesticides, manures, lime, and irrigation waters (Ross, 1994). Household waste, sewage, and wood burning also contribute metals to the environment (Ross, 1994; Ernst and Joosse, 1983, cited in Verkleij, 1993; Kleeman et al., 1999).

1.5 Description Of ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) had its beginnings in 1975 with the work of Gray, furthered by research by Houk et al. (Vandecasteele and Block, 1994). In 1983, the first commercially available ICP-MS heightened the interest in this instrument, and today ICP-MS instruments are used worldwide (Vandecasteele and Block, 1994).

Inductively coupled plasma mass spectrometers consist of three general components (Potts, 1987). The argon plasma, nebulizer, spray chamber, torch, work coils, and the associated power sources comprise the first component (Potts, 1987; Falkner et al., 1995). An interface which allows sampling of argon plasma gases and the transfer of the ion beam into the actual mass spectrometer is the second component (Potts, 1987). The third component is a quadrupole mass spectrometer and the associated data collection instrumentation (Potts, 1987).

Many techniques and devices exist which can transport solid, liquid, and gas samples into ICPs (Montaser et al., 1998). Use of gaseous samples is the most simplistic means of introducing samples into the plasma (Montaser et al., 1998). Laser ablation is a versatile technique used to sample a wide range of solid materials, a method which can yield a high spatial resolution (Montaser et al., 1998). Although techniques exist for introduction of solid and gaseous samples in ICP-MS, the introduction of dissolved samples is a common method utilized, thus necessitating carefully developed dissolution procedures (Vandecasteele and Block, 1994; Longerich et al., 1993). A nebulized solution is sprayed into an argon plasma where the dissolved solids are vapourized, dissociated, and become highly ionized (Strong and Longerich, 1985; Hasegawa et al., 1992). The mass spectrometer separates the ionized

atoms by their mass-to-charge ratio, and creates electrical impulses on a channel electron multiplier detector; then impulses are counted by simple digital electronics (Strong and Longerich, 1985). This is then followed by the appropriate data reduction which takes into account backgrounds, interferences, matrix, and instrumental drift (Longerich et al., 1993). Chapter 2 will provide more information about ICP-MS analysis.

ICP-MS has been applied to various areas of research, including the fields of geochemistry, environmental analysis, food science, medicine, and water resources (Date and Jarvis, 1989; Ward, 1989; Dean et al., 1989; Janghorbani and Ting, 1989; Taylor, 1989). There are several advantages which ICP-MS has over other methods (i.e. x-ray fluorescence, neutron activation, atomic absorption spectrometry) for geochemical analysis (Strong and Longerich, 1985). The major advantages of ICP-MS for geochemical and environmental analysis include: speed of analysis, multi-element capability, good sensitivity, and low detection limits (Longerich et al., 1990; Ward, 1989). As well, ICP-MS has low backgrounds and an absence of significant inter-element interferences (Potts, 1987; Strong and Longerich, 1985). These characteristics result in ICP-MS being a very useful tool in geochemical analysis (Longerich et al., 1990).

1.6 Literature Review

1.6.1 Digestion Literature

Although a great many digestion procedures exist for various sample types, there are a relatively limited quantity of procedures in the literature which are suited for dissolution of lichens for analysis with ICP-MS. Pertinent types of studies involving digestions are outlined below.

The preferred acid for sample digestion for ICP-MS analysis is nitric acid (HNO_3), with procedures involving HCl or H_2SO_4 being less desirable (Taylor, 1989; Horlick and Montaser, 1998). H_2SO_4 is problematic because of interferences (Horlick and Montaser, 1998). Reagents containing Cl are especially problematic as they cause interferences (by formation of molecular ions) with other elements (Friel et al., 1990); many dissolution procedures are unsuitable because of the utilization of H_2SO_4 or Cl-containing reagents (for example, the procedures outlined by Sloof and Wolterbeek (1991), Baxter et al. (1989), Satzger et al. (1984), and Satzger et al. (1982)).

A procedure by Yoshinaga et al. (1993) to dissolve hair for ICP-MS analysis involved simply placing the sample in nitric acid overnight. Based on trial digestions (involving wet ashings with nitric acid) prior to this study, it is unlikely that this procedure would be capable of breaking down the lichen samples (Tucker, 1995). Friel et al. (1990) used a microwave dissolution to prepare animal tissues for ICP-MS analysis. This procedure has the potential to digest lichens for ICP-MS analysis, but the necessary equipment (microwave and digestion bombs) was not readily available; similarly, a procedure detailed by Bettinelli et al. (1996) is suitable, however it also requires a microwave. The dissolution method of Ridout et al. (1988) involved placing the sample in nitric acid and heating in stages. It is possible that this procedure might work for lichens, but it may not be rigorous enough to ensure the complete destruction of the organic material. The procedure of Steinnes et al. (1993) involved bomb digestion (at 150 °C) of plant material for ICP-MS analysis. It is possible that this method could be suitable for lichens, but a similar type of bomb digest was attempted prior to this study and was found to be undesirable for the following reasons: the resulting solution was not clear and colourless, the number of bombs limits the number of

samples digested, and the bombs were not readily available (Tucker, 1995).

There are numerous dissolution procedures for rock analysis by ICP-MS, such as those found in Jackson et al. (1990) and Longerich et al. (1993). The chief concern with these procedures is whether or not all of the organic material from the lichen would be destroyed. As well, most of these procedures involve reagents which are best avoided if at all possible (HCl, HF, etc.).

The digestion procedure of Hill et al. (1986) for biological samples analyzed by atomic absorption spectrometry (AAS) was well-suited to lichen dissolution for ICP-MS analysis. The reagents used were nitric acid (the preferred acid for ICP-MS), and hydrogen peroxide, H_2O_2, (an additional oxidant for the organics) which degrades to water (i.e. causes no interferences). One of the most intriguing aspects of this technique was that it suggested that all carbon containing particles were digested. Also, all the equipment necessary for this procedure was readily available. This digestion method of Hill et al. (1986) served as the basis from which the digestion procedure of this research was developed.

1.6.2 Lichen Literature

There is a vast quantity of literature relating to various aspects of lichen research. For this work, of particular interest are lichen studies which examine trace metals, compare species and/or sites, and examine crystals and particles associated with undissolved lichen material. Some of the more important studies are summarized below.

Bennett and Wetmore (1999) examined the epiphytic lichens *Bryoria fremontii* (of the same genus as a lichen of the present study) and *Letharia vulpina* collected in Yellowstone National Park, Wyoming, U.S.A. The location of their study contained

geothermal features such as geysers, fumaroles, vents, and springs. They utilized acid digestion and ICP analysis to determine concentrations for over twenty elements, many of which are also of interest in this present study (e.g. Cd, Cu, Mg, Mn, V, Zn). These authors found that many of the elements had levels which were similar to levels in other national parks and wilderness areas in the region. They also found that some elements had concentration differences for different regions within the park.

The heavy metal content of lichens from Ireland and England were studied by Seaward et al. (1978). These authors used acid digestion (nitric and perchloric) and analysis by atomic absorption spectrophotometry (AAS) to examine Pb, Cr, Ni, Cu, Mn, Zn, and Fe in *Cladonia furcata* as well as in other species. They collected lichens from Ireland and England at sites that were considered by the authors to have background concentrations. As well, they collected samples from the location of spoil heaps of a disused Pb mine in England, which was considered to have elevated concentrations. This study is comparable to the current work in that lichens were sampled at sites of differing pollution levels and analyzed for heavy metal concentrations.

Nygard and Harju (1983) determined vanadium in the lichen *Hypogymnia physodes* at greater than twenty-five localities around a power plant in Finland. For these determinations, the authors utilized hydrochloric and nitric acids in a PTFE autoclave bomb at 120-140 °C and subsequent analysis by a DC plasma emission spectrometer. The power plant utilized a heavy fuel oil with a 70 ppm vanadium concentration. The authors determined the highest V concentrations in the lichen samples to be within 1 km of the plant (up to 57 ppm); at 50 km from the plant, the concentrations were determined to be at approximately background levels (2 ppm). This research is of particular interest since the

Come By Chance sampling site of the present study is the site of an oil refinery.

The work of Nieboer et al. (1972) examined different lichen species around a nickel smelter in Sudbury, Ontario. The authors used an acid digestion as well as a dry ashing with an acid digestion, and then performed analyses of all samples by atomic absorption spectrophotometry (AAS). Concentrations were reported for four Cladonia species, one of which, *Cladonia alpestris*, was also used in the present study. These concentrations were determined for metals which are also of interest in the present study (e.g. Cu, Ni, Zn, Fe). Their data indicated that within the same genus, some elements can have similar concentrations, while others can have a wide range of concentrations.

A study by Folkeson (1978) examined lichens and mosses in terms of their heavy metal content. The authors used a digestion with HNO_3 and $HClO_4$ and then analysis by flame AAS. Samples were collected around a brass foundry in SE Sweden. This study compared the data of different species and examined the concept of calculating calibration factors for comparison of different species. One of Folkeson's lichens, *Cladonia rangiferina*, is of the same genus as a lichen used in the present work (*Cladonia alpestris*). The metals examined by Folkeson (Fe, Cu, Zn, Pb, Ni, Cd) are metals which are also of interest to the current work.

Tomassini et al. (1976) carried out research on Cu, Fe, Ni, and S concentrations in lichens from the Sudbury area of Ontario, as well as from the Mackenzie Valley, Northwest Territories. They used dissolution in HNO_3 and $HClO_4$ with analysis by AAS, as well as preparation of pressed pellets and analysis by X-ray fluorescence (XRF). They studied the relationship between concentration and distance from the Copper Cliff smelter in Sudbury. They found that there was a linear relationship between concentration and the reciprocal of

the distance from the smelter. They also determined that the concentrations in the Arctic lichens were comparable to those of the periphery of the pollution zone in Sudbury.

The presence of particles and/or crystals in lichens has been the focus of considerable research. Aspects of lichen secondary metabolic products (also referred to as lichen substances or lichen acids) have been widely investigated (Galun and Shomer-Ilan, 1988). These substances can be in crystallized forms, and tend to be of an organic nature (Galun and Shomer-Ilan, 1988; Elix, 1996). The deposition of particular metallic fall-out accumulated in *Caloplaca aurantia* was studied by Garty et al. (1979). This study made use of a combination of SEM-EDX (used also by the present study), transmission electron microscopy-EDX, X-ray diffraction, X-ray fluorescence, and atomic absorption. They determined the mineralogy of the lichen trapped particles to be weddelite (calcium oxalate), quartz, calcium, magnetite, metallic iron with titanium, and amphibole. These authors also found that the particles incorporated by the lichens had a composition similar to that of dust particles from the sampling area.

CHAPTER 2: METHOD AND METHOD DEVELOPMENT

2.1 Sample Collection

Lichens were collected in a consistent manner at each sampling location. Much of the lichen sampling protocol had been developed previously for another project (Blake, 1998). Epiphytic lichens from four locations were used for this study. These sampling sites are described in Section 2.1.4, as well as in Appendix III. The principal lichen used in this study was *Alectoria sarmentosa*; *Bryoria sp.* and *Cladonia alpestris* were also used. These species are widespread in Newfoundland, relatively easy to identify, and generally present in abundance.

2.1.1 Sampling Protocol

During sampling, the utmost care was taken to avoid potential sources of contamination. Unpowdered vinyl gloves were used when picking the lichen; new gloves were used for different species and different locations. Collected samples were placed in paper sampling bags. A new sampling bag was used for a new location and/or a different species. Of course fragments of other unwanted material (lichen, twigs, needles, etc.) were unavoidably collected with the species of interest. Since the lichen of interest existed with these unwanted materials in the field before collection, no contaminants should arise from this (the twigs, etc. were later removed). The paper sampling bags from each site were then placed in labelled plastic Zip-loc bags, with a different Zip-loc bag used for each species.

Care was taken to sample only in areas where lichens were abundant, so as not to

endanger any lichen populations. To avoid the effects of automobile exhaust, dust, and residential or industrial emissions, sampling sites should ideally be located at least 200 m from roads and more than 1 km from towns.

At each sampling location, notes were taken describing the site, the species collected, the weather conditions, and any other pertinent information. An effort was made to collect the lichens when they were dry, as less time would be required to dry the lichen in the laboratory, and this would help to avoid mold growth.

After collection, lichen samples were stored at approximately 6 °C. The samples were dried as soon as possible after collection (as described in Section 2.2).

2.1.2 Collection Of Lichen From Trees

Alectoria sarmentosa and *Bryoria sp.* are epiphytic lichens which live on trees. *Alectoria sarmentosa* is generally yellow to greenish and hair-like in appearance. *Bryoria sp.* is generally brown and hair-like in appearance. Both species consist of branching strands, but the strands of *Bryoria sp.* tend to be smaller in diameter than those of *Alectoria sarmentosa*. Both of these species are relatively easy to identify in the field.

Alectoria sarmentosa and *Bryoria sp.* were collected from balsam fir trees only (for consistency). In order to avoid the potential effects of stem-flow from the trunks of trees, samples were collected from branches only, and at least 25 cm from the trunk of the tree. An effort was made to collect lichens at approximately shoulder height, but this was not always possible. Collection of lichens close to the ground was avoided. Lichens above reasonable reach when standing on the ground were also avoided. Lichens were collected from living trees in the age range of approximately 40-60 years old. Selection of trees in this

age range was achieved by choosing trees of similar diameter (10-15 cm) and height (3-4.5 m). (For this study, samples were not selected based upon a specific length, width, or age of the lichen.) Before visiting a sampling area, forest inventory maps were utilized to identify sites which have trees in the 40-60 year old age range. Lichens were collected from several different trees in each location and from all around each tree.

2.1.3 Collection Of Lichen From The Ground

Cladonia alpestris is generally off-white to pale yellow-green in colour and has a shrub-like appearance. It consists of a stalk-like lower portion and a bushier branched upper portion. *Cladonia alpestris* is an epiphytic lichen, however it grows on humus material and soil on the ground instead of on trees. *Cladonia alpestris* was always collected in patches where it existed in abundance.

2.1.4 Lichen Sampling Sites

Four lichen sampling sites were used for this work: Come By Chance, Bauline Line, Random Island, and Bonavista. Each of these four sites were located on the island of Newfoundland, located on Canada's eastern coast. The location of each site can be seen in Figure 2.1. Detailed site descriptions can be found in Appendix III. The descriptions of the Come By Chance, Random Island, and Bonavista sampling sites were taken from Evans (1996); these three sites form an approximately SW-NE transect. Further details about the rationale behind the sampling site selections are given in Section 2.3.2.

2.2 Sample Treatment Prior To Digestion

2.2.1 Storage And Drying

After the lichen was collected, it was kept in cold storage at a temperature of approximately 6 °C. As soon as possible after collection (ideally within a week), the samples were dried completely to avoid mold growth. Care was taken to avoid potential contamination during drying. The contents of each paper sampling bag was placed on low-lint (38-43 cm) Kimwipe paper towels, and covered by other Kimwipe paper towels. The samples were dried at room temperature in an area where they would not be disturbed. When dry, the samples were returned to paper sampling bags, placed in the zip-loc bags, and kept in cold storage (approximately 6 °C) until they were required. The sample treatment prior to digestion is summarized in the flow chart of Figure 2.2.

2.2.2 Cleaning

For cleaning, the sample was removed from the paper sampling bag and placed on Kimwipe paper towels on the lab bench. Teflon-coated tweezers were used to separate the desired lichen species from foreign material such as twigs, coniferous needles, and unwanted lichen species. An effort was made to clean some lichen from each "clump" of lichen from the sampling bag to ensure a representative sample was used.

In order to avoid the loss of elements of interest, the lichen samples used in this study were not washed. A study prior to this work suggested that not washing and air drying were the preferred methods for lichen sample preparation for elemental analysis (Tucker, 1995). Other researchers, such as Nimis et al. (1993), have also chosen not to wash lichen samples in order to avoid losing cations which can be leached during the washing process.

2.2.3 Crushing

The standard method used for this research was crushing in an agate mortar and pestle using liquid nitrogen. A small quantity of lichen was placed in the mortar, liquid nitrogen was poured in, and the sample was crushed using the pestle. This was repeated until a powder was obtained. The samples were then transferred to a plastic container and stored at approximately 6 °C when not in use.

For comparison, a mechanical crushing method was used in the first set of samples, in order to compare this more rapid method with the mortar and pestle. Samples were crushed in a tungsten carbide puck mill intended for crushing rock samples. The puck mill consisted of a tungsten carbide bowl into which a tungsten carbide ring was placed, and a puck of tungsten carbide was placed inside the ring. Samples were placed between the bowl and ring, and between the ring and puck. A tungsten carbide cover was placed on the bowl and locked into place. The puck mill was switched on for 30 seconds during which the bowl, ring, and puck were agitated and the sample crushed. The puck mill was switched on again for another 30 seconds so that a fine powder was obtained. The bowl, cover, ring, and puck were cleaned before and after each sample by first crushing sand, then by cleaning with alcohol and kimwipes. The crushed samples were sieved through a 60 mesh (0.250 mm) HDPE/polyester sieve; the lichen particles retained by the sieve were placed back into the puck mill and crushed again. (The sieve and pan were also cleaned between samples.) The crushed samples were transferred to plastic containers and kept in cold storage when not in use.

Upon examination of the trace element data for both methods of crushing, a number of elements had concentrations in which the mortar and pestle data differed notably from the

puck mill data (Table 2.1). The data for cobalt were different for each crushing method: the mortar and pestle method had values of hundredths of a ppm for each site, whereas the puck mill method had values of about ten ppm for each site. This indicated that the puck mill method had increased concentrations due to contamination. Differences of up to two orders of magnitude in the crushing methods were not acceptable for this research and the mortar and pestle were used for the remainder of this study. The complete data for the comparison of both crushing methods is presented in Chapter 3.

Other studies have also indicated that tungsten carbide crushing equipment can cause contamination. A study by Thompson and Bankston (1970), found that tungsten grinding equipment resulted in contamination from Co and Ti. A study in the Department of Earth Sciences at Memorial University of Newfoundland indicated that Ta and Nb were contaminants which resulted from crushing with the tungsten carbide puck mill, while it was assumed that W would also be a contaminant (this was the same puck mill utilized for this research) (King, pers. comm., 1999).

Table 2.1: Data (in ppm) for selected elements for the comparison of mortar and pestle crushing with puck mill crushing. These elements show marked differences between the two methods. A "*" next to a sample number indicates a duplicate (refer to Section 2.4 for the definition of a duplicate).

Sample	Ca	Mn	Co	Sr
jt-12 [2 CBC] mortar & pestle	1119	38	0.050	3.6
jt-13 [2 CBC] mortar & pestle	1243	38	0.050	3.8
jt-14 [2 CBC] puck mill	1116	44	9.8	3.4
jt-15 [2 CBC] puck mill	1089	44	9.6	3.4
jt-16 [Bauline] mortar & pestle	1648	88	0.047	5.9
jt-17 [Bauline] mortar & pestle	1709	92	0.052	6.4
jt-18 [Bauline] puck mill	2843	101	10.1	9.3
jt-19 [Bauline] puck mill	2806	101	10.0	9.4
jt-20 [4 Bona.] mortar & pestle	1030	130	0.029	8.8
jt-21 [4 Bona.] mortar & pestle	1048	136	0.027	8.8
jt-22 [4 Bona.] puck mill	1208	125	9.2	9.2
jt-23 [4 Bona.] puck mill	1217	122	9.2	9.3
jt-30* duplicate of jt-22	1242	123	9.2	9.2

Note: Sampling sites: 2 CBC = Come By Chance (Area 2)
Bauline = Bauline Line
4 Bona. = Bonavista (Area 4)

2.3 Sample Digestion

2.3.1 Development Of The Digestion Procedure

Experimental digestions were initially carried out to determine the most suitable digestion method. Various methods were explored: Parr high-pressure bomb digests, Krogh-type bomb digests, and different combinations of dry and wet ashings. The details of these trial digestions can be found in Tucker (1995).

The most successful digestion method was found to be two repetitions of dry and wet

ashings (i.e. dry ash, wet ash, dry ash, wet ash) (Tucker, 1995). This digestion method was developed by modifying the procedure of Hill et al. (1986). The developed procedure is a partial digestion. Since HF is not utilized in the dissolution, some residual particles remain after digestion. The steps of the developed dissolution procedure used in this present study are detailed in Appendix I; a summary of the procedure is provided in Figure 2.3. A partial digestion was found to be preferable for this work for several reasons. A complete digestion would necessitate the use of HF to dissolve silicates, thus making the laboratory procedures unnecessarily complicated. A dissolution method which involves fewer vessels, less reagents, and has fewer steps tends to cost less and presents fewer opportunities for sample contamination or loss. As well, the elements of interest for this study are chiefly those of environmental interest (e.g. Ni, Cu, Zn, Cd), so elements such as Si and Al which could exist in undissolved silicates were not of particular importance in this study. Partial digestions have been used by other researchers, and have yielded acceptable data (Chao, 1984; Coish, 2000).

After the initial development (Tucker, 1995), some refinements were made to the digestion procedure. One of these refinements was that the rate of increasing the muffle furnace temperature during the initial dry ashing was decreased from 50 °C per hour to 35 °C per hour. This was done to ensure that the temperature was not increased too rapidly. If the ramping process is too quick, samples with a high organic content can react violently (Longerich, pers. comm., 1999). If a sample ignites, there will be sample loss (due to the use of loosely capped test tubes), a dirty furnace and/or lab, as well as the obvious danger.

Another manner in which the digestion procedure was refined was that test tube caps were introduced into the dry ashing steps. There had been concern that contamination could

arise during dry ashing as vapours and soot evolved from the samples and the possibility existed that minute particles of one sample could fall into the test tube of another sample. Quartz caps were designed such that they were loose enough to allow vapours to escape freely from the test tubes and thus pressure build up was avoided. These caps allowed soot and vapours to leave the tubes but prevented particles from settling into the test tubes. Before these caps were used in the digestion of actual samples, a trial digestion was carried out to ensure that the use of the caps did not cause any adverse effects.

In order to reduce potential contamination, it was decided to reduce the contact which the samples had with metal objects such as tweezers and spatula. So before work began with the three sets of samples analyzed for this research, a change was made to Teflon-coated tweezers and spatula. Details of the procedures utilized for apparatus cleaning are provided in Appendix II.

2.3.2 Rationale Of Lichen Sample Selection

Sulphur isotopes are useful in environmental studies, including the identification of sources of atmospheric sulphur (Thode, 1991; Ryaboshapko, 1983). A major source of sulphur pollution is the burning of fossil fuels (Ryaboshapko, 1983). Fossil fuels from different sources can have different isotopic signatures (Ryaboshapko, 1983). Fossil fuels can also be a major source of metal pollution (Puxbaum, 1991). Since sulphur and metals are often associated together as pollutants, it is likely that areas with elevated sulphur pollutants will also have an associated elevation of metal pollutants.

For this research, it was desirable to digest and analyze lichens from sites with varying levels of pollution in order to determine whether or not the developed method of

digestion could be used to distinguish between relatively polluted and relatively pristine sites. Therefore, the findings of related research projects using sulphur isotopes in lichens in Newfoundland were used to select a relatively polluted site (Come By Chance), a somewhat polluted site (Bauline Line), an intermediate site (Random Island), and a relatively pristine site (Bonavista) (Evans, 1996; Blake, 1998). Sulphur concentration and the sulphur isotopic signature ($\delta\ ^{34}S$) were used to rank the sites according to the level of pollution. The graphical representation of these findings in Figure 2.4 indicates the relative levels of pollution for each of these sites. These are mean values only. The y-axis indicates the sulphur isotopic signature for $\delta\ ^{34}S$ in permil (‰), which is parts per thousand. The x-axis indicates the total S concentration in parts per million (ppm). In Newfoundland, a relatively high $\delta\ ^{34}S$ value is indicative of natural sources of sulphur (seaspray), and a relatively low value of $\delta\ ^{34}S$ is indicative of continental/anthropogenic sources of sulphur (Jamieson, 1995).

When the original research began (Tucker, 1995), no certified lichen reference materials were available, so certified SRM 1547, peach leaves, from the National Institute of Standards and Technology (N.I.S.T.) was selected as comparable to lichens than other available certified reference materials (CRMs). Later, two certified lichen reference materials were purchased and digested along with the peach leaves and the unknowns (i.e. collected lichen samples). These two lichen certified reference materials were: IAEA-336, the epiphytic lichen *Evernai prunasti (L.) Ach.*, from the International Atomic Energy Agency, and CRM 482, the lichen *Pseudevernia furfuracea*, from the Commission of European Communities, Community Bureau of Reference-BCR. Throughout this text, the SRM 1547 will be referred to as "the Peach Leaves CRM", the IAEA-336 will be referred to as "the IAEA Lichen CRM", and the CRM 482 will be referred to as "the BCR Lichen

CRM". The certified elemental concentrations for these CRMs are given in Appendix V.

There were three set of samples digested and analyzed for this research; a set of samples analyzed by ICP-MS is referred to as a Run. The samples included in each are outlined in Tables 2.2, 2.3, and 2.4. A "*" next to a sample number indicates a duplicate (refer to Section 2.4 for the definition of a duplicate).

Table 2.2: Sample numbers and types for the first set of digestions.

First Set Of Samples (Waters 120 Run)	
Sample #	**Sample Type**
jt-1 jt-2 jt-3	Peach Leaves CRM Peach Leaves CRM Peach Leaves CRM
jt-4 jt-5 jt-6 jt-7	IAEA Lichen CRM IAEA Lichen CRM IAEA Lichen CRM IAEA Lichen CRM
jt-8 jt-9 jt-10 jt-11	BCR Lichen CRM BCR Lichen CRM BCR Lichen CRM BCR Lichen CRM
jt-12 jt-13	*Alectoria sarmentosa*, Come By Chance (Area 2), mortar and pestle *Alectoria sarmentosa*, Come By Chance (Area 2), mortar and pestle
jt-14 jt-15	*Alectoria sarmentosa*, Come By Chance (Area 2), puck mill *Alectoria sarmentosa*, Come By Chance (Area 2), puck mill
jt-16 jt-17	*Alectoria sarmentosa*, Bauline Line (1996), mortar and pestle *Alectoria sarmentosa*, Bauline Line (1996), mortar and pestle
jt-18 jt-19	*Alectoria sarmentosa*, Bauline Line (1996), puck mill *Alectoria sarmentosa*, Bauline Line (1996), puck mill
jt-20 jt-21	*Alectoria sarmentosa*, Bonavista (Area 4), mortar and pestle *Alectoria sarmentosa*, Bonavista (Area 4), mortar and pestle
jt-22 jt-23	*Alectoria sarmentosa*, Bonavista (Area 4), puck mill *Alectoria sarmentosa*, Bonavista (Area 4), puck mill
jt-24 jt-25 jt-26	Reagent Blank Reagent Blank Reagent Blank
jt-27* jt-28* jt-29 jt-30*	Duplicate of jt-7 (IAEA) Duplicate of jt-10 (BCR) Deionized Water (acidified) Duplicate of jt-22 (*Alectoria sarmentosa*, Bonavista, puck mill)

Table 2.3: Sample numbers and types for the second set of digestions.

Second Set Of Samples (Waters 125 Run)	
Sample #	**Sample Type**
jt-1	Peach Leaves CRM
jt-2	Peach Leaves CRM
jt-3	Peach Leaves CRM
jt-4	IAEA Lichen CRM
jt-5	IAEA Lichen CRM
jt-6	IAEA Lichen CRM
jt-7	BCR Lichen CRM
jt-8	BCR Lichen CRM
jt-9	BCR Lichen CRM
jt-10	*Alectoria sarmentosa*, Bauline Line (1996)
jt-11	*Alectoria sarmentosa*, Bauline Line (1996)
jt-12	*Alectoria sarmentosa*, Bauline Line (1996)
jt-13	*Alectoria sarmentosa*, Bauline Line (1997)
jt-14	*Alectoria sarmentosa*, Bauline Line (1997)
jt-15	*Alectoria sarmentosa*, Bauline Line (1997)
jt-16	*Bryoria sp.*, Bauline Line (1997)
jt-18	*Bryoria sp.*, Bauline Line (1997)
jt-19	*Bryoria sp.*, Bauline Line (1997)
jt-20	*Cladonia alpestris*, Bauline Line (1997)
jt-21	*Cladonia alpestris*, Bauline Line (1997)
jt-22	*Cladonia alpestris*, Bauline Line (1997)
jt-23	*Cladonia alpestris*, Bauline Line (1997)
jt-24	Reagent Blank
jt-25	Reagent Blank
jt-26	Reagent Blank

Table 2.4: Sample numbers and types for the third set of digestions.

Third Set Of Samples (Waters 903 Run)	
Sample #	**Sample Type**
jt-1	IAEA Lichen CRM
jt-3 jt-4	BCR Lichen CRM BCR Lichen CRM
jt-5 jt-6	*Alectoria sarmentosa*, Bauline Line (1996) *Alectoria sarmentosa*, Bauline Line (1996)
jt-7 jt-8	*Alectoria sarmentosa*, Come By Chance (Area 3) *Alectoria sarmentosa*, Come By Chance (Area 3)
jt-9 jt-10	*Alectoria sarmentosa*, Random Island (Area 1) *Alectoria sarmentosa*, Random Island (Area 1)
jt-11 jt-12	*Alectoria sarmentosa*, Bonavista (Area 5) *Alectoria sarmentosa*, Bonavista (Area 5)
jt-13 jt-14	Reagent Blank Reagent Blank
jt-15* jt-16*	Duplicate of jt-1 (IAEA) Duplicate of jt-10 (*Alectoria sarmentosa*, Random Island)

In the first set of samples (Waters 120 Run), several things were examined. The IAEA and BCR lichen certified reference materials (CRMs) were digested for the first time, therefore 4 replicates of each were digested. The N.I.S.T. peach leaves CRM was also included, but since this CRM had been digested in previous trials, only 3 replicates were included. When using CRMs, it is necessary to show that the analytical methods used yield reproducible results of acceptable accuracy. All lichen samples (unknowns) in the first set of samples were *Alectoria sarmentosa*. It was necessary to determine whether or not the puck mill crushing method offered any advantages over the mortar and pestle crushing

method, so four samples from each of three sampling sites were digested. Two of the four samples from each site were crushed by each method. As well, this set of samples was designed to examine whether or not the developed digestion method could be used to demonstrate concentration differences for elements from a relatively polluted (Come By Chance), somewhat polluted (Bauline Line; samples collected 1996), and relatively pristine site (Bonavista). Three reagent blanks and several duplicates were also included.

In the second set of samples (Waters 125 Run), the objective being investigated was whether or not the developed digestion method could be utilized to distinguish between different lichen species using trace element profiles. Therefore, samples of three lichen species (*Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*) were digested from one site, Bauline Line (collected in 1997). Reagent blanks, as well as the IAEA, BCR, and peach leaves CRMs were also included in this run. Samples of *Alectoria sarmentosa* from Bauline Line (collected in 1996) were digested again in this second set of samples for comparison to the results from the analysis of the first set of samples.

In the third set of samples analyzed by ICP-MS (Waters 903 Run), samples of one species were selected from a relatively polluted site (Come By Chance), an intermediate site (Random Island), and a relatively pristine site (Bonavista). These samples were chosen in order to determine whether or not the elemental patterns would be similar to those from the first set of samples. Again, the *Alectoria sarmentosa* samples collected at Bauline Line in 1996 were included for comparison with the previous analyses. The BCR and IAEA lichen CRMs, reagent blanks, and duplicates were also included.

2.3.3 SEM-EDX Analysis

Characterization of the residual particulates from a partial digestion is an interesting and useful study, although it is not often mentioned in the literature. For the trial digestions (Tucker, 1995), the residual particles were examined by Scanning Electron Microscopy Energy Dispersive X-Ray analysis (SEM-EDX). This was used as a qualitative tool for evaluation of the success of a digestion since the most suitable procedure would result in a minimum of residual particles. Most of these particles were found to be dominantly Si in composition; many of these were alumino-silicates. The particles differed depending upon the sample type and the digestion procedure utilized. Further details of this can be found in Tucker (1995).

The residual particles of each of the following sample types were characterized by SEM-EDX: IAEA lichen certified reference material, *Cladonia alpestris*, *Bryoria sp.*, *Alectoria sarmentosa*, and BCR lichen certified reference material. The residual particles of the peach leaves certified reference material were examined previously by Tucker (1995).

The surface of strands of lichen were also examined for particles by SEM-EDX. Strands of *Alectoria sarmentosa* from Come By Chance (two sites) and Torbay (one site) were cut into 5-9 mm segments and mounted on aluminium stubs. This *Alectoria sarmentosa* had been collected for a related project (Blake, 1998). Six stubs were examined in total: three stubs (one from each site) with lichen strands that were not washed, and three stubs (one from each site) with lichen strands that had been rinsed in deionized water. These stubs were gold-coated before examination by SEM-EDX. The intent was to determine if there was any correlation between the particles observed on the lichen strands and those observed as residual particulates after partial digestion.

The residual particle slides were examined using a Hitachi Scanning Electron Microscope, with 20 kV as the accelerating voltage. The Energy Dispersive X-Ray analysis was done using beam spot mode on a Tracor Northern Energy Dispersive X-Ray Analyzer. The EDX was equipped with a Microtrace silicon X-Ray spectrometer, having a spectral resolution of 145 eV. Energy Dispersive X-Ray analysis yields graphical peaks at unique energy levels for all elements with an atomic number greater than eleven (Welton, 1984). The EDX graphs were not used to quantitatively represent the elemental concentrations, but to yield relative abundances. Peak identification was carried out by matching with known elemental peak energies. SEM photographs were made using secondary electrons. Minerals were identified by examining the visual appearances of particles along with the elemental peak patterns produced by the EDX.

2.4 ICP-MS Analysis

All digested (and filtered) samples were submitted for ICP-MS analysis using the Waters/Biologicals Package utilized in the Department of Earth Sciences of Memorial University of Newfoundland. The Waters/Biologicals Package is an in-house data acquisition and spreadsheet program which calculates elemental concentrations off-line for a suite of 39 elements in waters and biological samples; the package incorporates interference corrections as necessary (Tubrett, pers. comm., 2003). As mentioned previously, there were three sets of samples analyzed for this research; a set of samples analyzed by ICP-MS is referred to as a Run. The first set of sample solutions (Waters 120 Run), analyzed on a Perkin-Elmer SCIEX/ELAN 250 ICP-MS, required no 1:10 dilution before introduction into the instrument, and had a concentration of approximately 0.2 M

HNO_3. The second and third sets of samples (Waters 125 and 903 Runs) were analyzed on a Fisons VG Plasma Quad PQ2 PLUS "S" ICP-MS and a Hewlett-Packard HP 4500 ICP-MS respectively. These samples were diluted 1:10 (using 0.2 M HNO_3) before introduction to the ICP-MS; these samples had a concentration of approximately 0.2 M HNO_3. This 1:10 dilution was necessary to avoid detector shut-downs due to high count rates (tripping), since both the VG and HP ICP-MS have significantly higher sensitivities (and background signals for lighter elements) than the SCIEX ICP-MS. Each ICP-MS utilized an argon plasma, similar sample introduction systems, and the typical operating conditions for each instrument. The samples were analyzed on three ICP-MS instruments because prior to the analysis of the second set of samples, there was a decision made by the ICP-MS User Group members to replace the SCIEX ICP-MS with the HP ICP-MS, and in the interim the VG ICP-MS was used for solution analysis (usually this instrument is dedicated to laser ablation ICP-MS).

During analysis carried out previous to this research, it was found that the Peach Leaves CRM had unexpectedly high Y concentrations (Tucker, 1995). At that time, Y was one of the elements used as an internal standard in the ICP-MS Waters/Biologicals Package in the Department of Earth Sciences at Memorial University. One of the important assumptions made when selecting an element to use as an internal standard is that it is not present in any appreciable quantity in the samples to be analyzed. This Y problem, coupled with some problems with samples from a separate research project, led to a change from using Sc, Y, Tb, and Th as the internal standards in the Waters/Biologicals Package, to using Sc, Rh, Re, and Th.

The samples submitted for this research included: certified reference materials,

digested lichen (unknowns), sample duplicates, and reagent blanks. Internal standards, calibration standards, calibration blanks, and unknown waters reference materials were used by the technician in each of the three ICP-MS runs. Many of these terms are explained in the following paragraphs.

A certified reference material (CRM) is a bottle of homogenized powdered material purchased with a certificate of analysis. Certain elements are certified to have specific concentrations which are given on the certificate of analysis. The error for these concentrations is also provided. Concentrations for other elements present may be given as non-certified concentrations. The CRMs are digested (in separate test tubes) along with the unknown lichen samples. The CRMs can provide an indication of whether or not the digestion procedure is effective. Replicates of the same CRM can give a good indication of reproducibility.

A duplicate is a second tube of the same unknown sample solution. The purpose of a duplicate is to measure the reproducibility, to ensure that the sample solutions are homogeneous, and to ensure that the matrix and drift corrections of the data reduction package are working consistently.

A reagent blank contains only the reagents used in the digestion procedure and it is "digested" along with the unknown samples. The purpose of a reagent blank is to identify the portion of the final concentration which is due to the reagents and is not a part of the actual sample.

Internal standards are elements selected from across the mass range which are not likely to be present in the unknowns in any appreciable amount and are not of analytical interest. Four internal standards are used in the Waters/Biologicals Package: Sc, Rh, Re,

and Th. These internal standards are used in the matrix and drift corrections in the data reduction. Matrix and drift are changes in the instrument signal; drift is a change with time and matrix is a change in overall composition (Longerich, pers. comm., 1999). The four internal standards are added to every unknown, blank, standard, and flush by a y-tube during the ICP-MS analysis. The solution containing the four internal standard elements is prepared in-house from four SPEX compounds. Each compound is dissolved in HNO_3 (or water and HNO_3) to yield a stock solution of that element. A small quantity of each of the four stock solutions is added to more dilute HNO_3 to yield a stock solution of the desired concentrations of the four internal standard elements. For each run, this stock solution is then diluted 1:10 for the VG and HP ICP-MS (but did not require dilution for the SCIEX).

Calibration standards are solutions of one or more elements with known concentrations. Calibration factors are also used to determine interference factors and are included in the matrix/drift correction. The calibration standard solutions are referred to as: standard a, standard b, standard c, standard d, and standard e. These calibration standards are prepared by a technician in a manner similar to the internal standard solutions. A stock solution is prepared for each of the elements contained in the calibration standards. Each stock solution is prepared by dissolving a SPEX compound of the desired element in HNO_3 (some compounds may require initial dissolution in water or HCl). A small quantity of the solution of each element required for a particular calibration standard is then added together to yield a stock solution for that calibration standard. This stock solution (in nitric acid) is diluted 1:10 before analysis. The calibration standards are used to determine instrument sensitivity during a run. Sensitivity is the signal per unit concentration, counts per second (cps) per parts per million (ppm).

Calibration blanks (in the Department of Earth Sciences at Memorial University) consist of 0.2 N HNO_3 which is poured into test tubes and analyzed as a part of the data acquisition cycle. These blanks have not undergone digestion. It is from the calibration blanks that the background is calculated, a background correction applied, and the detection limit calculated. Background is the signal from the instrument when there is no sample present.

Reference materials are substances which are determined to be homogeneous and have been analyzed extensively for a particular suite of elements. The concentrations are known and accepted, but not certified. (It is possible that after a fee is paid and the proper analysis carried out, a reference material could become a certified reference material.)

The data acquisition cycle (ie. Run) consists of up to 56 tubes analyzed in approximately a 24 hour period (utilizing an autosampler). All of the solutions analyzed in one ICP-MS Run are grouped in cycles of 14 tubes. Each cycle is comprised of the following: 5 calibration standards (standard a, standard b, standard c, standard d, and standard e), 1 calibration blank, 7 unknowns (such as the samples submitted for this research), and 1 USGS (United States Geological Survey) waters reference material. Each Run ends with a partial cycle of the 5 standards (a, b, c, d, and e, as mentioned above), 1 blank, and then a tube of deionized water. This tube of deionized water is used only as an extra flush for the instrument and is not utilized in the data reduction in any way.

The ICP-MS operation was carried out by a technician. The majority of the data reduction was carried out by a technician. After receipt of the data (from the technician), the dilution factors were taken into account, the moisture content correction was applied, the data were converted from parts per billion (ppb) to parts per million (ppm), and the relative

standard deviations (RSDs) were calculated in order to determine whether samples were above or below the detection limit. Further details are given below.

For each ICP-MS Run, a technician monitored oxide formation using the ratio of ThO/Th. Oxide formation leads to interferences. Thorium oxide ratios were used as an indication of the magnitude of all the polyatomic interferences present in a run. Thorium oxide was monitored because there are no interferences on or by ThO or Th, and it is generally the oxide with the highest bond strength, with all other oxides forming to a lesser extent. It is assumed that if ThO/Th changes, then the other oxides change in a similar manner. In the data reduction, corrections were applied for the prominent interferences.

A moisture content correction was applied to all samples except the peach leaves certified reference material which had been dried prior to digestion (as per the instructions on the certificate of analysis). For each of the three sets of samples submitted for ICP-MS analysis, a moisture content determination was made in order to enable the moisture content correction to be calculated and applied. This determination was usually carried out a day before or after the start of the initial dry ashing of the digestion procedure. Porcelain crucibles were rinsed in deionized water and placed in an incubator to dry excess water. The crucibles were then placed in an oven for approximately 24 hours during which the crucibles were heated slowly to 1050 °C, the temperature remained at 1050 °C for 7 hours, and then the temperature decreased slowly. The crucibles were then removed to a desiccator to cool (at least 20 minutes). One of each sample type was weighed into a separate crucible (0.1 g of the BCR Lichen CRM; 0.5 g of the IAEA Lichen CRM and the lichen unknowns). These samples were placed into the oven to dry at approximately 100 °C for about 2 hours for all samples except the BCR Lichen CRM; the BCR Lichen CRM samples were kept the in oven

for about 3-4 hours (as per the instructions on the certificates of analysis). The samples were then removed from the oven and placed in a desiccator to cool (at least 20 minutes), and then weighed again. A calculation was then done to determine the percentage of moisture in the samples:

$$\%\text{moist.} = \{[(\text{wt. before drying}) - (\text{wt. dried sample})] / (\text{wt. before drying})\} \times 100\%$$

The moisture content correction was made according to:

$$Conc_{dry} = Conc_{wet} \times [1/(1-mc)]$$

Where: $Conc_{dry}$ = concentration (in ppm) of oven dried samples
$Conc_{wet}$ = concentration (in ppm) of samples before oven drying
mc = moisture content (as a fraction)

The detection limit is commonly defined as 3 times the standard deviation (σ) of the calibration blanks. The relative standard deviation (RSD) is the standard deviation divided by the average concentration, and is reported as a percentage. Another method of defining the detection limit would be to use the sample replicates. At the detection limit (D.L.), the average concentration will be 3σ. So, as in the equation below, the RSD will then be σ divided by 3σ, which equals ⅓ or 33%. This means that at the detection limit, the RSD is 33%. If the RSD of a group of sample replicates is less than 33%, then those samples are above the detection limit; if the RSD of a group of replicates is greater than 33%, then those samples are below the detection limit. If the samples are above the detection limit, it is not necessary to know the exact detection limit to interpret the data, therefore exact numerical

detection limits are not reported for this research. The following equation illustrates the detection limit definition explained above:

$$RSD = \text{std. dev. } (\sigma) / \text{ave. conc. } (3\sigma, \text{at D.L.}) = \sigma / 3\sigma = \frac{1}{3} \text{ or } 33\%$$

The above method of defining the detection limit has been used by other researchers, such as Prudnikov and Barnes (1998; 1999). This definition of the detection limit is the one used in this current work. An advantage of this definition of detection limit is that the contribution of the interferences to the detection limit is intrinsically encompassed. In other words, using replicate sample data takes into consideration the contribution of interferences on the detection limit, whereas choosing to calculate a detection limit based upon calibration blanks would also require a means of accounting for the increase in detection limit which is due to the additional uncertainty from interferences.

It should be noted that negative values can appear in the data. During the data reduction if a sample has a lower concentration of an element than the calibration blank, upon subtraction of the calibration blank, the concentration in the sample then becomes negative. These negatives occur in samples with very low concentrations of a particular element (e.g. Li, Be, Tl) and are interpreted as zero parts per million.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Results

As outlined in Chapter 2, this work yielded three sets of ICP-MS data of elemental concentrations. The complete data are presented in Appendix IV. Concentrations are given in parts per million (ppm). Means, sample standard deviations, and relative standard deviations (RSDs) are also provided in Appendix IV. Table 3.1 gives the means, standard deviations, and certified concentrations for the three certified reference materials from each ICP-MS Run. The ICP-MS data will be discussed in further detail in the subsequent sections.

Table 3.1: Means, standard deviations, and certified concentrations for the three certified reference materials from each ICP-MS Run (in ppm).

Sample Type (Waters 120 Run)		Li	Be	B	Mg	Al	Si	P	S	Cl	Ca
Peach Leaves CRM	mean	-0.096	-0.0167	14.1	3567	190	20.8	1310	1299	340	13955
Peach Leaves CRM	std. dev.	0.035	0.0010	1.1	48	3	4.3	39	213	50	117
Peach Leaves CRM	cert. conc.	-	-	29	4320	249	-	1370	-	360	15600
IAEA Lichen CRM	mean	0.378	-0.0029	-1.14	562	450	26.3	541	734	280	2066
IAEA Lichen CRM	std. dev.	0.090	0.0053	0.29	30	32	3.1	24	94	55	90
IAEA Lichen CRM	cert. conc.	-	-	-	-	-	-	-	-	-	-
BCR Lichen CRM	mean	0.661	0.0303	1.23	493	625	10.0	617	563	10	1959
BCR Lichen CRM	std. dev.	0.024	0.0024	0.08	17	30	2.5	13	65	13	56
BCR Lichen CRM	cert. conc.	-	-	-	-	1103	-	-	-	-	-
Sample Type (Waters 125 Run)		**Li**	**Be**	**B**	**Mg**	**Al**	**Si**	**P**	**S**	**Cl**	**Ca**
Peach Leaves CRM	mean	-0.189	0.0131	17.6	3681	204	13.7	1412	-	116	13425
Peach Leaves CRM	std. dev.	0.006	0.0029	0.8	47	3	2.4	36	-	43	135
Peach Leaves CRM	cert. conc.	-	-	29	4320	249	-	1370	-	360	15600
IAEA Lichen CRM	mean	0.410	0.0280	5.12	547	492	9.3	567	-	53	1449
IAEA Lichen CRM	std. dev.	0.199	0.0065	3.69	20	9	5.9	8	-	61	159
IAEA Lichen CRM	cert. conc.	-	-	-	-	-	-	-	-	-	-
BCR Lichen CRM	mean	0.539	0.0190	9.99	481	84	-1.3	675	-	-15	2226
BCR Lichen CRM	std. dev.	0.013	0.0010	10.78	4	12	1.3	4	-	21	27
BCR Lichen CRM	cert. conc.	-	-	-	-	1103	-	-	-	-	-
Sample Type (Waters 903 Run)		**Li**	**Be**	**B**	**Mg**	**Al**	**Si**	**P**	**S**	**Cl**	**Ca**
IAEA Lichen CRM	1 rep. only	0.696	0.0200	1.36	565	400	15.3	537	371	-6	2086
IAEA Lichen CRM	cert. conc.	-	-	-	-	-	-	-	-	-	-
BCR Lichen CRM	mean	0.571	0.0255	0.16	477	361	6.2	639	897	-54	1844
BCR Lichen CRM	std. dev.	0.009	0.0018	1.02	13	9	2.6	4	19	25	11
BCR Lichen CRM	cert. conc.	-	-	-	-	1103	-	-	-	-	-

Complete data given in Appendix IV.

Negative concentrations are interpreted as zero ppm (refer to Section 2.4).

Abbreviations: std. dev. = standard deviation, cert. conc. = certified concentration, and rep. = replicate.

A dash for the certified concentrations indicates that there is no certified concentration for that element.

A dash for S from Waters 125 indicates that there are no data for S in that Run (refer to Section 3.3.3.3.1).

Table 3.1 continued

Sample Type (Waters 120 Run)		Tl	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
Peach Leaves CRM	mean	3.88	0.261	0.830	83	181	0.093	0.52	3.47	16.5	0.085
Peach Leaves CRM	std. dev.	0.20	0.017	0.022	1	24	0.003	0.09	0.05	0.2	0.005
Peach Leaves CRM	cert. conc.	-	0.37	-	98	218	-	0.69	3.7	17.9	0.060
IAEA Lichen CRM	mean	6.75	1.05	0.819	57	345	0.260	0.88	3.36	30.0	0.286
IAEA Lichen CRM	std. dev.	0.49	0.05	0.043	3	18	0.014	0.03	0.40	3.2	0.017
IAEA Lichen CRM	cert. conc.	-	-	-	64.0	426.0	0.287	-	3.55	31.6	0.639
BCR Lichen CRM	mean	11.9	2.89	3.03	26	659	0.268	2.30	5.95	94.0	0.260
BCR Lichen CRM	std. dev.	0.7	0.06	0.15	1	21	0.006	0.22	0.14	3.6	0.016
BCR Lichen CRM	cert. conc.	-	-	4.12	-	-	-	2.47	7.03	100.6	0.85
Sample Type (Waters 125 Run)		**Tl**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**
Peach Leaves CRM	mean	5.38	0.281	0.940	85	86	0.061	1.14	3.14	16.8	0.883
Peach Leaves CRM	std. dev.	0.20	0.007	0.030	1	28	0.009	0.15	0.24	0.3	0.040
Peach Leaves CRM	cert. conc.	-	0.37	-	98	218	-	0.69	3.7	17.9	0.060
IAEA Lichen CRM	mean	8.30	1.2	1.48	58	374	0.262	1.49	3.34	31.5	0.303
IAEA Lichen CRM	std. dev.	0.20	0.0	0.42	0	20	0.013	0.09	0.13	0.1	0.026
IAEA Lichen CRM	cert. conc.	-	-	-	64.0	426.0	0.287	-	3.55	31.6	0.639
BCR Lichen CRM	mean	14.5	3.08	3.21	25	581	0.268	2.29	6.31	93.0	0.310
BCR Lichen CRM	std. dev.	0.5	0.03	0.32	0	7	0.003	0.06	0.15	0.4	0.027
BCR Lichen CRM	cert. conc.	-	-	4.12	-	-	-	2.47	7.03	100.6	0.85
Sample Type (Waters 903 Run)		**Tl**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**
IAEA Lichen CRM	1 rep. only	6.51	1.15	1.05	57	357	0.251	1.00	3.11	33.8	0.308
IAEA Lichen CRM	cert. conc.	-	-	-	64.0	426.0	0.287	-	3.55	31.6	0.639
BCR Lichen CRM	mean	12.6	2.94	3.14	25	631	0.262	2.21	5.25	88.2	0.254
BCR Lichen CRM	std. dev.	0.0	0.03	0.09	0	0	0.001	0.18	0.10	1.9	0.002
BCR Lichen CRM	cert. conc.	-	-	4.12	-	-	-	2.47	7.03	100.6	0.85

Table 3.1 continued

Sample Type (Waters 120 Run)		Br	Se	Rb	Sr	Mo	Ag	Cd	Sn	Sb	I
Peach Leaves CRM	mean	5.92	0.12	19.0	50.0	0.070	0.0024	0.027	3.94	0.005	-0.024
Peach Leaves CRM	std. dev.	1.60	0.03	0.4	1.6	0.007	0.0014	0.003	0.21	0.001	0.008
Peach Leaves CRM	cert. conc.	-	0.120	19.7	53	0.060	-	0.026	-	-	-
IAEA Lichen CRM	mean	2.27	0.09	1.27	7.77	0.050	0.0087	0.102	4.22	0.015	-0.039
IAEA Lichen CRM	std. dev.	0.97	0.13	0.06	0.32	0.002	0.0014	0.008	0.22	0.002	0.003
IAEA Lichen CRM	cert. conc.	12.9	0.216	1.72	-	-	-	0.117	-	0.073	-
BCR Lichen CRM	mean	1.14	0.27	8.09	8.88	0.319	0.0182	0.495	7.01	0.041	-0.020
BCR Lichen CRM	std. dev.	0.32	0.09	0.25	0.23	0.013	0.0059	0.003	0.41	0.004	0.008
BCR Lichen CRM	cert. conc.	-	-	-	-	-	-	0.56	-	-	-
Sample Type (Waters 125 Run)		**Br**	**Se**	**Rb**	**Sr**	**Mo**	**Ag**	**Cd**	**Sn**	**Sb**	**I**
Peach Leaves CRM	mean	3.13	2.67	17.5	50.4	0.048	0.0037	0.029	2.73	0.007	0.127
Peach Leaves CRM	std. dev.	0.16	0.25	0.1	0.4	0.002	0.0013	0.013	0.06	0.000	0.007
Peach Leaves CRM	cert. conc.	-	0.120	19.7	53	0.060	-	0.026	-	-	-
IAEA Lichen CRM	mean	0.01	0.06	1.31	8.24	0.047	0.0204	0.090	3.06	0.011	0.104
IAEA Lichen CRM	std. dev.	0.59	0.42	0.35	0.09	0.007	0.0007	0.018	0.09	0.004	0.004
IAEA Lichen CRM	cert. conc.	12.9	0.216	1.72	-	-	-	0.117	-	0.073	-
BCR Lichen CRM	mean	-0.16	0.98	8.25	9.00	0.324	0.0475	0.501	5.11	0.027	0.105
BCR Lichen CRM	std. dev.	0.10	0.40	0.08	0.08	0.011	0.0070	0.049	0.37	0.003	0.007
BCR Lichen CRM	cert. conc.	-	-	-	-	-	-	0.56	-	-	-
Sample Type (Waters 903 Run)		**Br**	**Se**	**Rb**	**Sr**	**Mo**	**Ag**	**Cd**	**Sn**	**Sb**	**I**
IAEA Lichen CRM	1 rep. only	1.00	-0.25	1.37	8.20	0.054	0.0150	0.098	3.47	0.017	0.006
IAEA Lichen CRM	cert. conc.	12.9	0.216	1.72	-	-	-	0.117	-	0.073	-
BCR Lichen CRM	mean	2.27	-0.57	7.66	8.73	0.324	0.0270	0.470	6.56	0.044	0.004
BCR Lichen CRM	std. dev.	0.13	0.15	0.13	0.09	0.017	0.0008	0.002	0.27	0.005	0.001
BCR Lichen CRM	cert. conc.	-	-	-	-	-	-	0.56	-	-	-

Table 3.1 continued

Sample Type (Waters 120 Run)		Cs	Ba	La	Ce	Hg	Tl	Pb	Bi	U
Peach Leaves CRM	mean	0.085	115	8.99	10.2	0.024	0.0173	0.839	0.0019	0.0087
Peach Leaves CRM	std. dev.	0.015	1	0.11	0.1	0.018	0.0014	0.016	0.0005	0.0015
Peach Leaves CRM	cert. conc.	-	124	-	-	0.031	-	0.87	-	-
IAEA Lichen CRM	mean	0.090	4.72	0.442	0.95	0.007	0.0059	4.27	0.0130	0.0302
IAEA Lichen CRM	std. dev.	0.005	0.39	0.036	0.08	0.006	0.0017	0.17	0.0010	0.0020
IAEA Lichen CRM	cert. conc.	0.110	-	-	1.27	0.200	-	-	-	-
BCR Lichen CRM	mean	0.182	9.76	0.543	1.14	-0.023	0.0266	33.1	0.0937	0.0336
BCR Lichen CRM	std. dev.	0.008	0.45	0.033	0.08	0.007	0.0015	0.7	0.0016	0.0019
BCR Lichen CRM	cert. conc.	-	-	-	-	0.48	-	40.9	-	-
Sample Type (Waters 125 Run)		**Cs**	**Ba**	**La**	**Ce**	**Hg**	**Tl**	**Pb**	**Bi**	**U**
Peach Leaves CRM	mean	0.034	110	8.81	10.26	-0.003	0.0171	0.340	0.0012	0.0072
Peach Leaves CRM	std. dev.	0.010	2	0.09	0.13	0.004	0.0018	0.009	0.0009	0.0023
Peach Leaves CRM	cert. conc.	-	124	-	-	0.031	-	0.87	-	-
IAEA Lichen CRM	mean	0.077	4.92	0.473	1.03	0.017	0.0052	1.82	0.0126	0.0275
IAEA Lichen CRM	std. dev.	0.032	0.13	0.008	0.02	0.010	0.0012	0.11	0.0009	0.0019
IAEA Lichen CRM	cert. conc.	0.110	-	-	1.27	0.200	-	-	-	-
BCR Lichen CRM	mean	0.182	9.71	0.569	1.19	0.012	0.0243	13.5	0.0986	0.0326
BCR Lichen CRM	std. dev.	0.008	0.23	0.021	0.03	0.016	0.0032	0.1	0.0014	0.0023
BCR Lichen CRM	cert. conc.	-	-	-	-	0.48	-	40.9	-	-
Sample Type (Waters 903 Run)		**Cs**	**Ba**	**La**	**Ce**	**Hg**	**Tl**	**Pb**	**Bi**	**U**
IAEA Lichen CRM	1 rep. only	0.092	5.12	0.490	1.07	-0.004	0.0059	4.14	0.0124	0.0302
IAEA Lichen CRM	cert. conc.	0.110	-	-	1.27	0.200	-	-	-	-
BCR Lichen CRM	mean	0.180	10.64	0.560	1.17	-0.001	0.0247	31.8	0.0925	0.0343
BCR Lichen CRM	std. dev.	0.005	1.53	0.006	0.01	0.000	0.0025	0.4	0.0026	0.0003
BCR Lichen CRM	cert. conc.	-	-	-	-	0.48	-	40.9	-	-

3.2 Digestion Procedure

3.2.1 In General

The procedure utilized in this study was a partial digestion consisting of a series of wet and dry ashings. The particles remaining after digestion were dominantly of a silicate composition (discussed in greater detail in Section 3.2.2). A complete digestion would necessitate the use of hydrofluoric acid to facilitate the dissolution of the silicates. Use of HF in the procedure used in this study would create some complications; this would necessitate the use of a vessel made of plastic or teflon, however a plastic or teflon vessel would not be suitable to undergo the dry ashing at temperatures up to 375 °C. If HF were to be used, then transfers between vessels would have to occur, and ensuring quantitative transfers would be difficult. For the purposes of this study, it was preferable to use a partial digest (bearing in mind that there would be silicate residual particles). Based on the SEM-EDX work, the only elements which are likely to reflect the incomplete digestion of the silicates are: Si, Fe, and potentially Ti. In fact, the titanium data produced by this study gave no indication that there was a notable problem, which is not surprising as Ti was a minor constituent of the residual particles; however, it should be kept in mind that none of the CRMs utilized had certified concentrations for titanium. (The certified values for the three CRMs used in this study are given in Appendix V.)

The intent of this study was to develop a procedure for lichen digestion which would yield data useful for environmental studies. The samples were analyzed by the Waters/Biologicals Package for the ICP-MS at Memorial University of Newfoundland which gives data for over 40 elements. The intent was not to develop a procedure which would yield good data for all the elements in the Waters/Biologicals Package. Some elements did

not yield acceptable data for a variety of reasons, for example, some elements were found to be in the residual particles (e.g. Si, Fe, Ti), and other elements were more volatile and would have been lost since the procedure was an open-vessel digestion (e.g. Cl, Br, I, Hg). The developed digestion procedure provided acceptable data for a suite of over 20 elements, many of which are of environmental interest (e.g. V, Cu, Zn); these elements are discussed later in this chapter.

3.2.2 SEM-EDX Analysis

3.2.2.1 Residual Particles

As described in Chapter 2, SEM-EDX analysis was carried out to characterize the undissolved particles remaining after digestion. (Some residual particle characterization was done in prior work, Tucker, 1995.) The sensitivity of SEM-EDX analysis is a function of the counting time and beam conditions (Shaffer, pers. comm., 2003). For the SEM-EDX analysis used in this study, an acceleration voltage of 20 kv and a beam current sufficient for acquiring a significant number of x-rays into the spectrum were used; the count times were long enough such that an element like Mn could most likely be seen above background when present at levels of 0.5 wt. % or greater (Shaffer, pers. comm., 2003). Residual particles were characterized for digestions of each of the sample types used. The EDX spectra indicated that the residual particles in all six of these sample types are broadly similar. That is, the dominant type of residual particle characterized had a high silicon content, with lower amounts of other elements (such as Fe, Ti, Al, K, Mg, Na, and/or Ca). This indicates that the majority of the particles are silicate minerals. These silicates are likely to be minerals such as: quartz, feldspars, olivines, garnets, micas, and clay minerals. The following

paragraphs describe the individual samples in more detail; the SEM-EDX component of this study was not undertaken to acquire quantitative information about the residual particles, therefore some of the observations are qualitative in nature.

The certified reference materials had more residue than either of the collected lichen samples. In general, the CRMs had a relatively large quantity of fine white/off-white residual particles covering the entire surface of the filter paper. These residues had the appearance of a whitish crust over the filter paper and had an appearance not unlike a low-profiled stucco ceiling. Table VI.1 in Appendix VI details the observations of the residual particles of the three CRMs. Most particles had a high Si content; some particles had high Si along with high Al or K, while others had high Si with low amounts of Al, K, Ca, Mg, Na, Fe, and/or Ti. The SEM photo in Plate 3.1 is an example of a smooth grain from the IAEA Lichen CRM residue; the relative composition of this particle is indicated by the SEM-EDX graph in Figure 3.1. The Peach Leaves CRM tended to have less variation in the residual particles. (The residual particles of the Peach Leaves CRM were characterized previously in Tucker, 1995.) The BCR Lichen CRM tended to be off-white/pale beige in colour, whereas the other CRMs were white. The BCR Lichen CRM also tended to have a noticeable quantity of particles which were not off-white/beige: many were black, some were deep red (possibly garnet), some were orange, one was yellow, and one green/blue. The IAEA Lichen CRM had some particles which were not white, but not as many as the BCR Lichen CRM: some black particles, and some pearly white particles. In general, the Lichen CRMs have a greater quantity of a more uniform residue than the lichen samples collected for this research. Perhaps the crushing method used for the Lichen CRMs produced finer particles with a more granular appearance which could explain the more uniform

appearance, but does not explain the relatively greater quantity of residue for the CRMs. The BCR Lichen CRM is the lichen species *Pseudevernia furfuracea*, and the IAEA Lichen CRM is *Evernai prunasti (L.) Ach.* Since the IAEA and BCR CRMs are lichens, it is puzzling that they have relatively more residue than the lichen samples collected for this study. It is possible that some species produce and/or entrap more particles than others, it is possible that more particles are deposited on the lichens in some areas than others, or perhaps there is another controlling variable which is less obvious.

The residual particles of *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris* lichens had characteristics in common. These lichens had much less residue than the CRMs and there were small areas of each where the filter paper was visible. As well, these lichen residues are not as uniform as the CRM residues. Each of *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris* had three dominant types of residual particles: clear colourless particles, white granular particles, and long thin particles which have a lint-like appearance. Examples of each of these particle types are illustrated in Plates 3.2-3.4, with the associated SEM-EDX spectra in Figures 3.2-3.4. Each of the collected lichens had some variability in the residual particles. Specific detailed observations for the residual particles of these three lichens are outlined in Table VI.2 in Appendix VI. Most of the observed particles had high Si; some had high Si with lower amounts of Al, Mg, Na, P, Ca, K, Fe, and/or Ti. There were some particles containing high K, Ca, Al, and/or Ti. The observed particles of *Alectoria sarmentosa* were all white or clear and colourless. The *Bryoria sp.* slide had more granular particles than the *Alectoria sarmentosa* slide. The *Cladonia alpestris* slide contained several pale orange particles and at least one red particle. The *Alectoria sarmentosa* and *Bryoria sp.* slides each had a lower quantity of particles than the *Cladonia alpestris* slide. The *Cladonia*

alpestris slide has many more long thin lint-like particles than either the *Alectoria sarmentosa* or *Bryoria sp* slide. Early developmental work done for the study of Tucker (1995) estimated that the quantity of residual particles for *Alectoria sarmentosa* and *Bryoria sp.* is 1 % (or less) of the original sample weight. For many elements, the concentrations determined by ICP-MS for the CRMs are generally higher than the concentrations for the collected lichens; this is particularly notable for Cr, Fe, Cu, Ba, La, Ce, and Tl. There appears to be a general relationship that more residual particles tend to be associated with higher concentrations.

Other researchers have noted particles in lichens. Much work has been done in the investigation of lichen secondary metabolic products, also referred to as "lichen substances" or "lichen acids" (Galun and Shomer-Ilan, 1988). These substances can be in crystallized forms, but tend to be organic in nature, whereas the SEM-EDX work for this study indicates that the residual particles remaining after digestion are inorganic (Galun and Shomer-Ilan, 1988; Elix, 1996). A study by Garty et al. (1979) examined particulate metallic fallout accumulated by the lichen *Caloplaca aurantia*. They used X-ray diffraction analysis to determine that the lichen trapped particles contained: weddelite (calcium oxalate), quartz, calcium, magnetite, metallic iron with titanium, and alkaline amphibole. They also found that the particles incorporated by the lichens were similar to the composition of dust particles from the sampling area.

The characterization of residual particles is an area which requires more work. At present the origin of the residual particles is unclear. The particles may have been deposited on the lichen in the field, they may be particles produced by the lichen, or they may be particles produced or altered during the digestion process. The study by Garty et al. (1979)

mentioned in the previous paragraph could suggest that at least some of the residual particles remaining after digestion might be particles which were deposited on the lichen prior to sampling. One of the challenges in doing this type of SEM-EDX work is that the coloured particles are difficult to distinguish by SEM-EDX. As well, it is generally difficult to determine the composition of very thin or very small particles because it is possible that elemental information is also acquired from the area surrounding the particle. Perhaps further work could eliminate some of these problems; this will be discussed in Chapter 4.

3.2.2.2 Surface Particles

An examination of the surface of strands of *Alectoria sarmentosa* was done by SEM-EDX, as was described previously in Chapter 2. Six stubs (A-F) were examined, with each stub having three strands of lichen (i.e. not digested). Table VI.3 in Appendix VI provides a brief outline of the six stubs and the strands on each. The lichen was from three different sites, two from Come By Chance (relatively polluted), and one from Torbay (relatively unpolluted). The lichen on the first three stubs was not washed, while the lichen on the last three stubs was washed by rinsing in deionized water. The intent of this study was to compare the surface particles with the residual particles. Also, surface particles from a relatively polluted site and a relatively unpolluted site could be examined, as well as the surface particles from washed and unwashed lichen.

Many different types of particles were observed on the surface of the lichen strands. Some of these particle types were observed frequently. There were many granular particles/areas, rounded/spherical particles, plate-like particles (or crystals), and there were also many long thin (lint-like) particles. Many other particles observed were more irregular,

and some particle morphologies were only noted once or twice. There were many particles which contained high Si, high Al, high Ca, and/or high Fe. Many areas/particles were observed which had some background elevation. Some particles observed had small amounts of other elements, such as: K, Na, Ti, S, and/or Cl (occasionally a particle was observed a with a higher quantity of one of these elements). It was assumed that any Au or Cu observed was due to the Au-coating.

After completing this surface examination, no clear trends seem evident. The spherical/rounded particles were observed on both the washed and unwashed strands. The plate-like particles/crystals were observed on strands from all stubs except Stub A (and were quite notable on Stub F). The long thin (lint-like) particles were observed in strands on all six stubs. The granular particles/areas were observed in all stubs with the exception of Stub A. The particles which contained Fe and the particles which contained Ca were each observed on both the washed and unwashed strands. Particles with Fe and particles with Ca were also observed on both strands from Come By Chance and strands from Torbay. Similarly, particles with high Si or high Al were observed on both washed and unwashed strands. Particles with high Si or high Al were also observed to be on both Come By Chance and Torbay strands. It is difficult to draw clear-cut conclusions from these observations.

Some comments can be made regarding the comparison of the surface particles with the residual particles which remain after digestion. In terms of morphology, both granular and long thin (lint-like) particles were observed on the lichen surface and in the residual particles. It is not known whether or not the residual particles were actually particles (or remnants of particles) which existed on the lichen surface prior to digestion; it is also not known if the long thin (lint-like) grains are related to the fungal hyphae of the lichen.

There were several difficulties that limited the information gained by the surface examination. Since the stubs are Al, it may be possible that some of the Al noted by SEM-EDX originated from the stubs. Contamination from the scissors used to cut the strand segments is a possibility. Cutting with scissors tends to flatten the strands, which could possibly alter the shape of some grains observed. Since the lower portion of the lichen strand is affixed to the double-sided tape on the stub, only the upper portion of the strand can be examined; perhaps the lower portion of the strand has useful information which is not accessible. As well, with this type of SEM-EDX work in general there are potential problems with adding or removing particles while preparing the stub/slide, while transporting the stub/slide, while carbon or gold-coating, and while examining with the SEM-EDX. Another of the challenges with this type of SEM-EDX study is that one must manually select which element corresponds to a peak on the EDX graph from a series of elements, and occasionally it can be difficult to make the appropriate selection because some elemental peaks overlap.

This surface particle examination by SEM-EDX was not used as the basis for deciding whether or not to wash the lichen samples prior to digestion. This decision was made based on other information, as outlined in Chapter 2. The surface particle examination of lichen has ample room for future work.

3.3 Evaluation Of Analytical Data

3.3.1 ICP-MS Instrument Data Quality

Three sets of samples were analyzed using solution ICP-MS. Complete data for these samples are presented in Appendix IV. These sets of samples were analyzed in three ICP-

MS Runs: Waters 120, 125, and 903. Since each Run was analyzed on a different instrument, it is important to assess the quality of data from each before further comparisons are attempted.

Background signals can be due to air entrainment into the system, material eroding from the sampler and skimmer, elements in the nitric acid, contaminants in the argon gas, and continuum background. For each instrument, the background signals for the heavier elements, with no significant polyatomic interferences, were on the order of approximately 100 cps or less. Some of the lighter elements have higher backgrounds due to polyatomic species. The backgrounds were within routine operating conditions for the tuning conditions employed for the Waters/Biological Package for each instrument (Tubrett, pers. comm., 2003). A background correction was employed using the calibration blanks (0.2 N nitric acid).

As outlined in Chapter 2, oxide formation for each set of samples analyzed was monitored using the ThO/Th ratio. Thorium oxide ratios are used as an indication of the magnitude of all the polyatomic interferences present in a given run. Thorium oxide is monitored because there are no interferences on or by ThO or Th, and it is generally the oxide with the highest bond strength, with all other oxides forming to a lesser extent. The oxides for each of the Waters Runs (120, 125, and 903) were all within the acceptable tuning specifications for each individual instrument. For the Waters Runs 120, 125, and 903, the respective oxides over the course of each run were: 8-10 %, 6-11 %, and 4-6 %; these oxides are on the same order of acceptable oxides as reported in Strong and Longerich (1985). Corrections were applied for the most important interferences.

The instrumental drift over the course of each run was within acceptable limits. The

matrix suppression/enhancement in each run was also within acceptable limits. Jackson et al. (1990) reported that instrumental drift (expressed as the maximum deviation of a single measurement on the calibration standard from the mean) was usually < 20 % in a 12.5 hour run. A matrix/drift correction applied using the internal standards further corrected for these effects. In the Waters 120 Run, for Sc the RSD of the signal over the entire run was in the order of approximately 13 % (considered low), which is an indication of instrument drift and/or matrix effects. In the same run, the approximate RSDs for the other internal standards were: Rh 10 %, Re 17 %, and Th 19 %. In the Waters 125 Run, the approximate RSDs were: Sc 4 %, Rh 2 %, Re 5 %, and Th 6 %. In the Waters 903 Run, the approximate RSDs were: Sc 9 %, Rh 12 %, Re 14 %, and Th 24 %. RSDs for internal standards in the three ICP-MS Runs of this present work are in ranges considered acceptable; the internal standard Th in the Waters 903 Run is slightly higher than typical RSDs, however, normalizing the data resulted in much lower RSDs of approximately 5 % (i.e. acceptable RSDs) (Tubrett, pers. comm., 2003). In each of the three Runs, after the data were normalized using the internal standards, the RSDs for most elements were about 5 %.

The instrument sensitivity during a run is determined from calibration standards. It is measured as counts per second per parts per billion (cps/ppb/abundance). The sensitivities for the data in the Waters 120 Run (using the SCIEX Elan ICP-MS) are approximately 1000 cps/ppb/abundance; in comparison, Jackson et al. (1990) utilized the same instrument and reported maximum sensitivities of approximately 300 cps/ppb (normalized to 100 % isotopic abundance). The sensitivities in the Waters 125 and 903 Runs (using the VG and HP ICP-MS instruments respectively) are about 10,000 cps/ppb/abundance. The signal difference is attributed to a great improvement in sensitivity; the SCIEX Elan ICP-MS was a first

generation instrument and its specifications were lower than the VG and HP instruments. Some elements with a higher ionization potential, for example Se, have lower (less) sensitivity. The sensitivities for all runs were acceptable (Tubrett, pers. comm., 2003).

3.3.2 Duplicates

Duplicates were analyzed in the Waters 120 and 903 Runs. Differences between the sample and duplicate could occur as a result of several factors: measurement noise, instability of sample solutions over time, instrument reproducibility, and/or inconsistency of matrix/drift corrections. The sample and duplicate data were examined by visual inspection for any obvious problems. Discrepancies for Si were attributed to the presence of Si in the residual particles; as HF was not used in the digestion procedure, most silicate minerals should not have been dissolved. Some elements with differences between samples and duplicates were also determined to have other problems with their data (such as poor precision and/or accuracy); these elements are discussed in Section 3.3.3. Other elements with differences are mentioned in the following paragraphs.

3.3.2.1 Waters 120 Run

In the Waters 120 Run, the data for the duplicate jt-27* of the IAEA Lichen CRM jt-7 are very similar to the data for the actual sample (jt-7) for most elements (see Figure 3.5). Beryllium shows a difference between the duplicate and sample, but the sample is below the detection limit. Sulphur also shows a difference. Since the sulphur data for both lichen CRMs had poor reproducibility, this suggests potential losses during digestion. (The sulphur data are further discussed in a subsequent section.)

The duplicate jt-28* and the corresponding sample jt-10 of the BCR Lichen CRM have no problematic differences (refer to Figure 3.6). There is a small difference for the sulphur determinations; the comments regarding sulphur in the previous paragraph are applicable in this case also.

In the Waters 120 Run, the sample jt-22 Alectoria Bonavista (Area 4) puck mill and its corresponding duplicate jt-30* show no prominent differences for most elements (refer to Figure 3.7). The elements vanadium, antimony, and bismuth have slight differences between samples and duplicates. These differences were noted, but have no obvious explanation.

3.3.2.2 Waters 903 Run

The data for the sample jt-1 IAEA Lichen CRM and its duplicate jt-15* have no problematic concentration differences for most elements (see Figure 3.8). Lithium and chromium have some differences, but not enough to warrant concern.

Most elements show no differences between the jt-10 Alectoria Random Island (Area 1) sample and the associated duplicate (jt-16*); refer to Figure 3.9. There are differences for beryllium and chromium, however in the case of these elements the samples are below the detection limit, so agreement is not expected.

3.3.3 Evaluation Of Individual Elements

The concentrations of thirty-nine elements were obtained using the ICP-MS Waters/Biologicals Package at Memorial University of Newfoundland. Some of the elemental data were suitable for interpretation, while some were not. It was not the intention

of this study to develop a method to determine the concentrations of all elements in the Waters/Biologicals Package, but to determine the concentrations of some elements of environmental interest. As a measure of accuracy for this study, the elements selected as having acceptable data were those which could be determined within ± 15 % relative difference of the certified concentrations for the CRMs. Reasons are given as to why the data of some elements were not suitable (e.g. poor accuracy or poor precision), and where possible, some explanations for the poor data are given. The following sections discuss all elements and the suitability of their data for the purposes of this research.

3.3.3.1 Unsuitable Elements

For various reasons, a subset of the thirty-nine elements was determined to be unsuitable for interpretation and these elements were eliminated from the list of useful elements. Some elements were volatile and would likely be lost during the open vessel digestion. Some elements had poor precision for a CRM between the ICP-MS Runs. Some elements had poor accuracy for the CRMs. Discussed below are the unsuitable elements and the reasons supporting the decisions to remove them from the list of elements suitable for interpretation.

3.3.3.1.1 Silver (Ag)

There was poor precision between the means of the determinations of silver in *Alectoria sarmentosa* from Bauline (1996) from each of the three ICP-MS Runs. Refer to Figure 3.10. This poor precision may be due to the tendency of silver to adhere readily to the interior of the ICP-MS, thus creating memory effects (Longerich et al., 1986). If silver

concentrations were of specific interest, the resolution of these difficulties would require further method development.

3.3.3.1.2 Bromine (Br), Chlorine (Cl), Iodine (I), and Mercury (Hg)

Volatile elements such as Br, Cl, I, and Hg yielded data which was not suitable for interpretation. These elements are likely to have been lost as gases during the digestion procedure. The specific accuracy and/or precision problems with each of these elements are discussed in the subsequent paragraphs.

The means of the bromine determinations in the IAEA Lichen CRM samples from each of the three ICP-MS Runs had both poor precision and poor accuracy. See Figure 3.11. The reason for this poor precision and accuracy is likely because reduced bromine is volatile (ex. Br_2) and would have escaped as a gas during the open-vessel digestion (Weast, 1975).

A number of factors were considered in making the decision to eliminate chlorine. The means of chlorine determinations in the Peach Leaves CRM in two sets of samples (peach leaves was not in the third set of samples) do not agree: for the Waters 120 Run, the determined mean agrees with the certified values (with a relative difference of only about -6 % between the certified and experimental values), but the mean for the Waters 125 Run is quite low compared to the certified values (with a relative difference of approximately -68 %). Refer to Figure 3.12. Chlorine is a gas, therefore the open vessel digestion method would likely cause much of the chlorine to be lost (Weast, 1975). Some forms of chlorine are readily lost as gases. Since bromine has been eliminated from the list of elements yielding useful data by the developed method, it is reasonable that chlorine would also be eliminated since it is expected that all the halides will behave in a similar manner.

Since neither of the three certified reference materials used in this work contained certified concentrations of iodine, an estimate of the accuracy of the experimental iodine concentrations could not be made. In general, the halides are expected to behave in a similar manner and since bromine and chlorine have been removed from the list of useful elements, then this would suggest that iodine should be removed also. It is expected that a portion of the iodine present in the samples would be lost during the open vessel digestion. In the Waters 120 Run, all the iodine data for the CRMs, lichen samples, and reagent blanks are negative; in the Waters 125 Run, all the data are positive; in the Waters 903 Run, some of the data are negative and some are positive. (An explanation of negative data was given in Section 2.4.) Since the data varies between positive and negative values, this is an indication of the unreliability of the iodine data. Since iodine volatilizes at ordinary temperatures, this element could reasonably be expected to be partially or fully lost by the completion of the digestion procedure (Weast, 1975). Therefore iodine was eliminated from the list of useful elements.

There were several reasons for choosing to remove Hg from the set of useful data. Mercury tends to adhere to the internal components of the ICP-MS and therefore has high memory effects which lead to unreliable data (Knight et al., 1999; Longerich et al., 1986). Mercury also has high backgrounds which is due, at least in part, to the presence of mercury in the argon used for ICP-MS analyses (Hirata and Nesbitt, 1995; Tubrett et al., 2001). Also, Hg is a volatile element and would likely be lost during the open vessel digestion (Weast, 1975). This loss of Hg is suggested by the fact that in each of the certified reference materials utilized, the Hg means for each set of samples were either negative (and did not plot on the log scale graphs) or were less than the certified values. See Figures 3.11, 3.12,

and 3.13. The data of this study indicate that Hg precision is poor. As well, Hg is below the detection limit for all samples with more than one replicate except for the *Alectoria sarmentosa* samples which were puck mill crushed in the Waters 120 Run (these samples were determined to be contaminated from the puck mill). Concentrations of Hg in the samples analyzed tend to be very low (0.045 ppm or less).

3.3.3.1.3 Arsenic (As) and Selenium (Se)

The elements arsenic and selenium both had similar problems with accuracy and precision. These problems are described in the subsequent paragraphs.

The means of the Peach Leaves CRM data for the Waters 120 Run and the Waters 125 Run indicated marked differences for arsenic (Peach Leaves was not in the Waters 903 Run). Refer to Figure 3.12. The mean of the determined concentrations for arsenic in the Waters 125 Run was more than an order of magnitude greater than the certified value (with a mean relative difference of about +1372 % between the experimental and certified values); the mean for the Waters 120 Run was much more accurate, but also high, with a mean relative difference between the certified and experimental values of about +42 %. Refer to Figures 3.14 and 3.15. Despite the relatively high experimental values for arsenic noted above for the Peach Leaves CRM, the means for the three sets of samples for both the BCR and IAEA Lichen CRMs were all low. See Figures 3.11 and 3.13. Since the data were inconsistent for the three CRMs, this indicated problems with the analysis of arsenic by this method, therefore a decision was made to remove arsenic from the list of elements suitable for interpretation.

As in the previous discussion of arsenic, the means of the Peach Leaves CRM data

for the Waters 120 Run and the Waters 125 Run yielded substantial differences in selenium concentrations (the Peach Leaves CRM was not in the Waters 903 Run). See Figure 3.12. The mean of the experimental selenium concentrations for the Peach Leaves CRM in the Waters 125 Run was more than an order of magnitude greater than the certified value (with an mean relative difference of about +2124 %). Refer to Figure 3.15. The experimental mean for Se in the Peach Leaves CRM in the Waters 120 Run was much closer to the certified value, with a mean relative difference of about +2 %; although this +2 % for the mean is reasonable, for the individual replicates the relative difference varies more widely from approximately -25 % to +23 %. Refer to Figure 3.14. Despite the selenium experimental values for the Peach Leaves CRM being higher than the certified values, the opposite was true for the IAEA Lichen CRM. The mean IAEA Lichen CRM data for each of the ICP-MS Runs yielded selenium concentrations which were less than the certified values. See Figure 3.11. Since the data are inconsistent for the Peach Leaves and IAEA Lichen CRMs, this indicates problems with selenium analysis using this method, therefore it is best to remove selenium from the list of useful elements. (Certified selenium values are not given for the BCR Lichen CRM.)

3.3.3.1.4 Aluminium (Al)

For the BCR Lichen certified reference material, the aluminium mean relative difference varies widely between the three ICP-MS Runs: -43% in the Waters 120 Run, -92% in the Waters 125 Run, and -67% in the Waters 903 Run. (Aluminium was above the detection limit in each case.) Refer to Figure 3.13. It was anticipated that aluminium would have lower experimental concentrations than the certified concentrations because it was one

of the elements that was observed by SEM-EDX to be present in the residual particles. Aluminium is not expected to be well-dissolved by the developed digestion method since HF is not used; silicates are dissolved by HF and aluminium is most likely present in alumino-silicates. As well, there could potentially be aluminium memory problems; rocks containing aluminium are frequently analyzed with the same ICP-MS instrument, so there is some potential for contamination.

3.3.3.1.5 Boron (B)

For boron, the relative differences for the Peach Leaves CRM for the two ICP-MS Runs indicate poor precision between the runs (the Waters 903 Run did not include Peach Leaves). For the Waters 120 Run, the mean relative difference was -51%, whereas for the Waters 125 Run, the mean relative difference was -39% (boron was well above the detection limit in each case). See Figure 3.12. As well, the accuracy is quite poor; boron is not one of the elements anticipated to be significant in the residual particles. As well, the ICP-MS instrument may be contaminated with boron due to the use of boric acid in some of the rock digests analyzed.

3.3.3.1.6 Lead (Pb)

Lead will not be included in the list of useful elements analyzed by this method since neither the precision nor accuracy is good. For the Peach Leaves CRM, the mean Pb concentration for the Waters 120 Run is very close to the certified value, however, for the Waters 125 Run, the mean Pb concentration is much lower than the certified value (a mean relative difference of -65%). For the BCR Lichen CRM, the mean Pb concentrations for the

Waters 120 and 903 Runs are similar and lower than the certified value (the mean relative differences are -19% and -22% respectively), however, the mean Pb concentration for the Waters 125 Run is much lower than the certified value (the mean relative difference is -67%). In the Waters 125 Run, there was a calibration problem with lead. Refer to Figures 3.12 and 3.13.

3.3.3.2 Suitable Elements

The remaining elements (ie. those not specifically mentioned in the list of eliminated elements) were retained as having data which was suitable for interpretation. These elements are:

Li, Be, Mg, Si, P, S, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, Mo, Cd, Sn, Sb, Cs, Ba, La, Ce, Tl, Bi, and U.

Some of the above elements have very good data; i.e. accuracy and precision are very good. This means that the average relative differences for the CRMs are generally less than ± 15 %, and the RSDs are less than 15 % for all samples (most are ≤ 5 %). Other elements have data that are good, but do not have the characteristics of very good data as described previously (these elements do not have specific problems with their data which would justify eliminating these elements). In general, this means that overall these elements have reasonable accuracy and precision, however some samples deviate beyond the criteria stated for the elements with very good data. Most of these elements with good data have average relative differences within approximately ±30 %, and RSDs that are dominantly ≤20 %.

There are also a number of elements for which there are no certified values in either of the three CRMs used in this research; these elements (with the exception of S discussed in the subsequent section) can only be evaluated by their precision and show no reason to be eliminated from the list of useful elements; these elements had RSDs which were mainly ≤20 %. (These elements could not have relative differences calculated, therefore accuracy could not be evaluated.) There are three elements (S, Ni, Mo) that could conceivably be eliminated, but will be kept; these elements are discussed in the subsequent section. These elements have average relative differences within ± 65 %, and RSDs ≤ 25 %; these elements have some problems with their data, but will be retained in the group of elements used for further interpretation. There are some elements with unsuitable data which have average relative differences up to ±2124 % and have RSDs ≤ 28 %; these elements each have specific problems with their data (as discussed previously) that justify these elements being eliminated from the group of elements providing suitable data. Table 3.2 illustrates the elements grouped into categories according to the evaluation of their ICP-MS data (based on the accuracy and precision of CRMs).

Table 3.2: Elemental groupings according to data quality. The first three columns are elements which have suitable data. The comments in brackets refer to whether or not the elements of that column were retained/removed from the list of elements which yield useful data. The first four columns of elements were used for the statistical analyses discussed later in this chapter. (CRM = Certified Reference Material)

Elements With Very Good Data (Will Retain)	Elements With Good Data (Will Retain)	Elements Not In Either Of The CRMs Used (Will Retain)	Elements With Data Which Could Be Deemed Unsuitable (Will Retain)	Elements With Unsuitable Data (Will Remove)
Mg	V	Li	S	B
P	Cr	Be	Ni	Al
Ca	Fe	Si	Mo	Cl
Mn	Cu	Ti		As
Co	Rb	Sn		Br
Zn	Cd	La		Se
Sr	Sb	Tl		Ag
Ba	Cs	Bi		I
	Ce	U		Hg
				Pb

3.3.3.3 Elements That Could Be Deemed Unsuitable

There are a few elements which will not be eliminated at this point, but which have data that could justify eliminating them from the list of useful elements. The problems with these elements will be kept in mind through the further interpretation of the data. These elements, S, Ni, and Mo, are discussed below.

3.3.3.3.1 Sulphur (S)

There are no certified values for sulphur in any of the three CRMs utilized in this research; therefore there is no measure of accuracy for sulphur. The S data have somewhat poor reproducibility for both the BCR and IAEA Lichen CRMs. See Figures 3.16 and 3.17. It is possible that some sulphur may be lost during the sample digestion (as sulphur gases); this could account for the poor precision. The removal of sulphur from the data set would be justified, however it was retained as perhaps some useful information could be obtained from the data, either for this present study or in future work.

There are no data for sulphur in Waters 125 Run. This was because the count rates for mass 34 were too high during the ICP-MS analysis. This was likely due to a polyatomic interference (i.e. summation of ion masses) from either H^2-O^{16}-O^{16}, O^{17}-O^{17}, or H^1-H^1-O^{16}-O^{16} (Tubrett, pers. comm., 2002). The Waters 125 Run was analyzed on the VG ICP-MS. With some instruments when the count rates are too high, the instrument switches from pulse counting mode to an analog mode, but with this VG ICP-MS this switching has always been problematic. As a result, there are no sulphur data in the Waters 125 Run.

3.3.3.3.2 Nickel (Ni)

For the Peach Leaves CRM, the mean for the Waters 120 Run data is very different from the mean for the Waters 125 Run data; the mean relative difference for the Waters 120 Run is -25 %, but for the Waters 125 Run it is +65 %. Refer to Figure 3.12. This indicates a problem with the Ni data. It is possible that some Ni could be in a silicate form (such as olivine), which would not be digested by the methods used in this work. Also, it is possible that there are high Ni backgrounds due to erosion of the ICP-MS sampler and skimmer as

the solutions are analyzed.

Ni was retained in the list of useful elements even though the data and criteria suggest it should be removed. This decision was made since Ni is an element of environmental interest and it was thought that the data could yield some worthwhile information even if there is a potential problem. Future work may determine that there is an analytical problem with nickel. The observed problem with Ni was noted and kept in mind throughout the data interpretation.

3.3.3.3.3 Molybdenum (Mo)

Molybdenum was retained in the list of useful elements even though for the Peach Leaves CRM, the mean relative differences for Waters 120 and 125 Runs are +16% and -20% respectively. See Figure 3.12. These values are not very different from the general criteria of ±15 % relative difference; the deviations for Mo are not as high as for some of the other elements discussed above. The problem with Mo was noted and kept in mind during the interpretation of the data.

3.3.4 Summary

The ICP-MS data were evaluated and a suite of seventeen elements was determined to have data suitable for interpretation. This element suite is comprised of: Mg, P, Ca, Mn, Co, Zn, Sr, Ba, V, Cr, Fe, Cu, Rb, Cd, Sb, Cs, and Ce. Nine elements were not certified in either of the CRMs utilized (thus accuracy could not be evaluated), but otherwise had acceptable data: Li, Be, Si, Ti, Sn, La, Tl, Bi, and U. Three elements had data of borderline acceptability: S, Ni, and Mo. This yields a potential suite of twenty-nine elements for which

acceptable data were obtained. Ten elements were determined to have unacceptable data for reasons discussed previously. The elements in the first four columns of Table 3.2 were the elements which were used for the statistical analyses in the interpretations of the ICP-MS data later in this chapter.

3.4 Comparison Of Sites

3.4.1 Introduction

One of the research questions of interest examined was whether or not the four lichen collection sites can be distinguished by their trace element data; and if so, which elements are useful in discrimination between the sites. This is of interest for a variety of reasons. It is useful to have a tool which could be used to establish elemental background levels or to monitor sites over time (Richardson, 1992). This is especially useful for monitoring events which involve a source of pollution starting or ceasing operations (for example, the opening of a smelter). Another application could involve establishing a calibration for individual elements in which different concentration ranges in a particular lichen species indicate whether a site is polluted or unpolluted for that particular element. It is of interest to determine relationships between concentration levels and distance from a pollution source (Tomassini et al., 1976). It is also useful to be able to determine information about the sources of trace elements at a particular site.

As stated in Chapter 2, the sites for this study were selected using the findings of related research which utilized sulphur isotopes of lichens in Newfoundland; the sites selected were (in general order of decreasing pollution): Come By Chance, Bauline Line, Random Island, and Bonavista. The sites Come By Chance, Random Island, and Bonavista

form a transect running from Come By Chance (the site of an oil refinery) in a NE direction to Bonavista (an area of no major industrial pollution). The dominant wind direction in this area is from the southwest or west (Banfield, 1981). It is logical to anticipate that for these three sites that pollutants from the oil refinery would decrease from Come By Chance to Random Island, to Bonavista. V and Zn are among the heavy metals most closely associated with the refining of petroleum, and Cd, Cr, Cu, and Ni are among the heavy metals that are also associated, to a lesser extent, with oil refining (Gov. of Newfoundland and Labrador, 1987).

3.4.2 Statistical Analysis Of Site Comparisons

The data utilized in the site comparisons (and for all the comparisons in this chapter) included all elements in the first four columns of Table 3.2 (with the exception of S). The analysis of site comparisons was done utilizing the Minitab statistical package. Pairwise comparisons of the four collection sites were done using t-tests. Only one species, *Alectoria sarmentosa*, was used in these site comparisons. Samples from all areas of each site were averaged in the t-tests. For Bauline Line, only the 1996 samples were used; a comparison between the 1996 and 1997 samples will be done later in this chapter. The flowchart in Figure 3.18 indicates the individual samples, the areas, and the sites utilized in the t-tests. Since there were not enough replicates from each site to determine with certainty whether or not the populations were normal, an assumption of normality was made. The t-tests were performed using a 95 % confidence interval. The p-values for the t-tests of the pairwise comparisons are given in tables in Appendix VII.

Prior to doing t-test analysis, F-tests were performed to determine whether or not the

populations of the four sampling sites had equal variances for each element. For those elements determined to have equal variances (as was the case for the majority of the elements), the t-tests were performed using the option assuming equal variances. For those elements with unequal variances, the option assuming equal variances was not used. The F-tests were performed using a 95 % confidence interval. Sulphur was omitted from the F and t-tests since the samples from the Waters 125 Run did not contain data for sulphur.

3.4.3 Trends

The relative concentrations of the four sampling sites are shown in Figure 3.19. This figure indicates that the general elemental character for each of the four sites is similar in nature, however, there are some elements which are quite different from site to site.

From the tables of p-values and the graph, it is clear that certain elements show differences in concentration for different sites. Other researchers have also found that there are concentration differences in lichens when examining sites which are differentially exposed to pollution (Tomassini et al., 1976; Richardson, 1992; Nimis et al., 1999; Seaward et al., 1978). For certain elements, the concentration increases from the less polluted sites to the more polluted sites (this will be referred to as an increasing trend). For other elements, the opposite was observed, i.e. the concentration tends to decrease from the less polluted sites to the more polluted sites (this will be referred to as a decreasing trend). Table 3.5 (in Section 3.4.3.3) groups the elements according to the general trend in concentration that they exhibit from site to site.

3.4.3.1 Increasing Trend

As mentioned above, many elements display an increasing trend, that is, concentrations increase from the less polluted to the more polluted sites. More specifically, this trend would be concentrations increasing from site to site in the following order: Bonavista, Random Island, Bauline Line, and Come By Chance.

For the four sites, this increasing trend is most notably observed in the metals that are known to be associated with anthropogenic pollution. V, Fe, Co, Ni, Cu, and Bi display this trend; each of these metals is an anthropogenic pollutant (Pacyna, 1986). Other elements also display a trend similar to the increasing trend, although the sites do not follow the exact order of increasing concentrations mentioned above. These elements with data that are similar to the increasing trend are: Ti, Cr, Zn, Mo, Sb, La, and Ce (refer to Figure 3.19). Other researchers have made similar findings. Tuba and Csintalan (1993) applied the technique of transplantation and measured elemental accumulation in the lichen *Cladonia convoluta* along a busy road south of Budapest, Hungary. They used a bomb digestion of HNO_3 and H_2O_2 with ICP-AES analysis and found that the contamination levels of Cu, Cr, Fe, Ni, and Al rapidly decreased with distance from the pollution source (the road).

It was anticipated that pollutants associated with petroleum refining (V, Zn, Cd, Cr, Cu, and Ni) would increase from Bonavista to Random Island to Come By Chance. For the three sites in this transect, V, Cu, and Ni follow the increasing trend, while Zn and Cr are similar to the increasing trend, and Cd shows no clear trend. This suggests that V, Cu, Ni, Zn, and Cr from Come By Chance are being transported away from the source in a northeast direction. Further sampling and analysis from more sites need to be carried out in order to determine if this is the case.

Table 3.3 compares some data from this study with data reported by Seaward et al. (1978) who analyzed the lichen *Cladonia furcata* from sites in England and Ireland. Seaward et al. used a digestion of nitric and perchloric acids with analysis by atomic absorption spectrophotometry. These researchers collected samples from amongst heather on an exposed hill top in County Wexford, Ireland; this site was considered by the authors to have background concentrations. They collected samples from a lowland heath in North Lincolnshire, England; this site was also considered to have background concentrations. They also collected lichen from spoil heaps at a disused lead mine in Yorkshire, England; this site was considered to have enhanced concentrations. In Nieboer and Richardson's (1981) examination of the data of Seaward et al. (1978), they state that the sample from the lowland heath is near a steelworks and may have contamination from air pollution, while the sample from Ireland is from a rural area and is considered a control sample with no known sources of pollution. When comparing the data of Seaward et al. with the data of this work, it is clear that the data of Seaward et al. have generally much higher concentration levels. For Cr, Fe, Ni, and Cu, the data of Seaward et al. are approximately 1-2 orders of magnitude more than that of this work. However, Mn and Zn are of the same of order of magnitude for both studies. For Cr, Mn, Fe, Ni, and Cu, the concentrations of the most polluted site of this work are all lower than the concentrations of the site in Ireland which was considered to have background concentration levels. For Fe it is understandable that the concentration from this research is lower since the partial digestion may not have dissolved all the iron from the samples. For Zn, the concentration of this study is approximately 2 ppm more at Come By Chance than the concentration reported by Seaward et al. at the Ireland site. If the *Cladonia furcata* and *Alectoria sarmentosa* yield comparable elemental concentrations, the comparison

between this study and that of Seaward et al. may suggest that eastern Newfoundland is generally less polluted than areas of England and Ireland. The work of Seaward et al. (1978) agrees with this study in that concentrations differ for different sites. The data of Seaward et al. also agree with this study in that both sets of data generally have lower concentrations of Cr, Fe, Ni, Cu, and Zn in the less polluted sites (Bonavista, Random Island, County Wexford) and higher concentrations in the more polluted sites (Bauline Line, Come By Chance, spoil heaps, lowland heath).

Table 3.3: a. Data from Seaward et al. (1978). The data (ppm) are for the lichen *Cladonia furcata* from sites in England and Ireland. The authors consider the spoil heaps site to have enhanced concentrations, while the other two sites are background concentrations. b. Data from this research. The data (ppm) are for the lichen *Alectoria sarmentosa* from sites in Newfoundland, Canada.

a.

Site Number And Description	Cr	Mn	Fe	Ni	Cu	Zn
2A. Spoil heaps, disused Pb mine, Yorkshire, England	3.7	13.0	651	3.3	12.5	50.3
5A. Lowland heath, N. Lincolnshire, England	5.3	45.6	6453	4.9	9.3	93.6
11. Amongst heather on exposed hill top, Co. Wexford, Ireland	2.2	33.9	652	2.1	5.6	35.0

b.

Site	Cr	Mn	Fe	Ni	Cu	Zn
Bonavista Mean (relatively pristine)	0.102	94	9.9	0.20	0.73	23
Random Island Mean (intermediate)	0.046	39	13.2	0.29	0.86	21
Bauline Line Mean (somewhat polluted)	0.261	82	14.0	0.55	0.90	33
Come By Chance Mean (relatively polluted)	0.152	31	19.3	1.22	0.98	37

Nygard and Harju (1983) studied V in the lichen *Hypogymnia physodes* at more than twenty-five sites surrounding a power plant in Finland. These authors used hydrochloric and nitric acids in a PTFE autoclave bomb with subsequent analysis by a DC plasma emission spectrometer. The power plant used heavy fuel oil with a vanadium content of 70 ppm in the sample that the authors analyzed (the content could have been quite variable). The findings of these authors generally are in agreement with the findings of this study. Nygard and Harju found the highest concentrations, up to 57 ppm, to be within 1 km of the power plant, while the lower concentrations were further away from the plant, at 50 km away the concentrations were less than 2 ppm (approximately background levels). For this research, the mean V data were as follows: 2.71 ppm in Come By Chance, 0.35 ppm in Bauline Line, 0.32 ppm in Random Island, and 0.08 ppm in Bonavista. The transect away from Come By Chance (mentioned above) shows concentrations that decrease with distance from the pollution source, similar to the observations made by Nygard and Harju. The values for the Newfoundland sites are generally much lower than those reported by Nygard and Harju, even the more polluted Come By Chance site is only about 1 ppm more than the background levels in the area of Finland studied by Nygard and Harju.

Table 3.4 includes data from this present study as well as data from Bennett and Wetmore (1999) for comparison purposes. Bennett and Wetmore analyzed the epiphytic lichen *Bryoria fremontii* from Yellowstone National Park in Wyoming, U.S.A. These authors used an acid digestion with ICP analysis. The area they selected to study contained many geothermal features such as geysers, fumaroles, vents, and springs. They found that levels of most elements were comparable to levels in other national parks and wilderness

areas in the region. Many of the elements have higher concentrations in Bennett and Wetmore's data than in the data for any of the sites of this research. These elements for which Bennett and Wetmore's data are highest are: P, Ti, Cr, Fe, Co, Cu, Mo, and Cd. There are only two elements for which there is a lower concentration at Yellowstone than at Bonavista: Mg and Mn. Unlike the comparison made with the data of Seaward et al. (1978), the concentrations of Bennett and Wetmore are closer to those of this study. The following elements have the same orders of magnitude for both studies: Mg, P, Ca, V, Cr, Mn, Ni, Zn, and Mo. The remaining elements have a difference of only approximately one order of magnitude: Ti, Fe, Co, Cu, and Cd. Differences between the two sets of data could be due to the geothermal features in Yellowstone National Park, or any of the reasons mentioned previously during the comparison with the Seaward et al. (1978) data. Bennett and Wetmore found that some elements were significantly higher in the southern regions of the park, while other elements were significantly higher in the northern regions. This concentration difference between sampling areas is in agreement with the findings of this study.

Table 3.4: Data (ppm) for comparison between this study and that of Bennett and Wetmore (1999). The first four rows of data are the means of *Alectoria sarmentosa* samples from this research, while the last row is the mean concentrations reported by Bennett and Wetmore for samples of *Bryoria fremontii* (an epiphytic lichen) collected in Yellowstone National Park, Wyoming, U.S.A.

Site	Mg	P	Ca	Ti	V	Cr	Mn
Bonavista	681	398	1033	0.79	0.08	0.10	94
Random Island	322	173	682	1.01	0.32	0.05	39
Bauline Line	290	216	1811	1.68	0.35	0.26	82
Come By Chance	203	202	886	1.33	2.71	0.15	31
Yellowstone	441	937	1585	12.04	0.29	0.59	52

Site	Fe	Co	Ni	Cu	Zn	Mo	Cd
Bonavista	9.9	0.019	0.20	0.73	23	0.043	0.040
Random Island	13.2	0.022	0.29	0.86	21	0.059	0.033
Bauline Line	14.0	0.042	0.55	0.90	33	0.073	0.046
Come By Chance	19.3	0.048	1.22	0.98	37	0.068	0.041
Yellowstone	168.3	0.14	0.94	2.09	24	0.09	0.23

3.4.3.2 Decreasing Trend

Magnesium is the only element whose concentration decreases from Bonavista, to Random Island, to Bauline Line, to Come By Chance. However, there are several elements which tend to be higher in the less polluted sites: Li, Si, P, Mn, and Sr. The decreasing trend may be indicative of the influence of other sources, such as the ocean. Magnesium is one of the major constituents of seawater (Nozaki, 2001). Lithium, silicon, phosphorus, manganese, and strontium are also present in seawater, but to a much lesser degree (Nozaki, 2001). It is possible that some of these elements could be present at the lichen collection

sites due, in part, to seaspray. This may be the case for strontium and silicon particularly since they are more abundant in seawater than lithium, phosphorus, or manganese (Nozaki, 2001). Thus, it is possible that each of the sites studied is influenced by a combination of elemental sources, including both natural (e.g. oceanic) and anthropogenic sources. Perhaps the Random Island and Bonavista sites are dominantly influenced by oceanic sources. Seaward et al. (1978) had findings similar to that of this research for Mn. As mentioned previously, these authors used acid digestion with AAS analysis. The Mn data of Seaward et al. given in Table 3.3 are lowest for the spoil heaps site (considered to have enhanced concentrations) and higher for the two sites that were considered to have background concentrations. Perhaps this is related to the oceanic influence on these sites in England and Ireland; the sites of Seaward et al. and those of this study are all located on islands surrounded by the Atlantic Ocean. The work of Evans (1996) supports the interpretation of Mg originating from a marine source. Evans studied trace metals in rain samples collected in the town of Bonavista, Newfoundland. The trends noted by Evans indicated that Mg had a marine origin.

3.4.3.3 Other

Elements such as Be, Ca, Rb, Cd, Sn, Cs, Ba, Tl, and U, neither increase nor decrease consistently away from the two major sources described above (i.e. anthropogenic and natural sources). It is possible that these elements have more than one source and thus represent a mixture of influences. Perhaps some of these elements have both anthropogenic and natural (e.g. oceanic and/or geological) sources. Calcium is one of the major constituents of seawater and perhaps a significant portion of the Ca in the lichen originated

from a marine source (Nozaki, 2001). It is possible that with more data, some of these elements may display one of the trends described above.

Evans (1996) examined sulphur isotopes and sulphur concentrations in lichen (*Alectoria sarmentosa*) from Come By Chance, Random Island, and Bonavista. As well, this author determined trace elements in rain samples collected at Bonavista. Evans found that the Bonavista rain samples contained elements of an anthropogenic or continental origin (Cd, Ni, Cu, Mn, V, and for some samples, Pb and Zn), while Mg had a marine origin. Figure 3.20 shows the volume-weighted mean for Evans' Bonavista rain data plotted with the Bonavista lichen data of this current study, indicating that the rain and the lichen samples have a similar elemental pattern. The same work by Evans used sulphur isotopes to show that Random Island could be influenced by a mixture of anthropogenic and marine sources. Although Evans primarily studied sulphur, trace metal pollution can be associated with sulphur pollution. Since Evans suggested that Bonavista, Random Island, and Come By Chance could each be influenced by more than one source of trace elements, this may explain why some elements of this current work display no clear trend in their data.

Table 3.5: Grouping of elements according to the general trend for the sites. The increasing trend is the concentrations increasing from the least polluted site to the most polluted site. The decreasing trend is the concentrations decreasing from the least polluted site to the most polluted site.

Elements with data displaying increasing trend	Elements with data similar to increasing trend	Element with data displaying decreasing trend	Elements with data similar to decreasing trend	Elements with data that are neither the increasing nor the decreasing trend
V	Ti	Mg	Li	Be
Fe	Cr		Si	Ca
Co	Zn		P	Rb
Ni	Mo		Mn	Cd
Cu	Sb		Sr	Sn
Bi	La			Cs
	Ce			Ba
				Tl
				U

3.4.4 Distinguishing Between Sites

Elements showing concentration differences (as indicated by the t-tests) have the potential to be useful for distinguishing between sites. If an element was below the detection limit for the two sites compared, then that element could not be reliably used to distinguish between those sites. However, if an element was below the detection limit for only one of the two sites, then there could be a suitable difference in concentration such that the element in question could still be used to distinguish those sites. Table 3.6 lists the elements which are useful to distinguish between sites, with the elements removed which are at or near the

detection limit. Compilation of the list of all elements which are potentially useful for distinguishing between two or more of the sampling sites yields nineteen useful elements. These elements are: Ca, Sr, V, Cs, Mo, Mg, Rb, La, Ti, Co, Ni, Ce, Bi, Zn, Cu, Mn, P, Cd, and Sn. Figures 3.21 and 3.22 display x-y plots of some of these elements. Figure 3.21 shows Ni versus Cu; these are elements which suggest the increasing trend discussed previously. Figure 3.22 shows elements which suggest the decreasing trend. The data of this research indicate that the following nine elements are not useful in distinguishing between any of the sampling sites: Fe, Si, Be, Sb, Ba, Cr, Li, Tl, and U. There is a group of elements that are useful in distinguishing between the sites in several (or more) of the pairwise comparisons; the twelve elements in this group are: Ca, Sr, V, Cs, Mg, Rb, La, Co, Ni, Ce, Mo, and Zn. It is these elements that have very good potential to distinguish between sites of varying pollution exposure. Table 3.7 lists the number of comparisons for which each element is useful.

Table 3.6: Elements which are useful to distinguish sites using pairwise comparisons. The elements have been removed which have some samples at or near the detection limit (Tl, Li, and U were removed, as well as Bi from the Bonavista and Bauline Line comparison).

Elements with differences between Bonavista and Random Island	Elements with differences between Bonavista and Bauline Line	Elements with differences between Bonavista and Come By Chance	Elements with differences between Random Island and Bauline Line	Elements with differences between Random Island and Come By Chance	Elements with differences between Bauline Line and Come By Chance
Ca	La	Ni	Ca	Ni	Ni
Sr	Ca	Sr	Cs	Co	Mn
V	Sr	Cs	Mn	Mg	Cs
Cs	V	Ce	Sr	V	Mg
Mo	Mo	Mo	Zn	Ce	Sr
Mg	Ti	Mg	Rb	La	Rb
Rb	Co	V	La	Zn	V
	Ni	Bi	Co	Rb	Ca
	Mg	La	P		Sn
	Cs	Co	Cd		
	Ce	Zn	Ti		
		Cu	Ce		
		Rb	Ni		

Table 3.7: The useful elements from Table 3.6 above and the corresponding number of pairwise comparisons for which that element is useful (arranged in order of increasing number of comparisons for which the element is useful).

Useful Elements	Number of pairwise comparisons in which the element is useful for distinguishing between the two sites
Cu	1
Sn	1
Cd	1
P	1
Bi	1
Mn	2
Ti	2
Mo	3
Zn	3
Co	4
La	4
Ce	4
Ca	4
Ni	5
Rb	5
Mg	5
Cs	5
V	5
Sr	5

In order to reduce the list of twelve elements with very good potential to distinguish sites (as given in the latter part of the previous paragraph), a comparison of this list was made with Table 3.2. Based on this comparison, Ni and Mo were removed from the list as they did not have optimal data for the certified reference materials (even though these elements were not eliminated from the data set completely as they could still potentially yield worthwhile information). As well, La was removed from the list as there were no La values given in either of the CRMs used in this study, so an evaluation of the accuracy could not be made. This then produced a list of nine elements which have the greatest potential for utilization for distinguishing between sites of varying pollution exposure, using the developed method: Mg, Ca, V, Co, Zn, Rb, Sr, Cs, and Ce. These elements are plotted as bar graphs with a logarithmic scale in Figure 3.23. In order to show in more detail, these nine elements are also shown as bar graphs with linear scales in Figures 3.24, 3.25, 3.26, and 3.27.

Although all of the nine elements mentioned in the previous paragraph are useful to distinguish between the sites, two elements were selected which, when used in conjunction with one another, could be used to distinguish between all four sites. These elements were vanadium and zinc. Vanadium is very useful in distinguishing between the sites since: it is useful in five of the six comparisons between the sites, the p-values in these five comparisons are quite low (0.01 or less), V is well above the detection limit for all the samples, V was one of the elements with very good accuracy and precision (refer to previous Section 3.3), and V is an element known to be an anthropogenic pollutant (Seaward and Richardson, 1990). Similarly, zinc is very useful in distinguishing between the sites since: it is a useful indicator element in three of the six comparisons (one of which is the comparison for which V is not

useful), the p-values in these three comparisons are quite low (0.026 or less), Zn is well above the detection limit for all samples, Zn is one of the elements with very good accuracy and precision (refer to previous Section 3.3), and Zn is known to be an anthropogenic pollutant (Seaward and Richardson, 1990). Figure 3.28 shows each site plotted as a function of its mean Zn and V concentrations. If additional samples were collected from these sites in the future, their V and Zn concentrations should be near the respective values on this graph. However, if pollution levels at a site change significantly over time, the new samples may plot further away from the samples of this study. Further sampling and analyses for these sites, as well as other sites, is needed to confirm the findings of this work.

3.5 Comparison Of Species

3.5.1 Introduction

One of the goals of this research was to apply the digestion method to different lichen species to determine whether or not different species from the same sampling site have the same elemental concentrations. This is of interest to know whether or not direct comparisons of data from different species can be made. If data of different species are directly comparable, then data could be obtained from other species in areas where the species of choice is not present. This research question may also be of interest if a study was planned to investigate the efficiencies of different lichen species in retaining trace elements.

The species selected for this comparison were: *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*. These three species were collected from Bauline Line in 1997. Details of the sampling site are given in Appendix III. The samples for these species will be referred to as: Alectoria Bauline 97, Bryoria Bauline 97, and Cladonia Bauline 97. For the

explanation of the elements included in the comparison of species, refer to Section 3.4.2 of the sites comparison.

3.5.2 Statistical Analysis For Species Comparison

The statistical package Minitab was utilized for the species comparisons between *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*. The t-test was used to perform pairwise comparisons between the species. These comparisons were done in a manner similar to the sites comparisons outlined previously. A 95 % confidence interval was used for the t-tests. The p-values for these pairwise comparisons are given in tables in Appendix VII. The data for the species comparisons are from the Waters 125 ICP-MS Run. Since there were only three or four replicates (i.e. samples of the same type) for each species, it was not possible to determine with any degree of certainty whether or not the populations of each species were normal, so an assumption of normality was made.

Prior to use of the t-test, the F-test was performed to determine whether or not the populations of the three species had equal variances for each element. For those elements with equal variances (as was the case for the majority of the elements), the t-tests were performed using the option to assume equal variances. For those elements with unequal variances, the option to assume equal variances was not used. The F-test was performed using a 95 % confidence interval.

3.5.3 Comparison Of Lichen Species

The relative elemental concentrations of the three lichen species can be seen in Figure 3.29. From this figure, it can be seen that the general elemental character of the three lichen

species is similar. This is not surprising as they are similar organisms collected from the same area at the same time. The differences in concentrations observed for each species could be a result of such factors as: differences in the ion-uptake capacity, differences in particulate trapping efficiency due to the surface morphology, and differences in the exposure to dry and wet deposition and wind (Nieboer and Richardson, 1981). This latter factor may be particularly important in the differences between *Cladonia alpestris* and the two tree-dwelling lichens.

From the graph of Figure 3.29, along with the t-test results, it is clear that there are certain elements which show concentration differences for different species (e.g. Cs), whereas other elements are present at similar concentrations in the three species (e.g. Cr). For many elements, *Bryoria sp.* has the highest concentrations of the three species (e.g. Mg, P, V, Co, Ni, Cu, Zn). For some elements, *Alectoria sarmentosa* has higher concentrations than *Cladonia alpestris* (e.g. Ca, Co, Mo, Sb), for other elements the opposite is true (e.g. Ti, Rb, Cs, Ba, Ce).

From this work, it is apparent that one species of lichen could not be readily substituted for another when sampling for the purpose of determining elemental concentrations. It may be possible that a general concentration trend in one species may suggest a similar trend in another species; for example, if Cu is found to be elevated above background in *Bryoria sp.*, then perhaps it will also be elevated in *Cladonia alpestris*. Further analyses would have to be done to establish such relationships.

This research found that different species sampled from the same site differ in concentration for some elements while other elements can have similar concentrations. This is in general agreement with other studies found in the literature. Nieboer et al. (1972) and

Folkeson (1978) both had similar findings, as discussed below.

Nieboer et al. (1972) analyzed different species of lichens around a nickel smelter in Sudbury, Ontario. They reported concentrations of metals for four *Cladonia* species 30 miles from the Copper Cliff Smelter. One of these species, *Cladonia alpestris*, is the same one utilized in this study. Table 3.8 gives their data for Cu, Ni, Zn, and Fe. It is apparent that even within the same genus some elements such as Ni can have similar concentrations over all four species, yet other elements, such as Cu can have a greater range of concentrations. The data of Nieboer et al. (1972) can be compared with data from this present research, as given in Table 3.9. For Cu, Ni, and Fe the concentrations of Nieboer et al. are 1-3 orders of magnitude greater than those of this work; this is not surprising since Nieboer et al. collected samples in an area near a nickel smelter whereas the three species for this work were collected outside a small city where there are no major sources of industrial air pollutants. However, the Zn of both studies are somewhat similar in concentration; there is no evident explanation for this similarity.

Table 3.8: Heavy metal contents of *Cladonia* species as reported by Nieboer et al. (1972), expressed as ppm. Samples were collected 30 miles from the Copper Cliff Smelter.

Species	Cu	Ni	Zn	Fe
C. deformis	87	109	36	1316
C. mitis	183	112	28	1489
C. alpestris	95	113	27	1615
C. rangiferina	56	101	23	1456

Table 3.9: Mean concentrations in ppm for selected elements for the three species of this study (from Bauline Line).

Species	Cu	Ni	Zn	Fe	Cd
Alectoria sarmentosa	0.90	0.71	28	38	0.031
Bryoria sp.	2.46	1.56	48	99	0.032
Cladonia alpestris	1.10	0.60	11	46	0.023

Folkeson (1978) studied metals in five mosses and four lichens around a brass foundry in Sweden in order to compare the species and explore the possibility of calibration between species. The lichen data for the mean concentrations of Fe, Cu, Zn, Ni, and Cd from that study are presented in Table 3.10. Folkeson observed that the *Cladonia* in his study accumulated less Cu and Zn than the other species analyzed. Similarly, in this present study, *Cladonia alpestris* accumulated less Zn than the other two species, however, the Cu concentration in *Alectoria sarmentosa* was slightly lower than in *Cladonia alpestris*. This could be the result of a variety of factors including: differences in metal uptake, differences in growth rate, and differences in climate conditions such as environmental exposure, humidity, wind, and deposition (Folkeson, 1978; Nieboer and Richardson, 1981). This higher Cu in *C. alpestris* is not altogether surprising after examination of the data in Table 3.8 of Nieboer et al., since *Cladonia alpestris* tends to have concentrations that are higher than those of *Cladonia rangiferina*. Folkeson found that different species from the same

sites can differ in metal concentrations; this is in agreement with the results of this research using *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*. His data also show that different species can have the same concentrations for some elements, for example, Cd in *Pseudevernia furfuracea* and *Usnea filipendula*. Folkeson (1978) also calculated calibration factors between the species studied so that if a desired species was not present at a sampling site an alternate species could be collected. He notes that the calibration factors may change depending upon the conditions (sample substrate, sample preparation, and concentration intervals). The concentrations for this study reported in Table 3.9 are generally lower than those found by Folkeson, which is not surprising as his sites were near a prominent source of metal pollution.

Table 3.10: Mean concentrations in ppm for selected elements for the four species of lichen in the study by Folkeson (1978).

Species	Cu	Ni	Zn	Fe	Cd
Cladonia rangiferina	14.5	1.5	102	442	0.5
Hypogymnia physodes	28.2	2.6	232	832	1.1
Pseudevernia furfuracea	35.0	2.8	237	926	0.6
Usnea filipendula	22.4	2.6	182	614	0.6

3.6 Comparison Of Alectoria Bauline Line 1996 Samples Analyzed Separately

A comparison was made between the samples of the lichen *Alectoria sarmentosa* from Bauline Line (1996) which were analyzed in the Waters 120 Run, the Waters 125 Run, and the Waters 903 Run (different ICP-MS instruments). Ideally, since these samples originated from the same site and were collected at the same time, there should be no significant differences between the samples.

A statistical analysis was carried out in a manner similar to that discussed previously for the species comparisons. Minitab t-tests were used to perform pairwise comparisons between the three sets of samples. Prior to the t-tests, F-tests were performed to determine whether or not the data displayed equal variances. Both of these statistical tests were done using a 95 % confidence interval. The p-values from the t-tests are reported in tables in Appendix VII. From these tables, it can be seen that there are elements which show some differences between the ICP-MS Runs.

The average concentrations for each of the three sets of Alectoria Bauline Line 1996 samples are plotted in Figure 3.10. From this line graph, it can be seen that the general elemental character for each of the three ICP-MS Runs is similar.

Table 3.11: Elements that show differences for the Alectoria Bauline Line 1996 samples in each of the pairwise comparisons (compiled from the tables of p-values in Appendix VII).

Comparison of Waters 120 and 125 Runs	Comparison of Waters 120 and 903 Runs	Comparison of Waters 125 and 903 Runs
Ce	Be	Sn
Sn	Ce	Ce
La	V	La
Ca	Cr	Ti
Be	Si	Co
Mg	P	
Mo	Li	
Mn	Co	
Co	Sn	
	Ca	

From the examination of the p-values as well as the graph in Figure 3.10, it is shown that there are some elements which have differences for the samples of Alectoria Bauline 1996 that were analyzed in the Waters 120, 125, and 903 Runs. These elements are given in Table 3.11. The compiled list of elements that show differences in one or more of the comparisons is as follows: Ce, Sn, La, Ca, Be, Mg, Mo, Mn, Co, V, Cr, Si, P, Li, and Ti. However, not all of these elements are necessarily a cause for concern. Li, Be, Si, Ti, Sn, and La have no certified values in either of the CRMs utilized in this study, so it cannot be determined whether or not these elements can yield good data with the procedure used in this study. Mo had precision which was bordering on poor, as discussed earlier in this chapter. This leaves a smaller list of elements which show differences for the data of this study.

Potential reasons for these differences are explored below.

The differences between the three ICP-MS Runs could potentially be explained by one of more different factors. Sample storage, homogeneity, and/or contamination prior to or during digestion may be some of these factors. These are discussed in the following paragraphs.

Another possibility is that the lichen samples were affected by the length of time and/or conditions of storage. Since the Alectoria Bauline Line samples in the later ICP-MS Runs were in storage longer than the earlier Runs, the samples did have differing storage times. The details of the conditions of storage are given in Chapter 2. The potential for contamination to occur during storage is estimated to be minimal. Since the samples of Alectoria Bauline Line 1996 lichen for this study were cleaned and crushed separately for each run, cleaning and crushing all of this lichen type at once for all three ICP-MS Runs would be preferable.

It is possible that the powdered lichen samples were not homogeneous. This may be due to the lichen sample not being adequately crushed to a fine powder. Homogeneity may be improved if all samples had been sieved; only the samples which were puck mill crushed were sieved for this study (as described in Chapter 2). However this particular type of sieve is difficult to clean thoroughly and tiny fragments of powdered lichen can remain in crevices even after cleaning. The containers with the crushed lichen were rolled manually before the individual one gram samples were weighed into test tubes; perhaps the samples could be stored in cleaned glass bottles and then rolled on the mechanical roller for approximately two minutes as was done for the lichen certified reference materials. If glass bottles were to be used, plastic caps might be preferable, or perhaps parafilm could be used to prevent a metal

cap from directly contacting the bottle and sample. When a sample was weighed into a test tube, static electricity was often a problem; perhaps static electricity causes particles of a certain size, shape, or composition to be preferentially attracted to or repelled from the spatula and thus included or excluded from a sample. A static gun was used to help reduce static electricity, but this was not adequate. The degree to which static electricity was a problem varied from day to day due to humidity, etc. Solving the problem of static electricity effects during sample weighing and transfer into test tubes would make this part of the procedure less difficult and could also reduce the risk of inhomogeneous samples. Perhaps a static mat on the floor would help to remedy this problem. These homogeneity issues seem to be the most likely explanation for the observed differences. Cleaning and crushing all the Alectoria Bauline Line 1996 lichen required for the three ICP-MS Runs at once (as mentioned in the previous paragraph) would reduce the possibility of having varying sample homogeneity between ICP-MS Runs.

Another possible explanation for the differences is contamination during the sample pre-treatment prior to digestion and/or during digestion itself. Although a conscientious effort was made to avoid contamination, it is possible that some particles of dust, dirt, or an insect settled on the samples while they were exposed to the air. This exposure to contamination could have occurred during the cleaning, crushing, weighing, dry ashing, or wet ashing. Several steps could be undertaken to reduce the potential for this type of contamination in the future. Another type of oven/furnace could be used for the dry ashing; this oven should be easily cleaned and should be dedicated to this type of work only. Perhaps a different method could be used to shield the samples during the wet ashing in the fumehood; these shields could reduce the air movement over the test tubes, but still allow

the vapours to escape readily and safely to the fumehood. Perhaps a shield over the samples during cleaning and crushing could also reduce the risk of particles falling onto the samples. Perhaps the caps used during the dry ashing could be used (or modified to be used) during the wet ashing; the caps would have to facilitate the escape of any gases evolved during the wet ashing. The test tube blocks used could be new, not corroded, sized appropriately to the test tubes, and each block should be made of the same composition (explore alternatives, perhaps a Teflon-coated metal block would be appropriate). Ensure that the test tube blocks adequately space the tubes apart such that the reagents can be added with a minimum of passing the pipet or one's hand over the adjacent tubes (the caps during the wet ashing would be helpful for this). Cleaning and crushing all the Alectoria Bauline Line 1996 necessary for all three ICP-MS Runs at once would likely reduce the variability of contamination between ICP-MS Runs.

It bears mentioning again that a conscientious effort was made to avoid contamination throughout the sample collection, storage, cleaning, crushing, digestion, and submission for ICP-MS analysis. One would hope that the above potential sources of error would be insignificant. More samples should be collected and analyzed to determine whether the inconsistencies noted in the t-tests are confirmed.

3.7 Comparison Of The Alectoria Bauline Line 1996 And 1997 Samples

A comparison was made between the Alectoria Bauline Line 1996 samples and the Alectoria Bauline Line 1997 samples (both from the Waters 125 Run). Based upon the differences noted in Section 3.6 between the Alectoria Bauline Line 1996 samples from the Waters 120, 125, and 903 Runs, a decision was made to use only the Alectoria Bauline Line

1996 samples from the Waters 125 Run to compare with the Alectoria Bauline Line 1997 samples. This decision was made in order to reduce any potential effects caused by the differences noted between the ICP-MS Runs in Section 3.6.

A statistical analysis was carried out in a similar manner as described previously for the species comparisons. Minitab was used to preform F-tests and t-tests to compare the two sample types. These tests were carried out at a 95 % confidence level. The p-values from the t-tests are tabulated in Appendix VII.

The *Alectoria sarmentosa* samples used to examine temporal difference were collected one year apart from the same location. The time frame of this study (ie. one year) has been found by other researchers to be sufficient for observation of potential concentration changes (Loppi et al., 1998; Wiseman, 1999). Figure 3.30 shows the average concentrations for both the Alectoria Bauline Line 1996 and 1997 samples. This graph shows that the characters of both the 1996 and 1997 samples are similar. However, there are some elements which show differences between these two samples, as is indicated by the p-values.

From the p-values, the following twelve elements show differences between the 1996 and 1997 samples of *Alectoria sarmentosa* from Bauline Line: Ce, Ca, P, Mn, Mg, Sr, V, Mo, Ba, La, Zn, and Sb. (Since Be was below the detection limit for both the 1996 and 1997 samples, it will not be considered an element which shows differences between the samples even though it had a p-value of less than 0.05.) Of these elements, P, Mg, V, Mo, and Sb were higher in the 1997 samples, whereas Ca, Mn, Zn, Sr, Ba, La, and Ce were higher in the 1996 samples. Elements from the list of twelve which are also elements that displayed differences for the Alectoria 1996 Bauline Line samples between the three ICP-MS Runs (Section 3.6) may be elements with a problem such as inhomogeneity and/or contamination

(e.g. Ce, Ca, La). Elements from this list which did not display differences for the Alectoria 1996 Bauline Line samples between the three ICP-MS Runs are likely to be elements that have differences due to the year of sample collection. Elements which are likely to display differences due to year of collection include Sr, Ba, Zn, and Sb. It is also a possibility that some elements may show differences due to both inhomogeneity/contamination and year of collection. Further work with more sample analysis would have to be carried out to confirm the findings of this study and to determine with more certainty the causes of the observed differences.

Differences between samples collected in different years could be due to a number of influencing factors. The differences could be due to an increase or decrease in pollution which originates from sources at distance from the sampling site. Some elements such as V, Ti, and Ni which are commonly associated with anthropogenic pollution (Byerrum, 1991; Whitehead, 1991; Sunderman and Oskarsson, 1991) have higher concentrations in the 1997 samples, which may suggest an increase in pollution. Other possible causes of differences from year to year are changes in local activities that produce pollution such as: changes in the local traffic patterns, changes in the heating fuels used by the nearby households, changes in the nearby peat excavation, and changes in utilization of the area by hikers, animals, etc. It is also possible that some changes occurred which increased concentrations while others occurred which decreased concentrations such that the effects cancelled each other out and the data of this study show only the net difference.

Since the comparison of lichens from the same area collected at different times comprised only a minor part of this study, there is much more work that could be done in analyzing lichens over an extended period of time. A study could be designed which

examines changes in lichen concentrations over extended periods of time in the same location (i.e. sampling annually). A study such as this would ideally analyze one species of lichen from the same group of trees at regular time intervals, and under the same weather conditions (e.g. one day after a rainfall). This type of study could be conducted using each of *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris* (as well as other species).

CHAPTER 4: SUMMARY AND CONCLUSIONS

4.1 Summary

4.1.1 Introduction, Objectives, And Methods

This study was undertaken to develop and refine a partial digestion procedure for lichen digestion which could be used for ICP-MS analysis of trace elements. This procedure was then applied to lichen samples collected from sites of varying degrees of environmental exposure to pollution in order to determine whether or not these sites could be distinguished by the trace element data. This procedure was also applied to analyze different lichen species from the same location in order to determine whether different species were equivalent in their geochemical representation of the site. The lichen species of interest for this study were *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*. Three certified reference materials (CRMs) were used in this study: IAEA-336 Lichen CRM, BCR Lichen CRM 482, and NIST Peach Leaves CRM 1547. Two different methods of crushing were used: crushing with liquid nitrogen in a mortar and pestle, and crushing in a puck mill. Also, samples of *Alectoria sarmentosa* were collected from the same area approximately one year apart and the trace element data were compared. In addition, analysis of *Alectoria sarmentosa* collected from one sampling site was repeated in each of the three separate sets of samples analyzed by ICP-MS.

4.1.2 Evaluation Of Data Quality

The ICP-MS data were evaluated and a suite of seventeen elements was determined to have acceptable data. This element suite is comprised of: Mg, P, Ca, Mn, Co, Zn, Sr, Ba, V, Cr, Fe, Cu, Rb, Cd, Sb, Cs, and Ce. Nine elements were not certified in either of the CRMs utilized (thus accuracy could not be evaluated), but their data did not show any reason for them to be eliminated from the group of useful elements: Li, Be, Si, Ti, Sn, La, Tl, Bi, and U. Three elements had data which could be deemed unsuitable (but were retained in the group of useful elements): S, Ni, and Mo. This yields a potential suite of twenty-nine elements for which useful data were obtained.

4.1.3 Comparison Of Sites

The application of the developed method to lichens from sites of varying pollution levels yielded the result that the sites could be distinguished by their trace element content. Samples from four sites were analyzed: Come By Chance (relatively polluted), Bauline Line (somewhat polluted), Random Island (intermediate), and Bonavista (relatively pristine). Only the species *Alectoria sarmentosa* was used in these comparisons. The elemental character of each site was broadly similar in nature, however there were some elements which had quite variable concentrations from site to site. Some elements displayed a trend in which concentrations increased from the less polluted sites to the more polluted sites, for example V, Fe, Co, Ni, Cu, and Bi. This trend is likely due to changes in exposure to anthropogenic pollution. Magnesium displayed a trend in which elemental concentration decreased from the less polluted sites to the more polluted sites. This trend is likely due to the influence of seaspray. Other elements do not display patterns which fit either the

increasing or decreasing trend. These elements may originate from more than one source, with their concentrations a result of a mixture of influences. Nineteen elements were found to be useful for distinguishing between two or more of the sites: Ca, Sr, V, Cs, Mo, Mg, Rb, La, Ti, Co, Ni, Ce, Bi, Zn, Cu, Mn, P, Cd, and Sn. Of these elements, two of environmental interest were selected, V and Zn, which could distinguish between all four sites when used in conjunction with one another.

4.1.4 Comparison Of Species

Different lichen species (*Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*) collected from the same site (Bauline Line) displayed differences in their trace element content. For many elements, *Bryoria sp.* had the highest concentrations (for example Mg, P, V, Mn, Co, Ni, Cu, Zn, and Rb.) For a number of elements, *Alectoria sarmentosa* had higher concentrations than *Cladonia alpestris*, such as: Mg, Ca, Cr, Mn, Co, Ni, Zn, Sr, and Sb. While for other elements such as V, Cu, Rb, Cs, Ba, La, and Ce, the opposite was true. Elements which had concentration differences between species could potentially be used to distinguish between the species. Thirteen elements were found to be potentially useful in distinguishing all three of the species: P, Ti, Zn, Rb, Mo, Co, La, V, Cu, Mn, Mg, Cs, and Ce. Since there are elemental differences between species, care must be taken if a comparison is to be made between the data of two different species for environmental monitoring.

4.1.5 Comparison Of Alectoria Bauline Line 1996 From The Three ICP-MS Runs

Samples of *Alectoria sarmentosa* from Bauline Line (1996) were analyzed in three separate ICP-MS Runs. The general elemental concentration pattern for each of the three ICP-MS Runs was similar, but there were elements which displayed concentration differences. These are explained by one or more of: sample storage, homogeneity, and/or contamination prior to or during digestion. Sample homogeneity seems to be the most likely explanation for the observed differences. If the samples utilized for each of the three ICP-MS Runs had been collected, cleaned, crushed, and stored together as one large sample, and then analyzed in separate ICP-MS Runs, the inconsistencies might have been eliminated.

4.1.6 Comparison Of Alectoria Bauline Line 1996 And 1997 Samples

Samples of *Alectoria sarmentosa* collected at Bauline Line in 1996 and 1997 showed concentration differences for some elements. Elements that showed differences between these samples were: Ce, Ca, P, Mn, Mg, Sr, V, Mo, Ba, La, Zn, and Sb. Of these elements, P, Mg, V, Mo, and Sb were higher in 1997, while Ca, Mn, Zn, Sr, Ba, La, and Ce were higher in 1996. Some elements which showed differences between these samples also showed differences between the three ICP-MS Runs and this may be explained by inhomogeneity or contamination (e.g. Ce, Ca, La). Elements which did not show differences for the Alectoria Bauline Line 1996 samples between the three ICP-MS Runs were more likely to be elements that have differences due to year of sample collection; elements such as Sr, Ba, Zn, and Sb display differences that can be attributed to collection year. Some elements could have shown differences due to both inhomogeneity/contamination and collection year. Elements (e.g. Sb) which were observed to have higher concentrations in

the 1997 samples, suggested a pollution increase. Differences between samples collected in different years could be caused by a number of factors. The differences could be due to a change in pollution which originated from sources at distance. Another possible cause of year to year differences could be changes in local activities which produced pollutants, such as: local traffic, use of heating fuels, peat excavation activities, and use of area by hikers/animals. It is also possible that some changes occurred which increased concentrations, while others occurred which decreased concentrations, with the data showing only the net difference.

4.1.7 SEM-EDX Examination

An SEM-EDX analysis was carried out to characterize the undissolved particles remaining after digestion. The dominant type of residual particle is characterized by a high silicon content, with lower amounts of other elements (e.g. Al, K, Na, Mg, Ca, Fe, Ti). This indicated that the majority of the particles were silicate minerals, likely quartz, feldspars, olivines, garnets, micas, and/or clay minerals. It is as yet unclear whether the residual particles existed on the lichen in the field, were produced by the lichen, and/or were produced or altered during digestion.

SEM-EDX examination of the surface of washed and unwashed strands of *Alectoria sarmentosa* from Come By Chance and Torbay did not yield any meaningful conclusions. Many types of particles were observed on the surface of the lichen strands. There were many granular particles/areas, rounded/spherical particles, plate-like particles (or crystals), and there were also many long thin particles. Other more irregular particles were observed. There were many particles which contained high Si, high Al, high Ca, and/or high Fe. Some

particles observed had relatively small quantities of K, Na, Ti, S, and/or Cl. It was expected that the washed lichen strands would not have surface particles and that certain elements would only be observed in more polluted sites, but no clear trends were observed. Some general comparisons could be made between the surface particles and the residual particles. In terms of morphology, both granular and long thin particles were observed on the lichen surface and in the residual particles. A future study could examine whether or not the origin of the long thin (lint-like) grains is related to the fungal hyphae.

4.2 Conclusions

A partial digestion procedure was successfully developed for lichens such that useful elemental concentrations could be determined using Inductively Coupled Plasma Mass Spectrometry. This procedure was desired for biomonitoring purposes. The procedure consisted of a series of dry and wet ashings and was demonstrated to provide acceptable data for the following suite of elements: Mg, P, Ca, Mn, Co, Zn, Sr, Ba, V, Cr, Fe, Cu, Rb, Cd, Sb, Cs, and Ce. Two methods of crushing were investigated, and it was determined that crushing with liquid nitrogen in an agate mortar and pestle yielded less contamination than crushing in a tungsten carbide puck mill. Research in the areas of sample digestion and analysis is dynamic and will require much attention in the future.

Using the developed procedure, it was found that sites with different levels of pollution could be distinguished by their trace element content. Elements which were useful in this regard included vanadium, zinc, and copper. Two trends were observed among these elements which were shown to be useful in distinguishing between sites. Elements displaying the first trend increased in concentration from the less polluted sites to the more

polluted sites; these were elements known to be anthropogenic pollutants. Elements displaying the second trend decreased in concentration from the less polluted sites to the more polluted sites; these elements were considered to be from natural sources, such as seaspray. This second trend may be indicative of proximity to natural sources, such as the ocean. Another question of interest in this study was whether or not the developed digestion procedure could be utilized to determine if different species have different concentrations of trace elements. It was concluded that different species do not have the same trace element concentrations. If inter-species comparisons are to be made, some method of calibration between species may be necessary.

A comparison was made between lichens collected a year apart. These lichens had different concentrations for some elements. These differences could be manifestations of changes in pollutants emitted from sources in the local area or at distance from the sampling site, and/or changes in local activities. Comparison between lichens which were collected together and then cleaned, crushed, and analyzed separately indicated that there were some concentration differences. These differences were attributed to inhomogeneity of the powdered samples.

In general, concentrations reported for lichens from areas outside of Newfoundland tend to be higher than those presented in this research. Newfoundland is generally less polluted than other areas, particularly areas of dense population and major industrial activity.

The success of this research indicates that a complete digestion is not always necessary. A partial digestion can provide useful data while avoiding some of the problems that a complete digestion can involve, such as the use of hydrofluoric acid in a procedure that yields solutions for ICP-MS analysis. The developed digestion procedure provides

acceptable data for many elements and has useful applications in environmental analysis.

4.3 Future Work

4.3.1 Pollution Monitoring With Lichens

There is substantial room for more work to be done using lichens as biomonitors in Newfoundland and Labrador as it has only been in recent years that much work has been carried out in this area. In terms of environmental monitoring, it would be useful to have metal concentration data from lichens for all areas of the province. It would also be useful to have background lichen concentrations before substantial new industrial activities are begun. Maps of lichen species coverage in Newfoundland and Labrador could be produced. Studies could then be carried out to monitor lichen coverage over time and to determine if more sensitive species are disappearing in polluted areas or if species are re-inhabiting areas where pollution levels have decreased. It would also be useful to produce maps of elemental concentrations in lichens for the province.

4.3.2 Sample Treatment Prior To Digestion And Digestion Itself

Sample preparation and digestion are wide and variable fields which will undoubtedly continue to develop and improve over time. Future work could be carried out in these areas. Work could be done to reduce the time required to clean laboratory equipment without compromising cleanliness. During the weighing of the crushed samples into test tubes, static electricity could be reduced. The main area which requires future work after this study is reducing the total time required for digestion. With this goal in mind, experiments could be designed to explore optimization of the digestion procedure (times in

furnace, test tube dimensions, temperatures, etc.). Digestion and analysis of a statistically significant number (i.e. >30) of samples of the same type should be done.

4.3.3 Residual Particle Characterization

Residual particle characterization is an interesting area and offers much opportunity for future work. Particularly, it would be useful to have an understanding of the exact mineralogy and origin of the residual particles. The type of SEM-EDX work done in this study has several challenges associated with it. It is not easy to determine the composition of very small or thin particles since elemental information is likely to also be acquired from the area surrounding the particles. Sometimes a particle may move substantially while an EDX graph is being acquired. Another challenge is that a coloured particle observed by dissection microscope can be difficult to locate by SEM-EDX because the image is not in colour. The clarity of the image can vary; at times even the ink "map" on the filter paper cannot be seen clearly. Using a more modern SEM-EDX or another type of instrument could alleviate some of these problems. Since the residual particles are not fixed into place, this reduces the potential number of instruments which may be utilized. A variable pressure SEM-EDX would have improved imaging capabilities and also would not require the sample to be carbon or gold-coated. Examination with a transmission electron microscope or a confocal microscope are other options that may also be worthwhile.

4.3.4 Surface Particle Characterization

Although the examination of lichen surface characteristics was not one of the main objectives of this research, this is an area which could be studied in the future. Potential

sources of alteration/contamination of the sample could be investigated and eliminated. Also, attention could be focused on each type of particle individually. This would involve determining the exact mineralogies and the origins of each particle type observed. Perhaps a future study could examine the internal portions of the lichen and characterize any particles observed. Future studies could also include *Bryoria sp.*, *Cladonia alpestris*, and other species. Surface particle examination with SEM-EDX faces some of the same challenges mentioned previously for the residual particle analysis, so this area may also benefit from another study with a newer instrument.

4.3.5 Further Applications

The sample preparation and digestion procedures used in this study have the potential to be utilized for different applications. The digestion procedure could potentially be adapted for other sample types such as: other lichen species, plants (including aquatic plants), foods, and animal tissues (including organisms which can be used as biomonitors, such as mussels). Some experimentation with the digestion of foods (powdered milk, flour, wheat germ, and parmesan cheese) was carried out in the past (Tucker, 1995). It would be useful to have a digestion procedure which is robust enough to dissolve many different sample types with little modification to the procedure. If the digestion procedure used in this study evolved into a more universal digestion procedure, it is possible that it may have applications outside of the field of environmental work. Perhaps there could be applications in the use of biological material for mineral exploration, or in trace elemental characterization of a number of materials, such as wood, paper, or leather.

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PLATES

Plate Captions

Plate 3.1: SEM photo of a smooth particle. It has the general appearance of many of the high silicon particles observed except that it is somewhat smaller than many of the observed particles. This particle is from the residue of an IAEA Lichen CRM digestion from the Waters 120 Run. The SEM-EDX spectra for this particle is Figure 3.1.

Plate 3.2: SEM photo of a white, granular particle. This particle has the typical appearance of the granular particles, however this is one of the larger examples observed. This particle is from residue of a *Bryoria sp.* digestion from the Waters 125 ICP-MS Run. The SEM-EDX spectra for this particle is Figure 3.2.

Plate 3.3: SEM photo of a typical clear, colourless, smooth, flat, vitreous particle. This particle is from residue of a *Bryoria sp.* digestion from the Waters 125 ICP-MS Run. The SEM-EDX spectra for this particle is Figure 3.3.

Plate 3.4: SEM photo of a typical long, thin particle. This particle is from residue of a *Bryoria sp.* digestion from the Waters 125 ICP-MS Run. The SEM-EDX spectra for this particle is Figure 3.4.

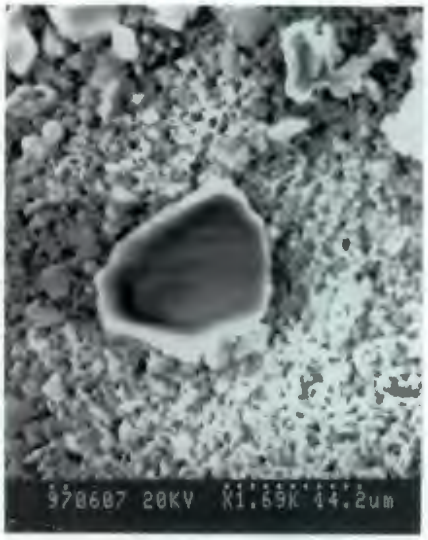

Plate 3.1

Plate 3.2

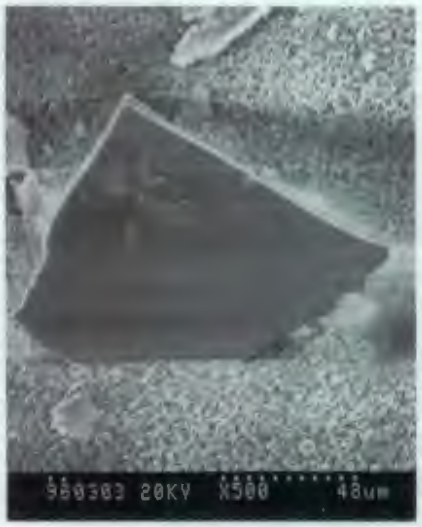

Plate 3.3

Plate 3.4

FIGURES

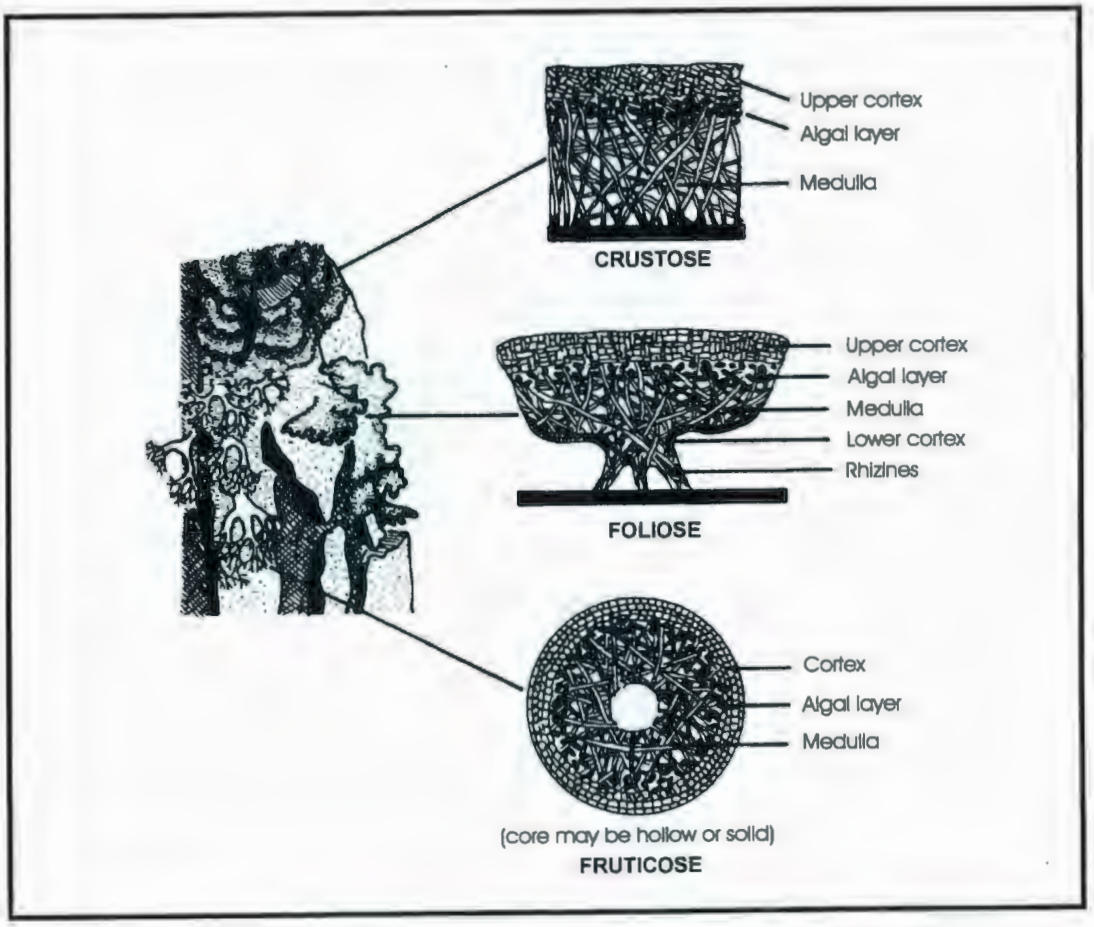

Figure 1.1: The three main types of lichen thalli (from Ahmadjian and Paracer, 1986).

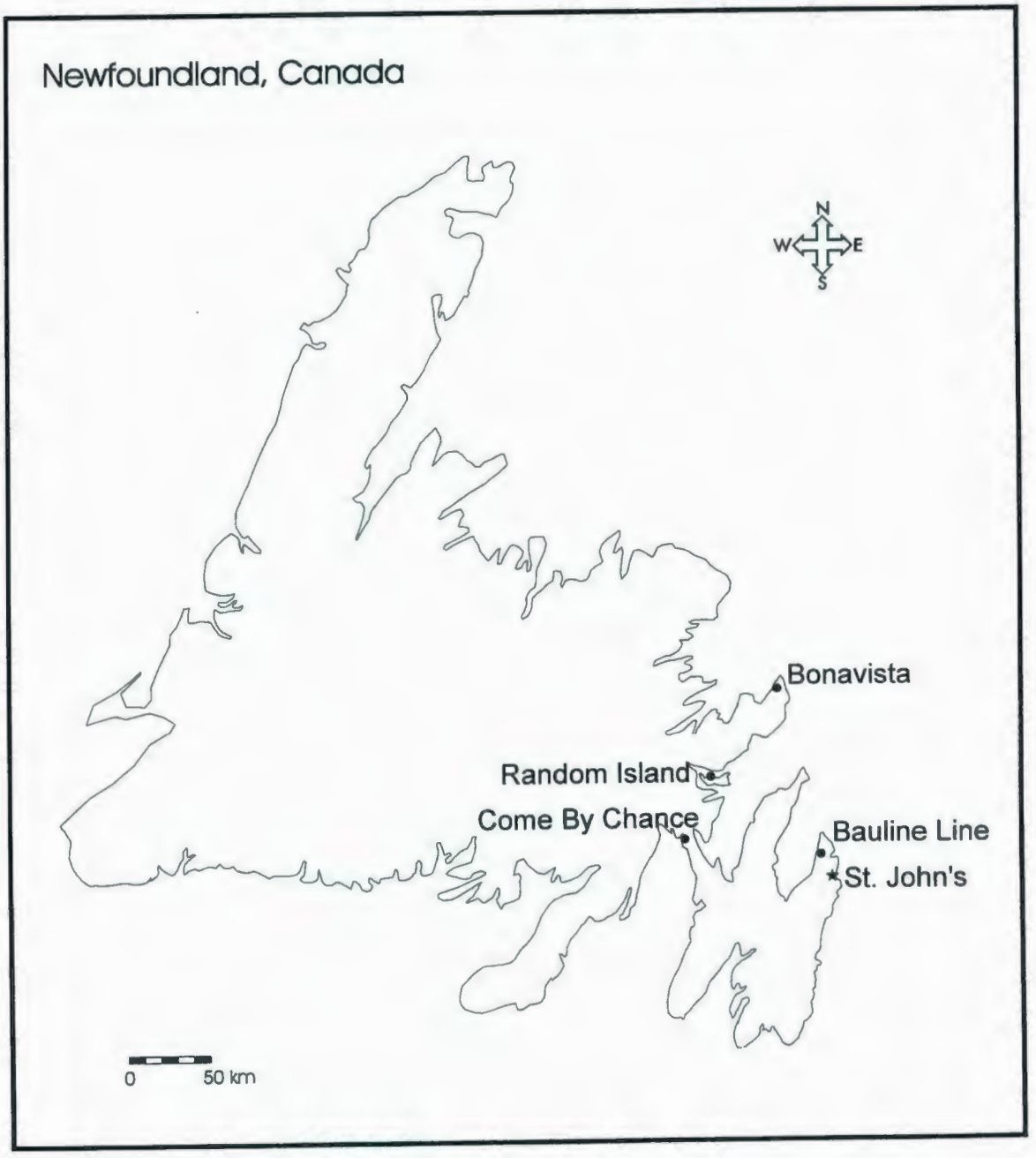

Figure 2.1: Location of lichen sampling sites in Newfoundland, Canada.

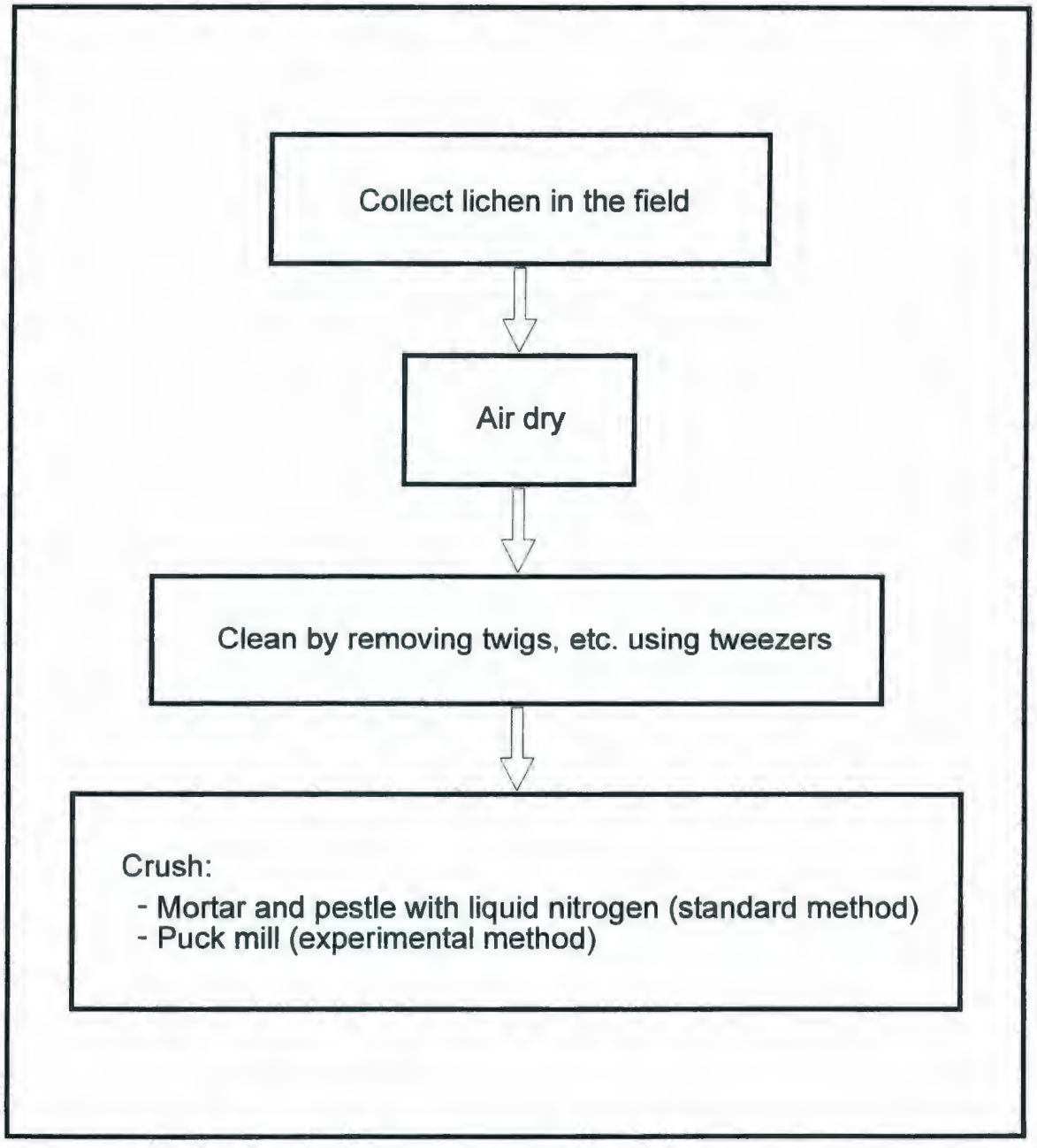

Figure 2.2: Summary of lichen sample treatment prior to digestion.

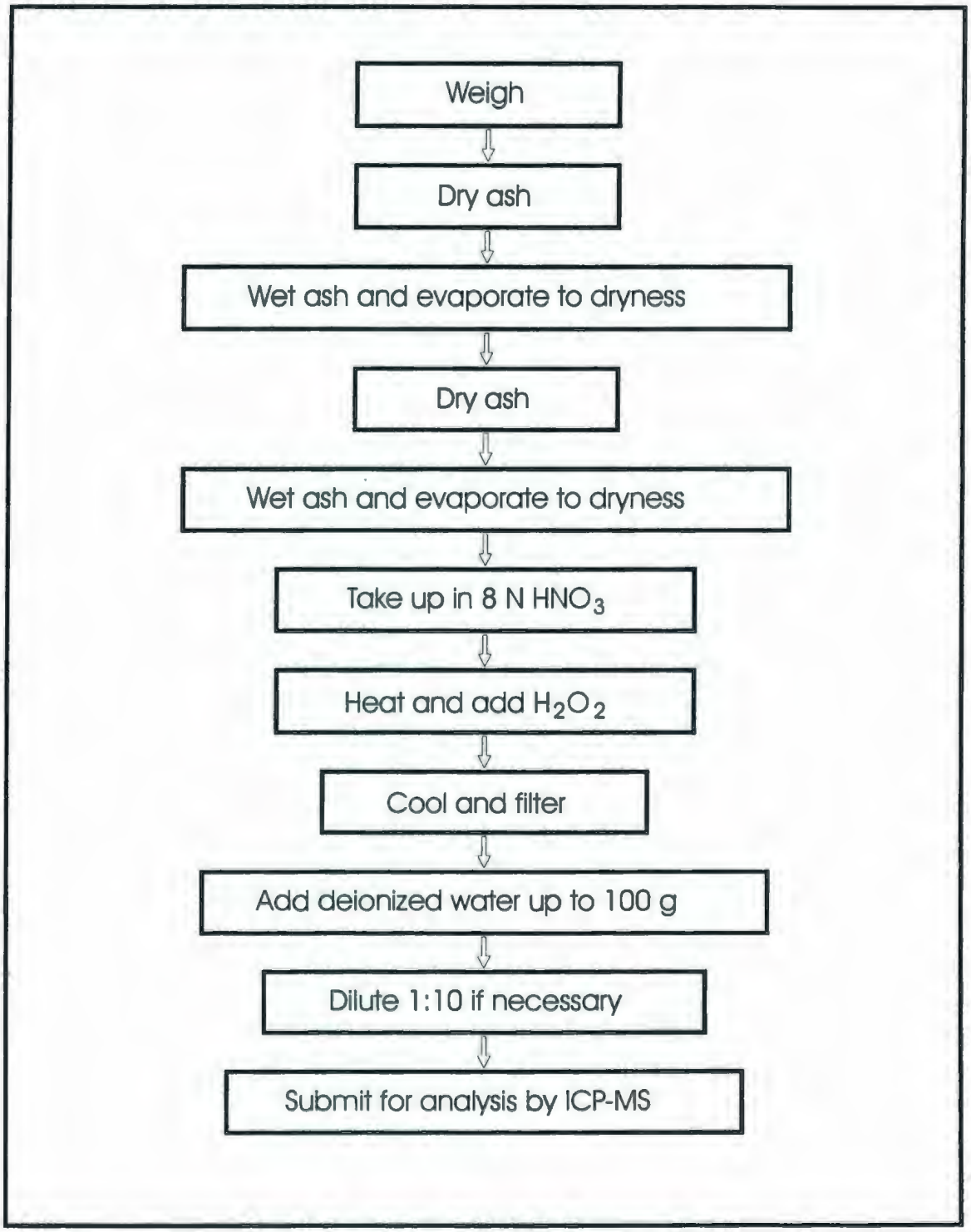

Figure 2.3: The developed digestion procedure

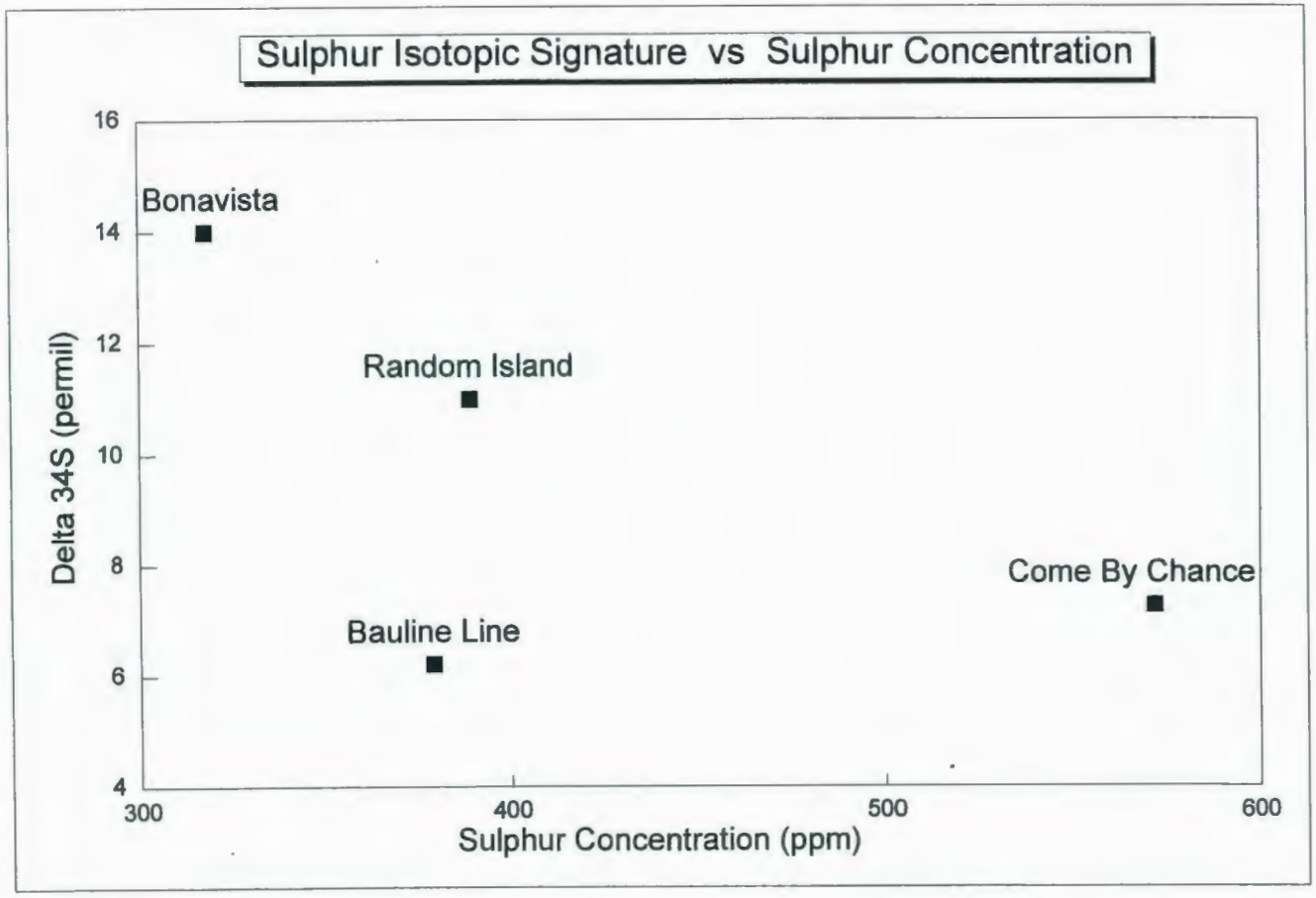

Figure 2.4: Graphical representation of the relative pollution levels at the four sites used in this research, as interpreted from sulphur data. After Evans (1996) and Blake (1998). In Newfoundland, a relatively high Delta 34S value is indicative of natural sources of sulphur (seaspray), and a relatively low value of Delta 34S is indicative of continental/anthropogenic sources of sulphur (Jamieson, 1995).

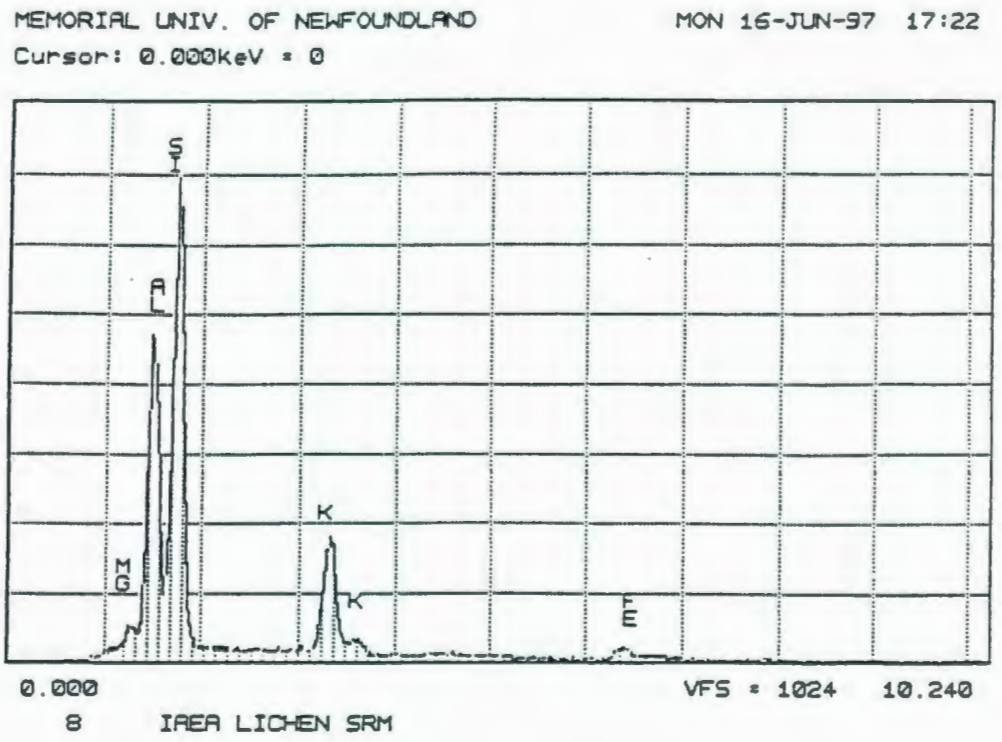

Figure 3.1: SEM-EDX spectra of the relative composition for Plate 3.1. This is a common composition of a high silicon particle containing iron.

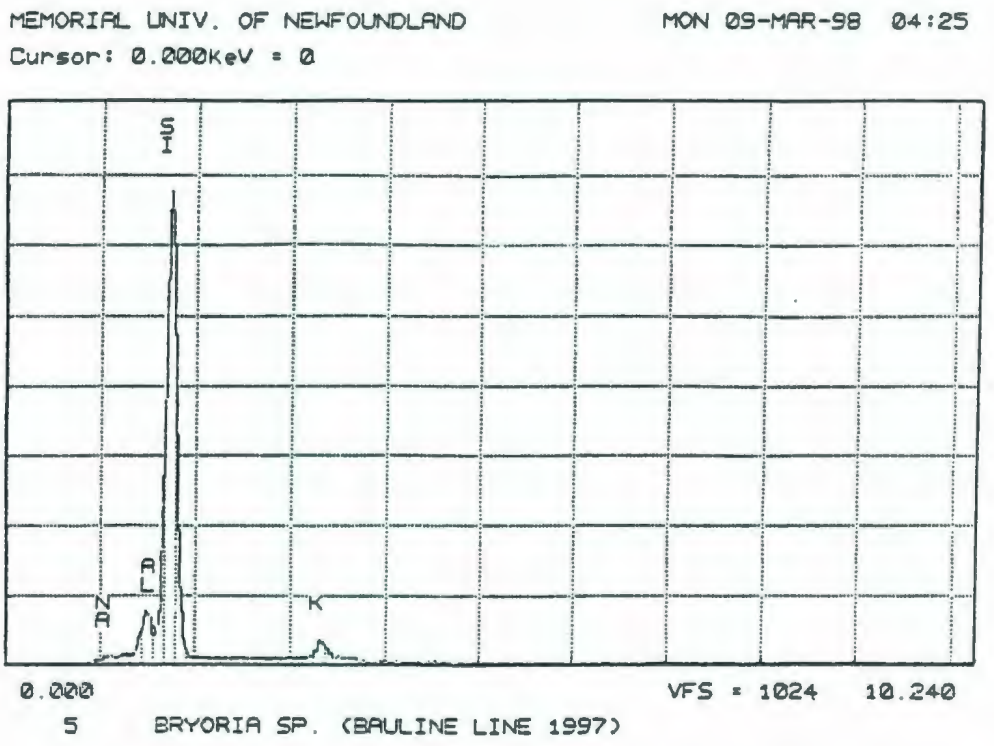

Figure 3.2: SEM-EDX spectra of the relative composition for Plate 3.2. This is a common composition of a high silicon particle.

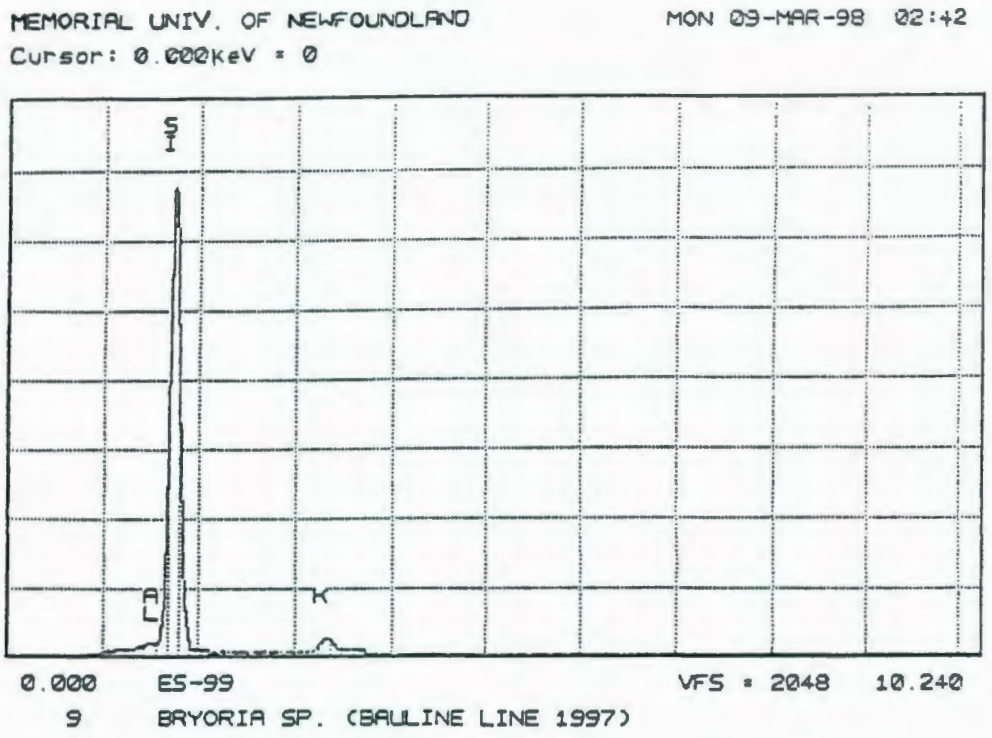

Figure 3.3: SEM-EDX spectra of the relative composition for Plate 3.3. This is a common composition of a high silicon particle.

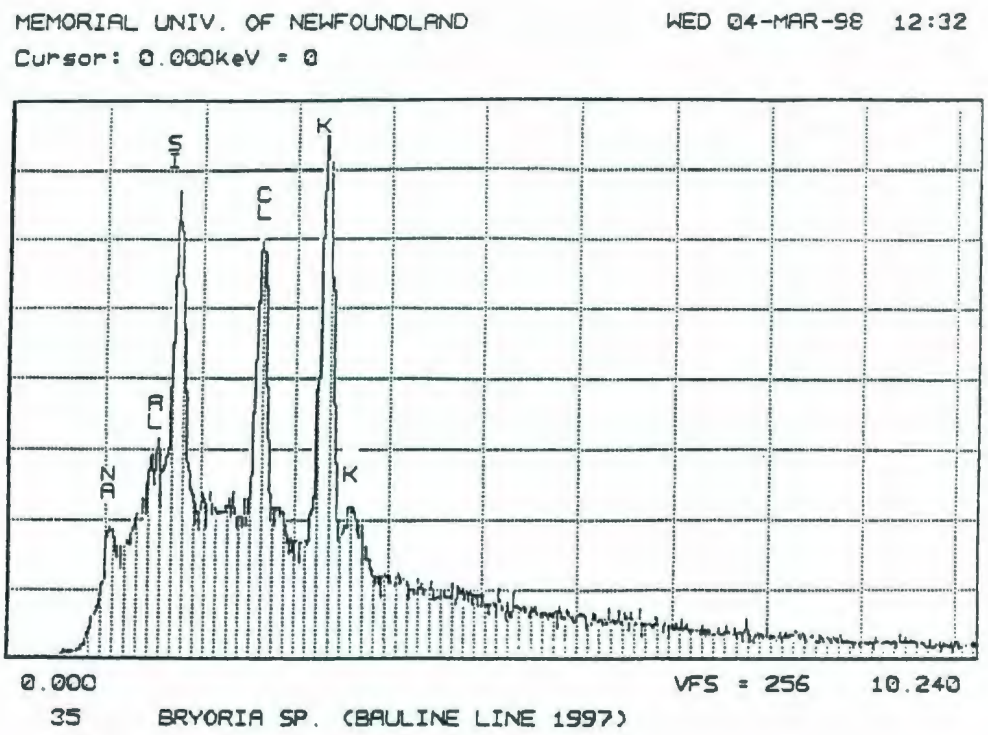

Figure 3.4: SEM-EDX spectra of the relative composition for Plate 3.4. This is an uncommon composition, especially the high chlorine. As this is a relatively thin particle, it is likely that elemental information has been collected from the area around the particle as well. The general elevation of the background is most likely due to the filter paper.

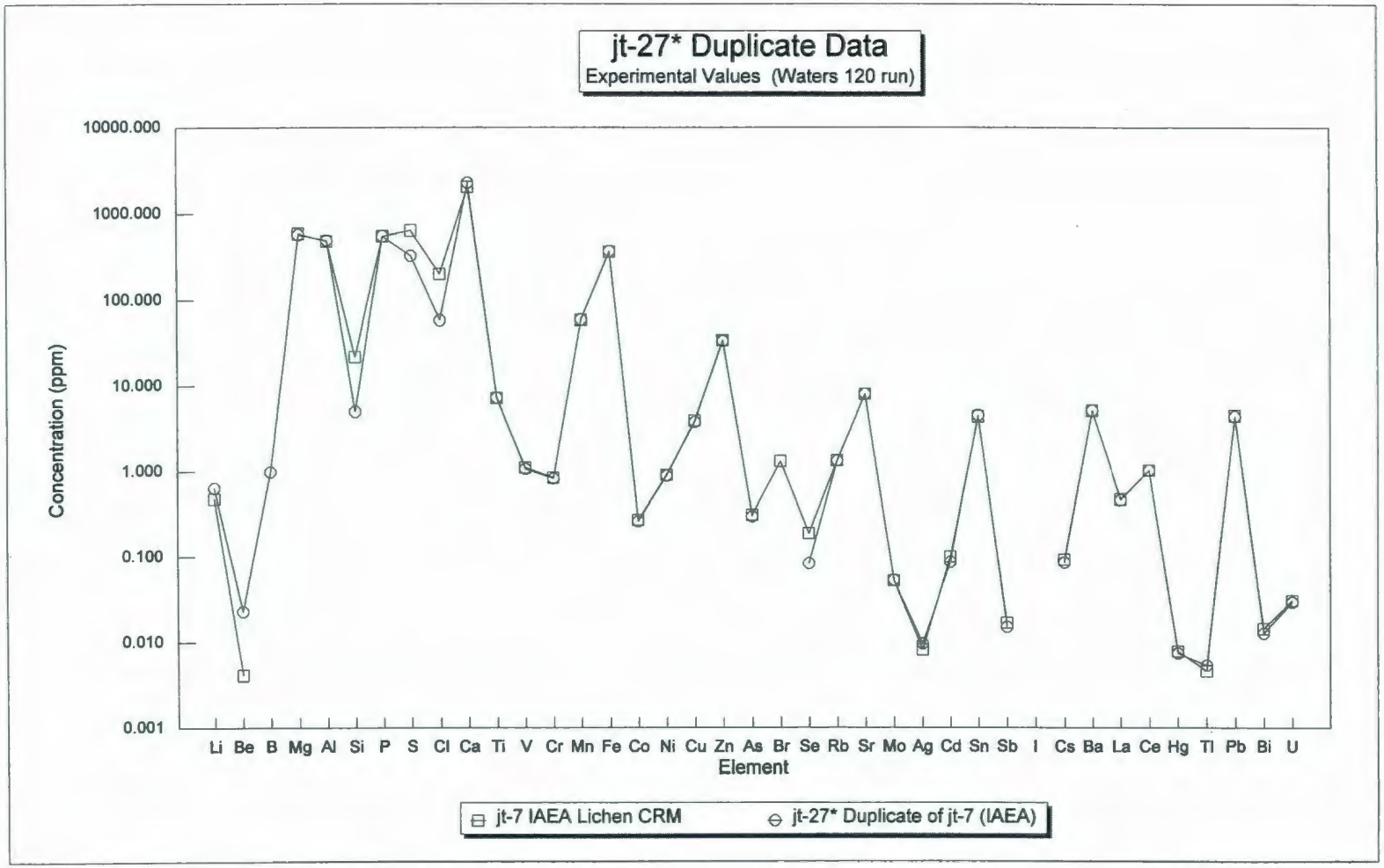

Figure 3.5: Elemental concentrations (in ppm) for the IAEA Lichen CRM duplicate and the corresponding sample from the ICP-MS Waters 120 Run (in ppm).

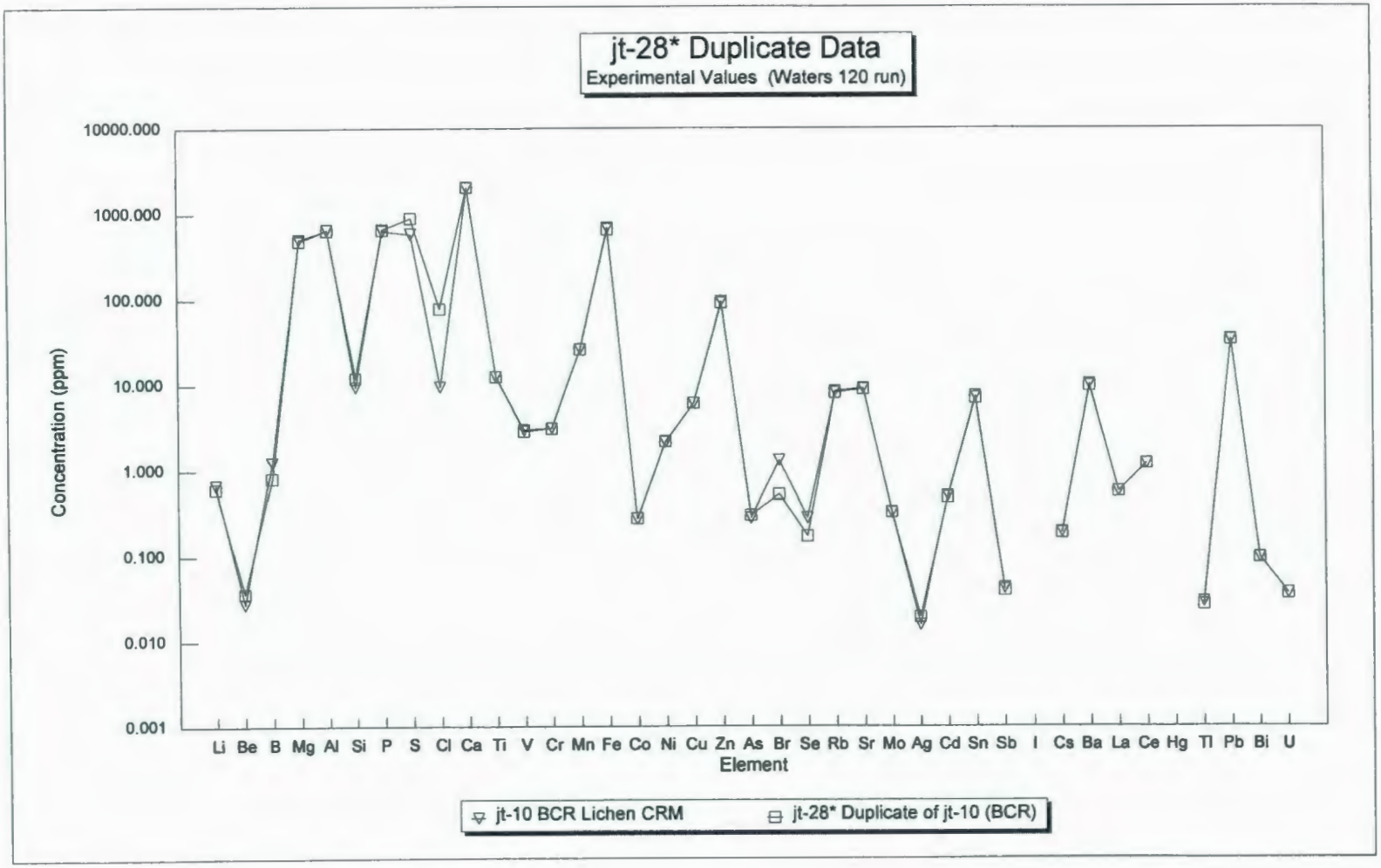

Figure 3.6: Elemental concentrations (in ppm) for the BCR Lichen CRM duplicate and the corresponding sample from the ICP-MS Waters 120 Run.

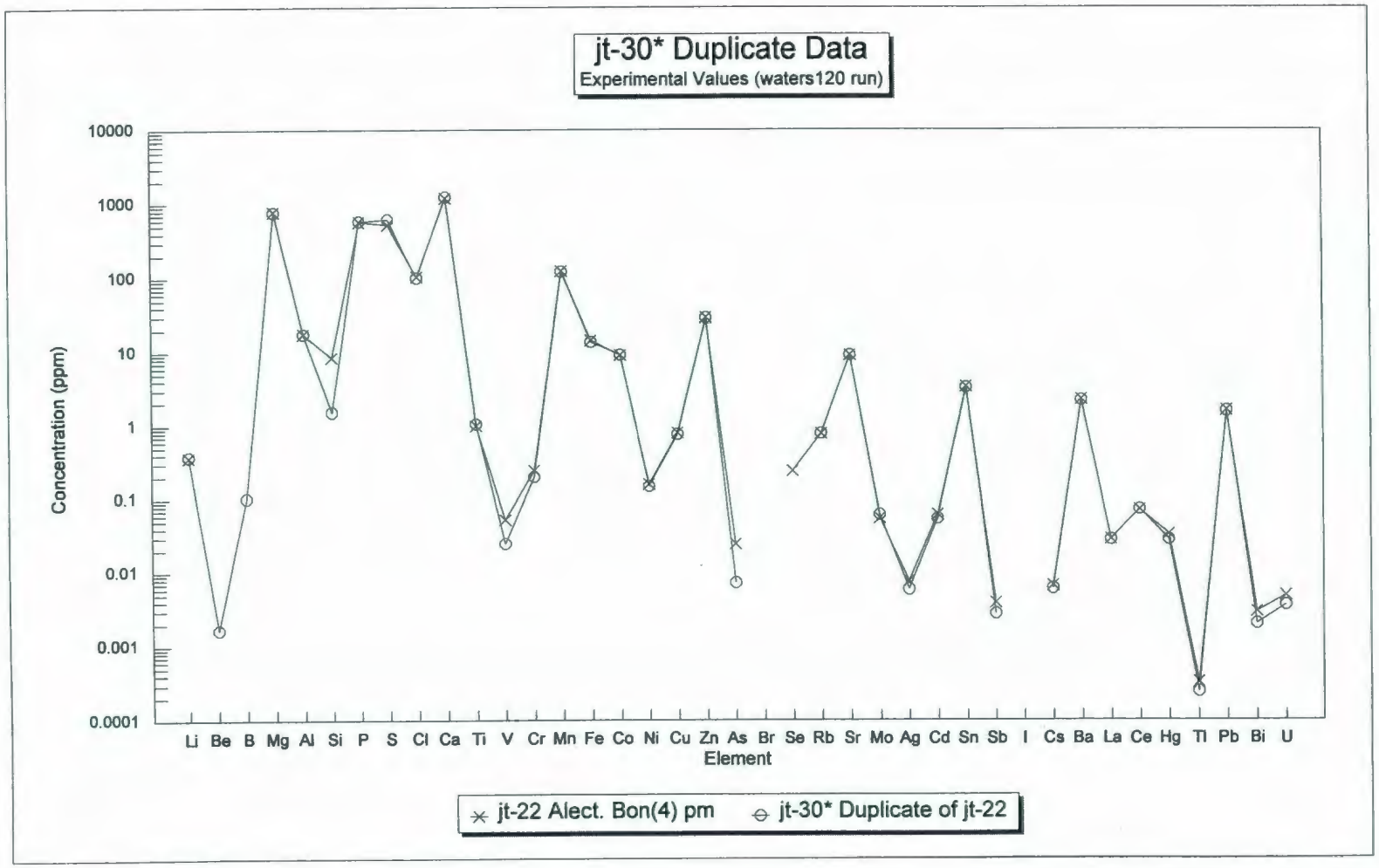

Figure 3.7: Elemental concentrations (in ppm) for the Alectoria Bonavista (Area 4) puck mill duplicate and the corresponding sample from the ICP-MS Waters 120 Run.

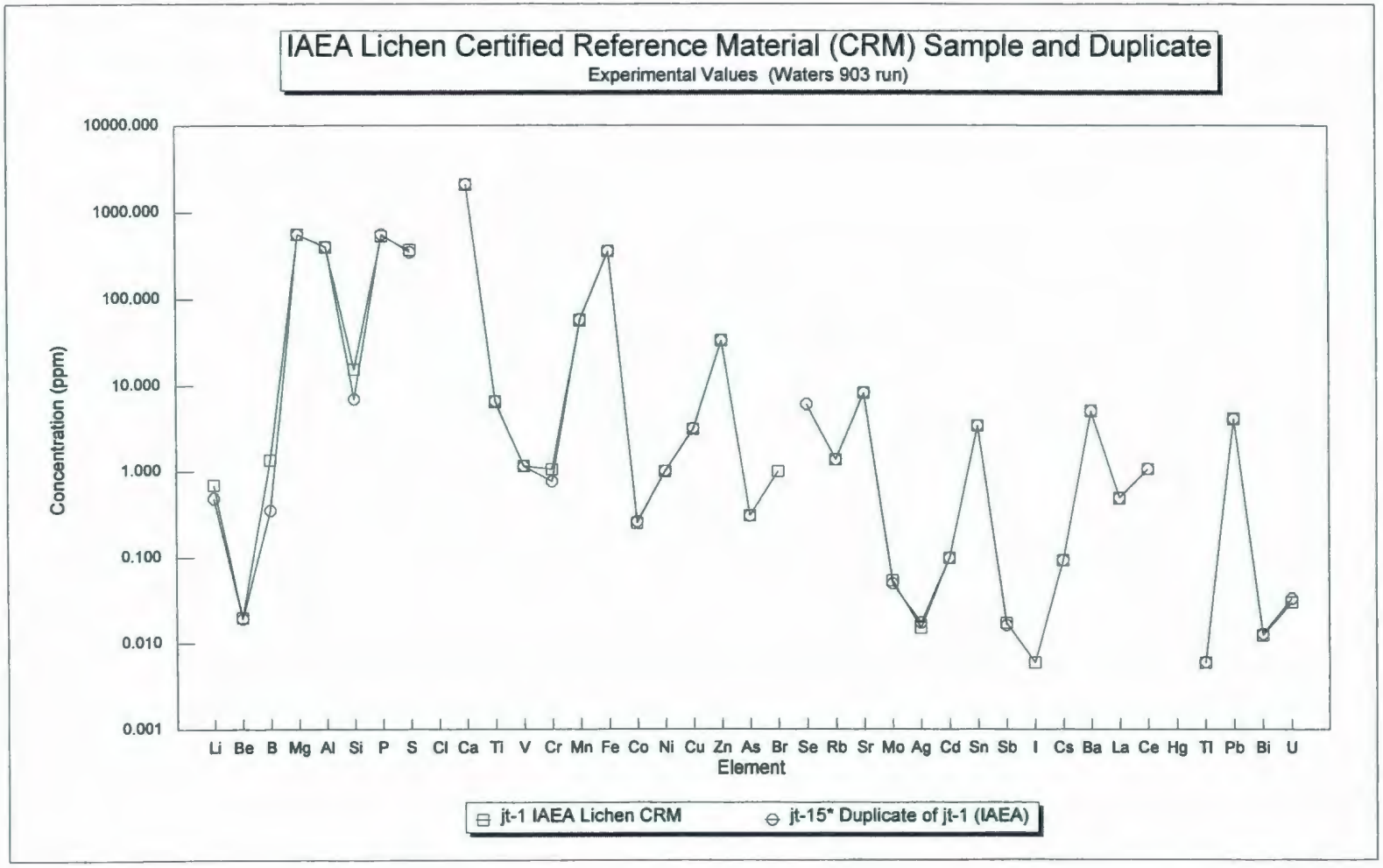

Figure 3.8: Elemental concentrations (in ppm) for the IAEA Lichen CRM duplicate and the corresponding sample from the ICP-MS Waters 903 Run.

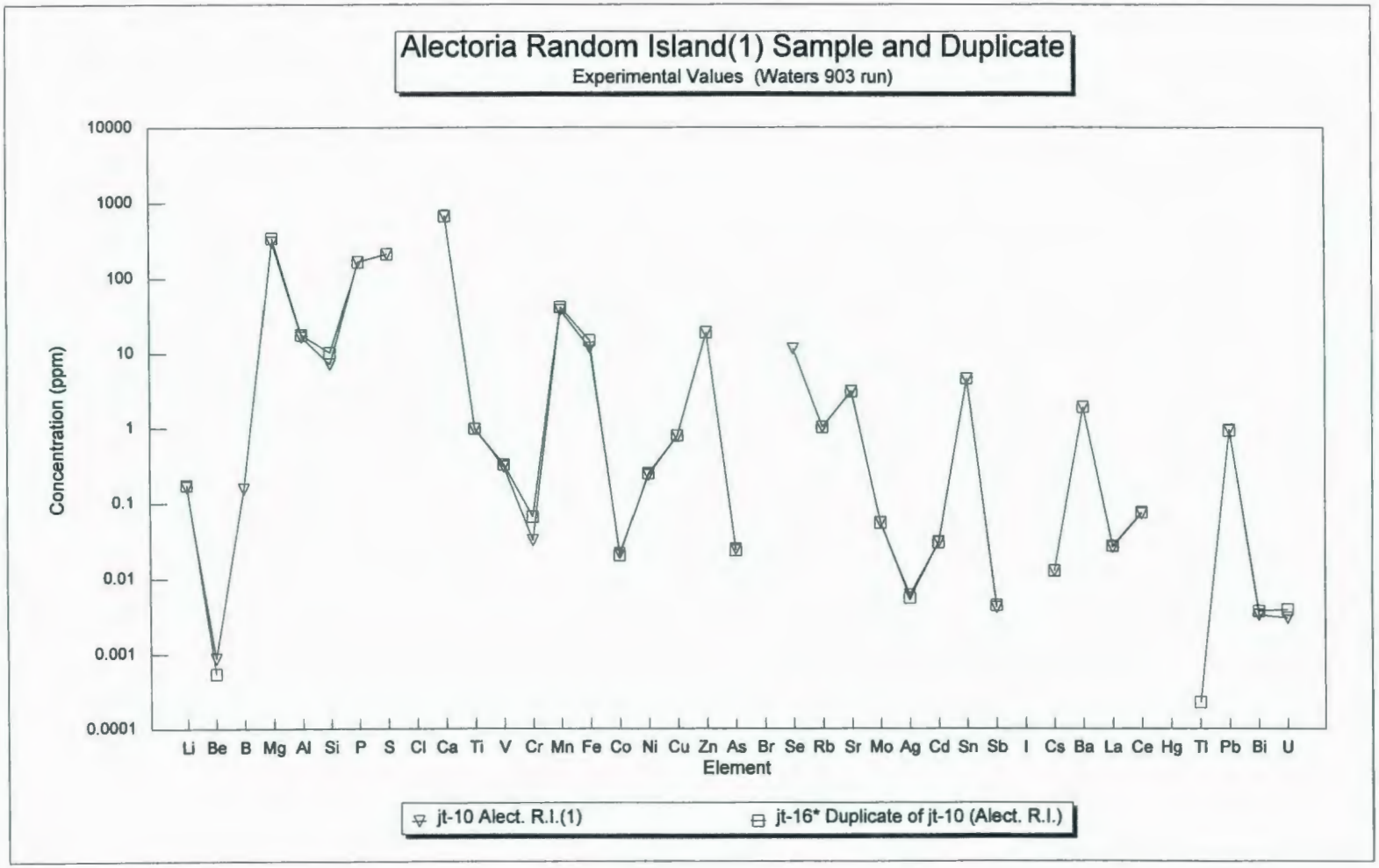

Figure 3.9: Elemental concentrations (in ppm) for the Alectoria Random Island (Area 1) duplicate and the corresponding sample from the ICP-MS Waters 903 Run.

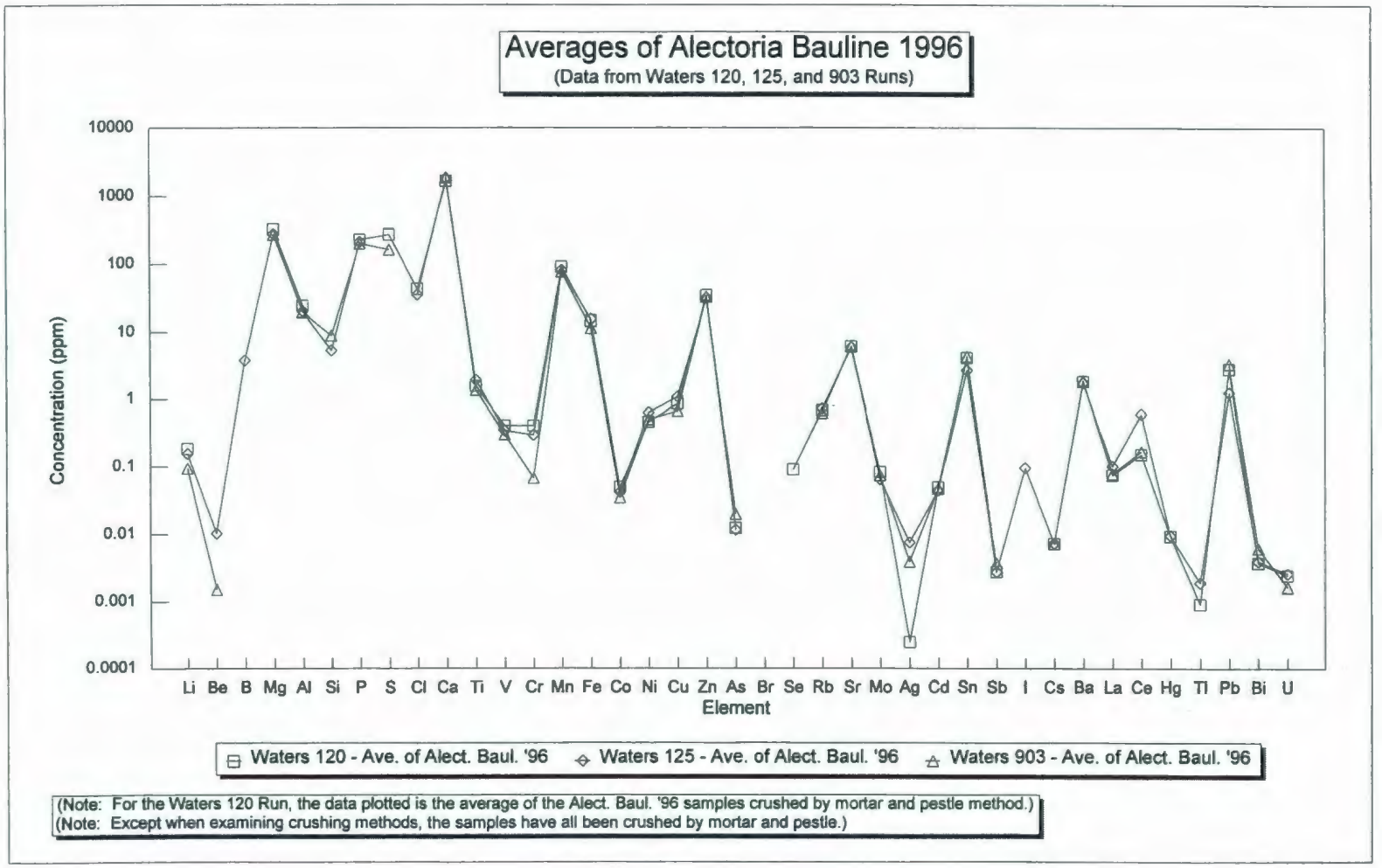

Figure 3.10: Mean concentrations (in ppm) of the Alectoria Bauline 1996 samples from each of the three ICP-MS Runs.

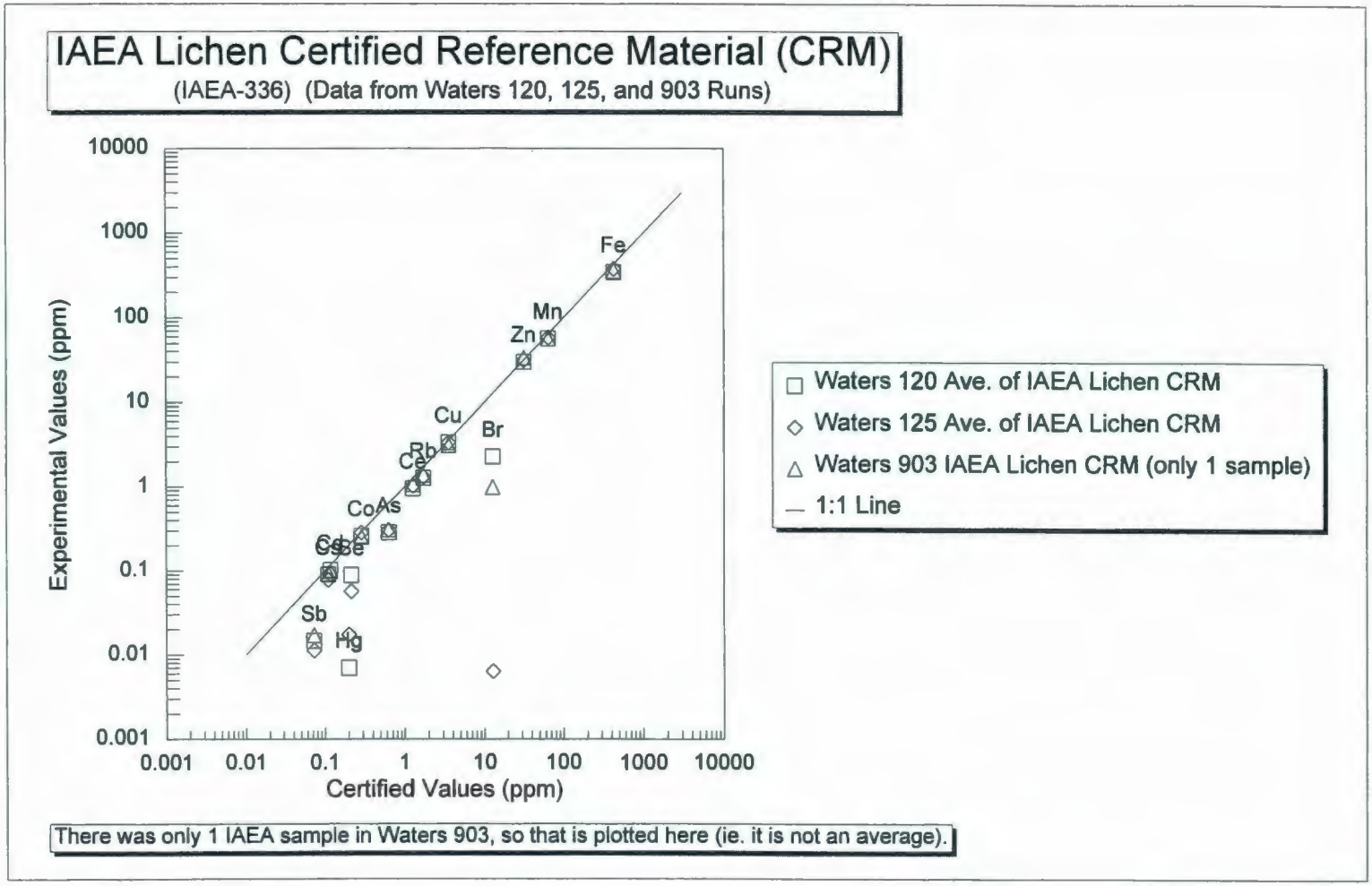

Figure 3.11: Determined (i.e. experimental) versus certified concentrations (in ppm) for the IAEA Lichen CRM. The mean concentrations for each of the three ICP-MS Runs are shown.

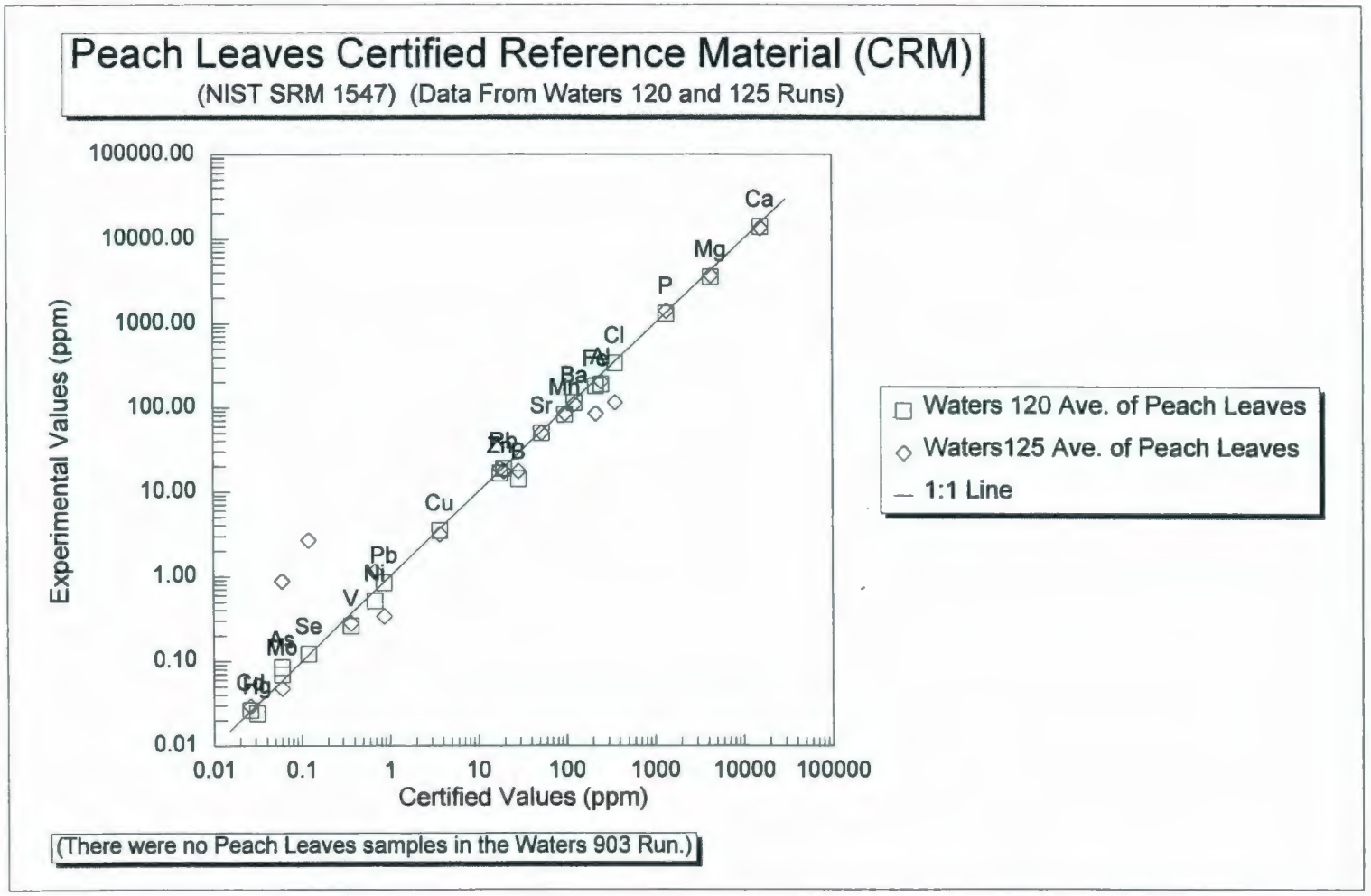

Figure 3.12: Determined (i.e. experimental) versus certified concentrations (in ppm) for the Peach Leaves CRM. The mean concentrations for each of the ICP-MS Waters 120 and 125 Runs are shown (there were no Peach Leaves CRM samples in the Waters 903 Run).

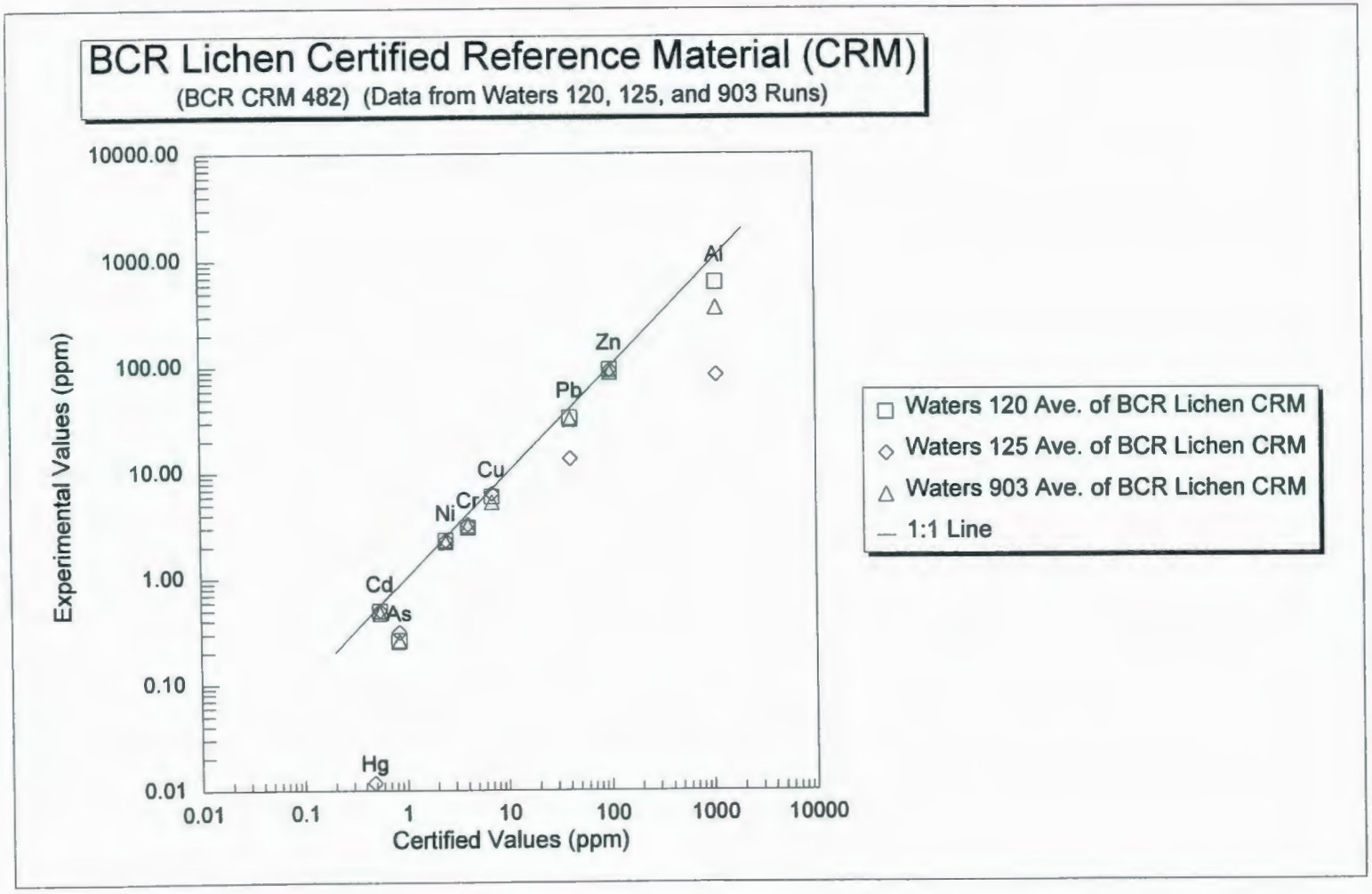

Figure 3.13: Determined (i.e. experimental) versus certified concentrations (in ppm) for the BCR Lichen CRM. The mean concentrations for each of the three ICP-MS Runs are shown.

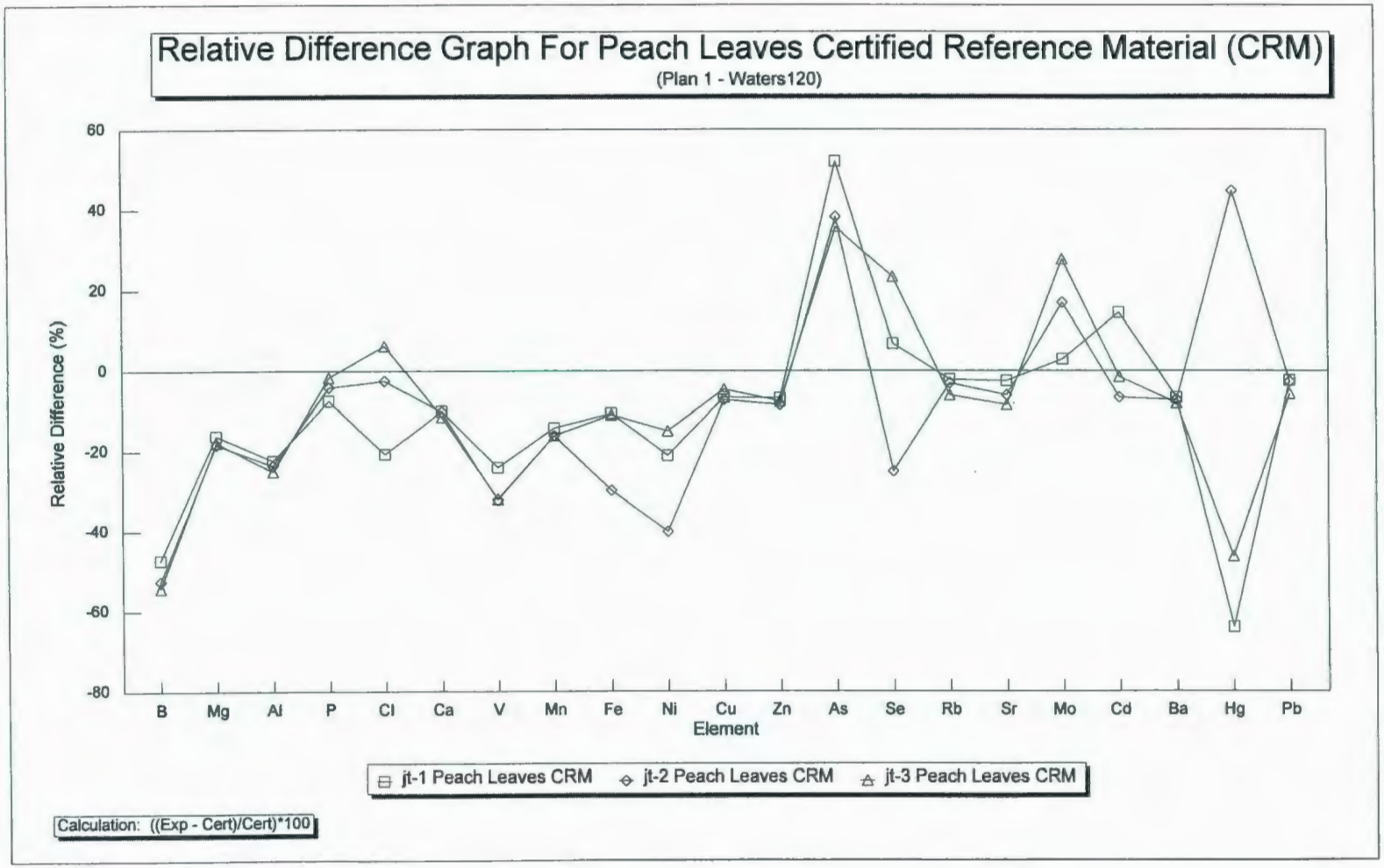

Figure 3.14: Plot of relative difference for the Peach Leaves CRM from the ICP-MS Waters 120 Run. Relative difference is a comparison between the certified and the determined (i.e. experimental) concentrations; the equation for relative difference is given below the plot.

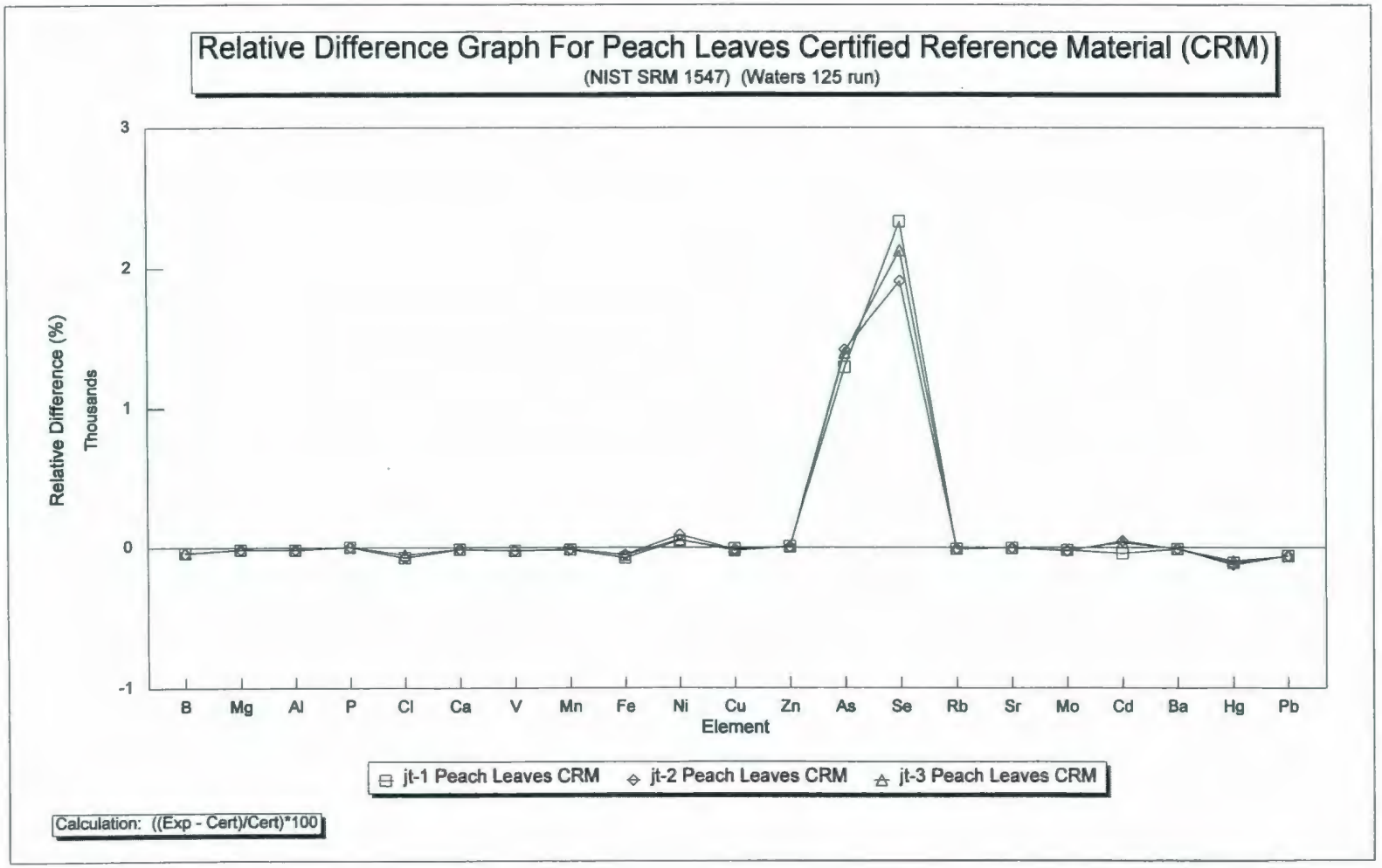

Figure 3.15: Plot of relative difference for the Peach Leaves CRM from the ICP-MS Waters 125 Run. Relative difference is a comparison between the certified and the determined (i.e. experimental) concentrations; the equation for relative difference is given below the plot.

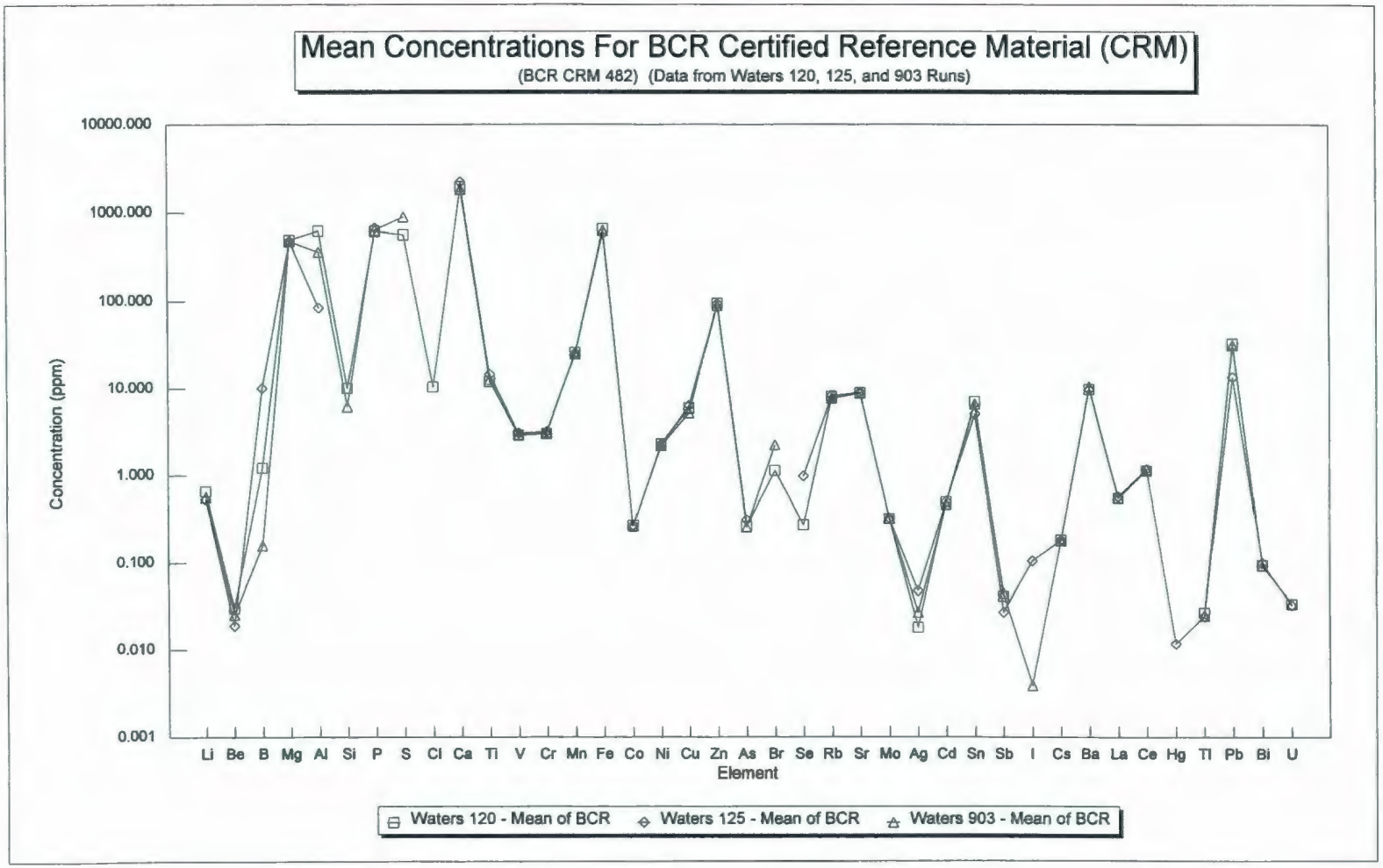

Figure 3.16: Mean concentrations (in ppm) of the BCR Lichen CRM samples from each of the three ICP-MS Runs.

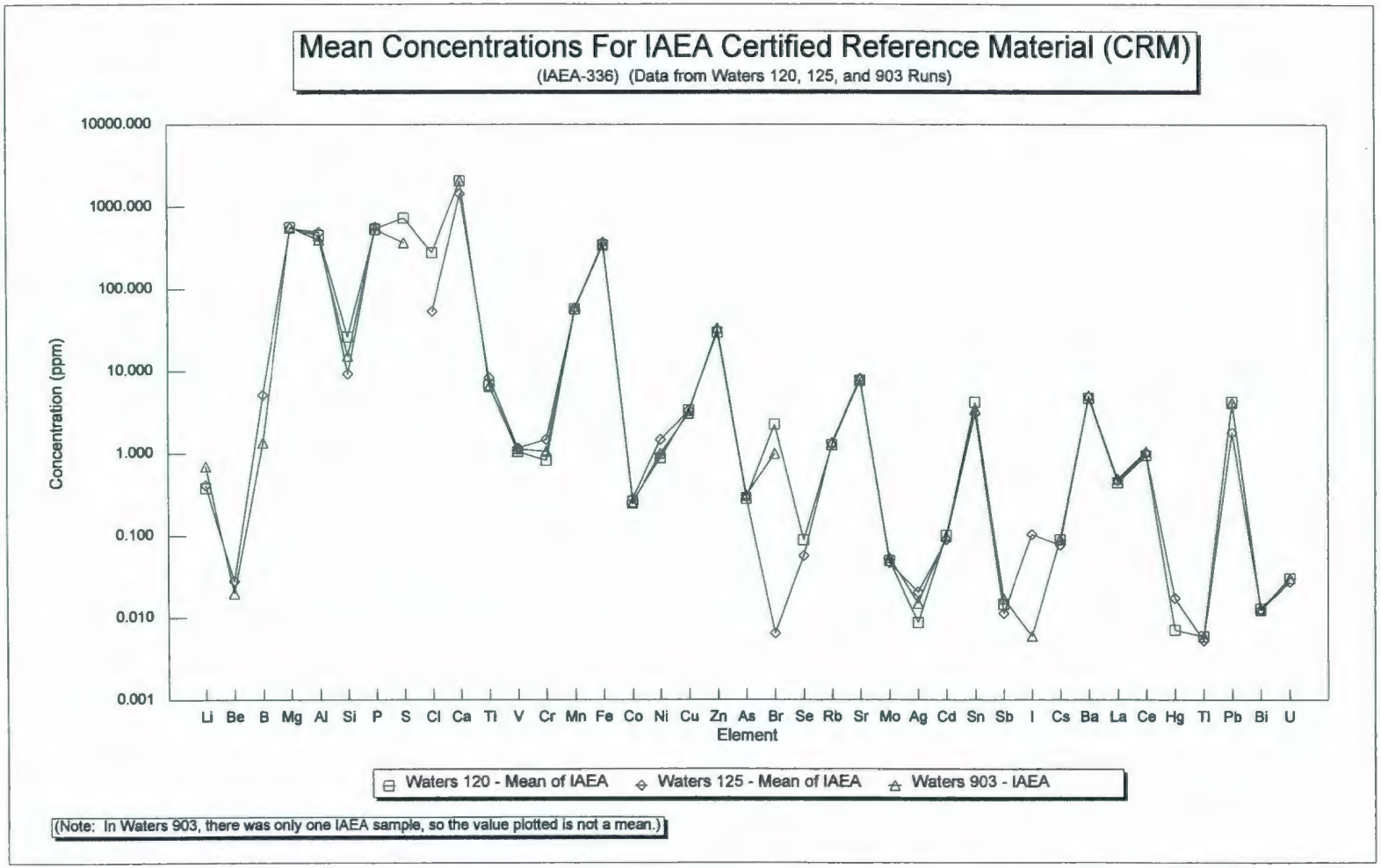

Figure 3.17: Mean concentrations (in ppm) of the IAEA Lichen CRM samples from each of the three ICP-MS Runs (the Waters 903 Run contained only one IAEA Lichen CRM sample).

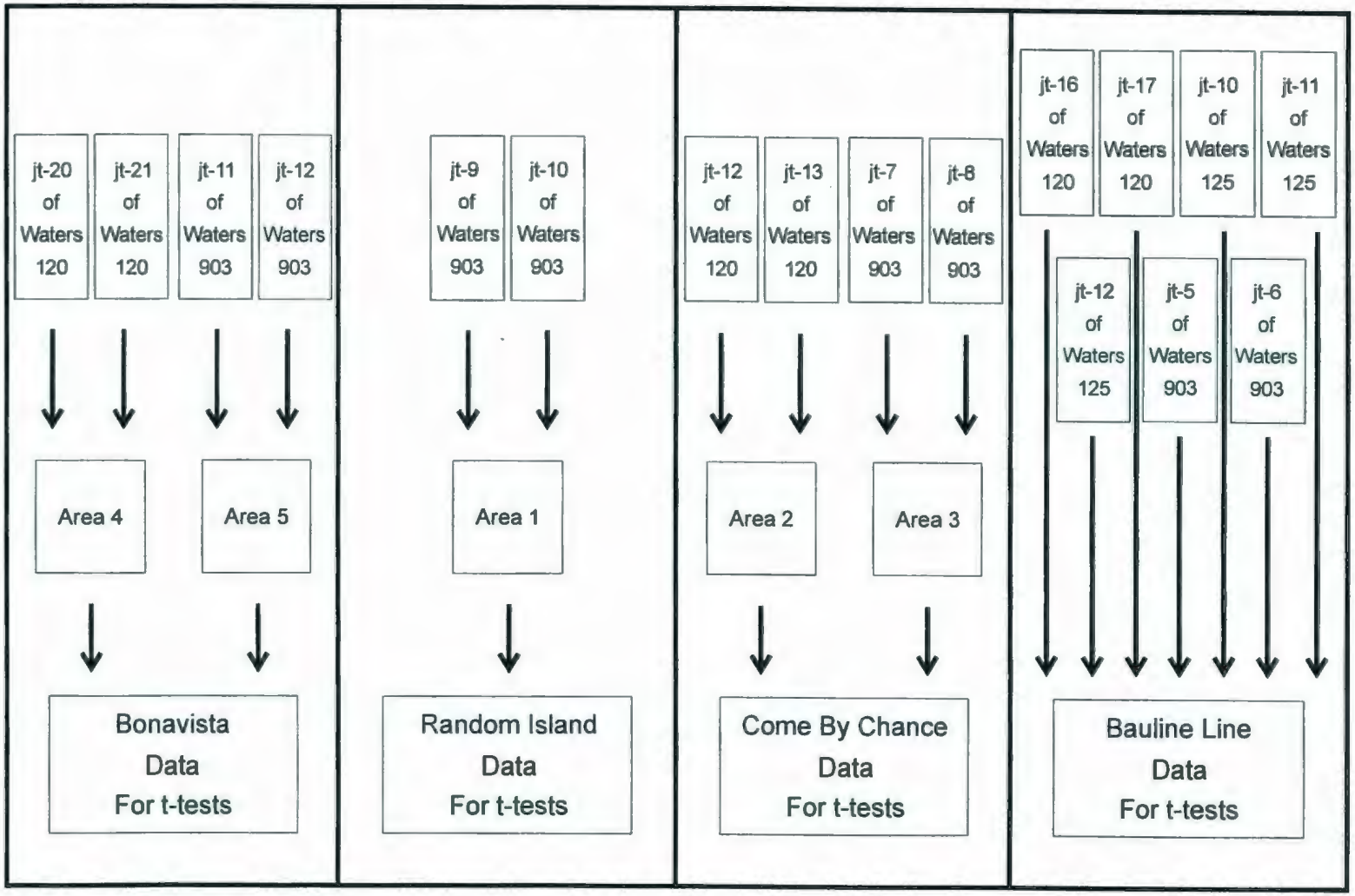

Figure 3.18: Compilation of the samples used in the t-tests for the pairwise comparisons of sampling sites. For the complete keys of the sample names, refer to Tables 2.1 - 2.3.

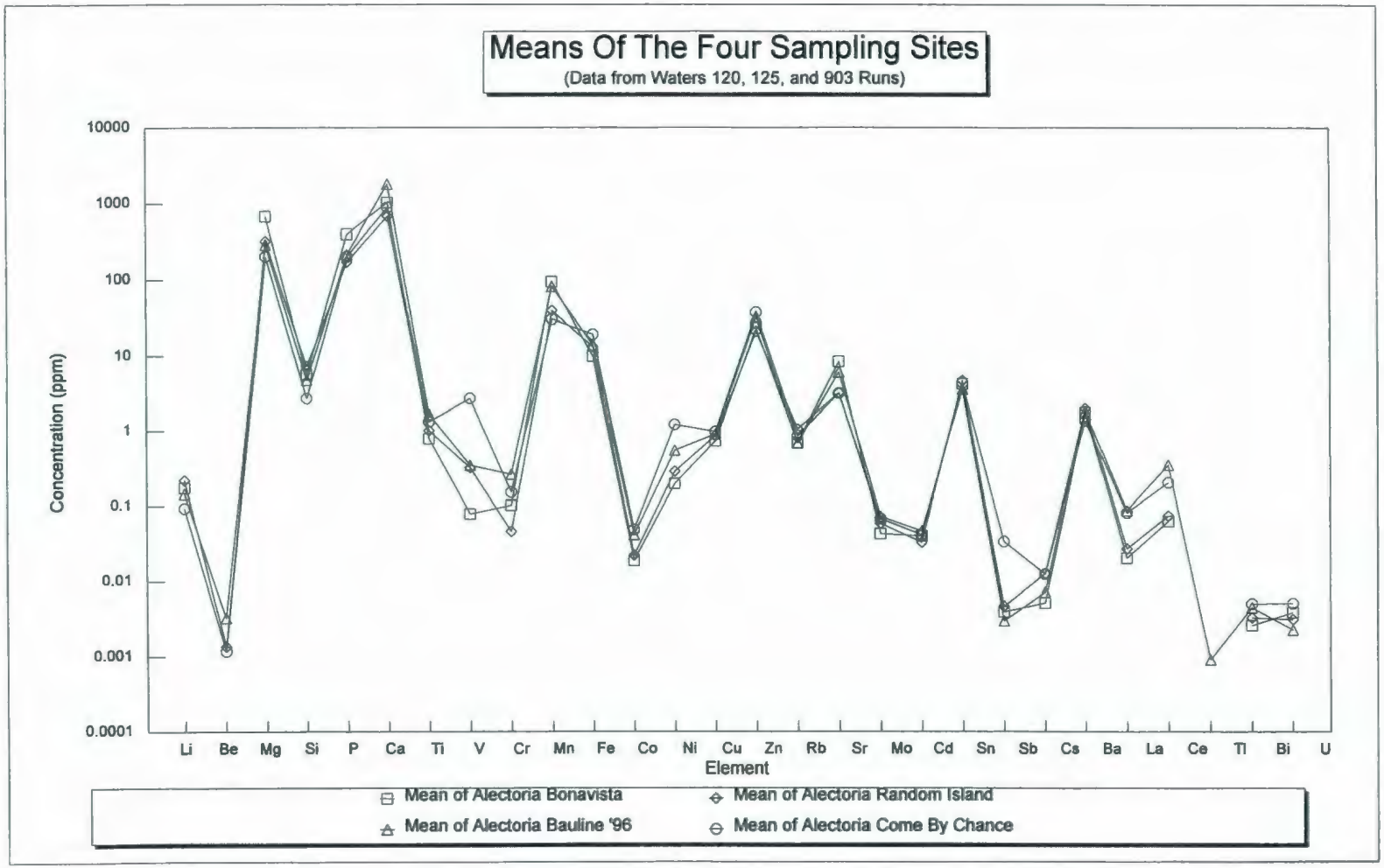

Figure 3.19: Mean concentrations (in ppm) of the *Alectoria sarmentosa* samples from each of the four sampling sites.

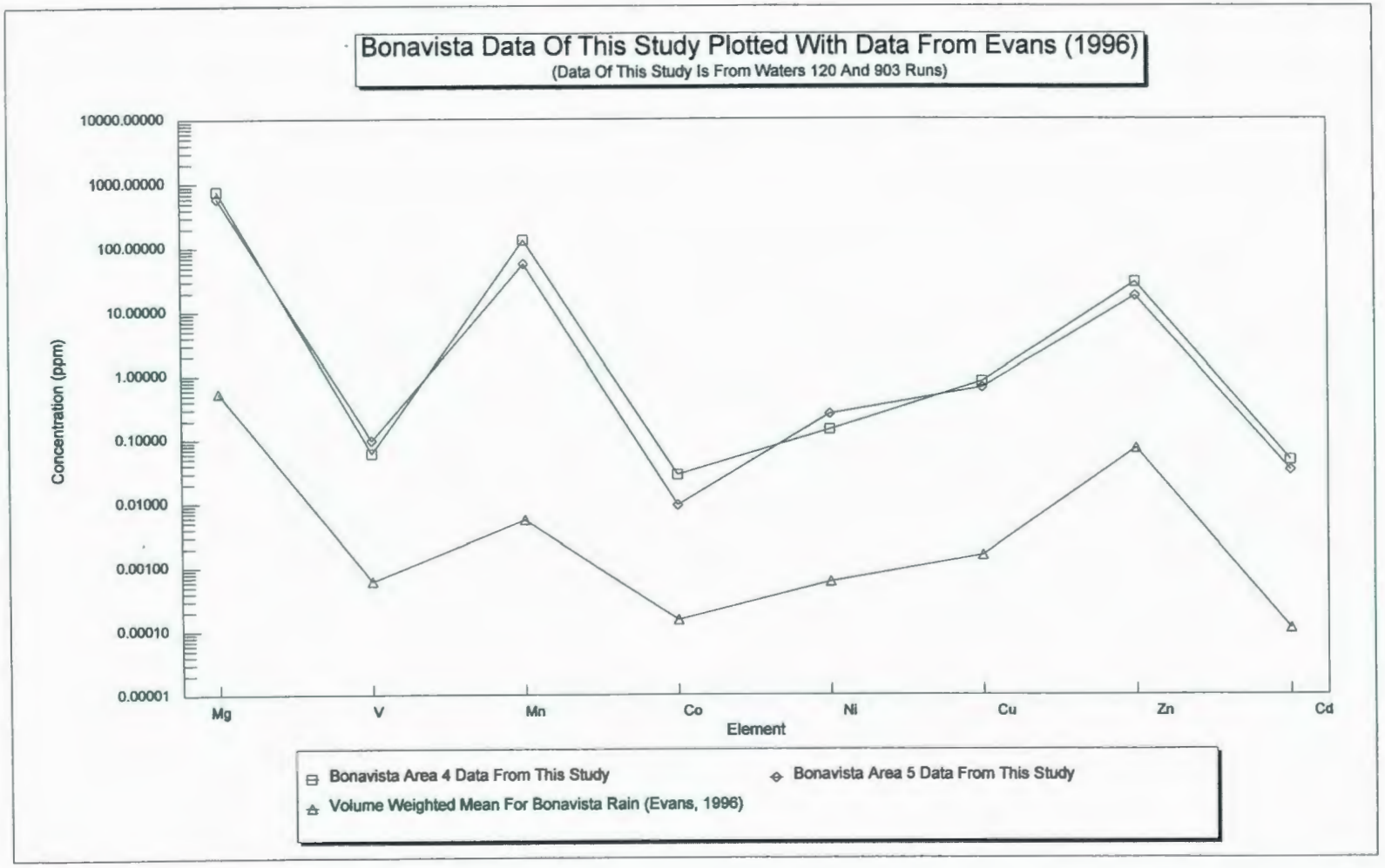

Figure 3.20: The *Alectoria sarmentosa* concentration data from Bonavista for this study plotted with rain data from Bonavista as reported by Evans (1996). The concentrations are shown in ppm.

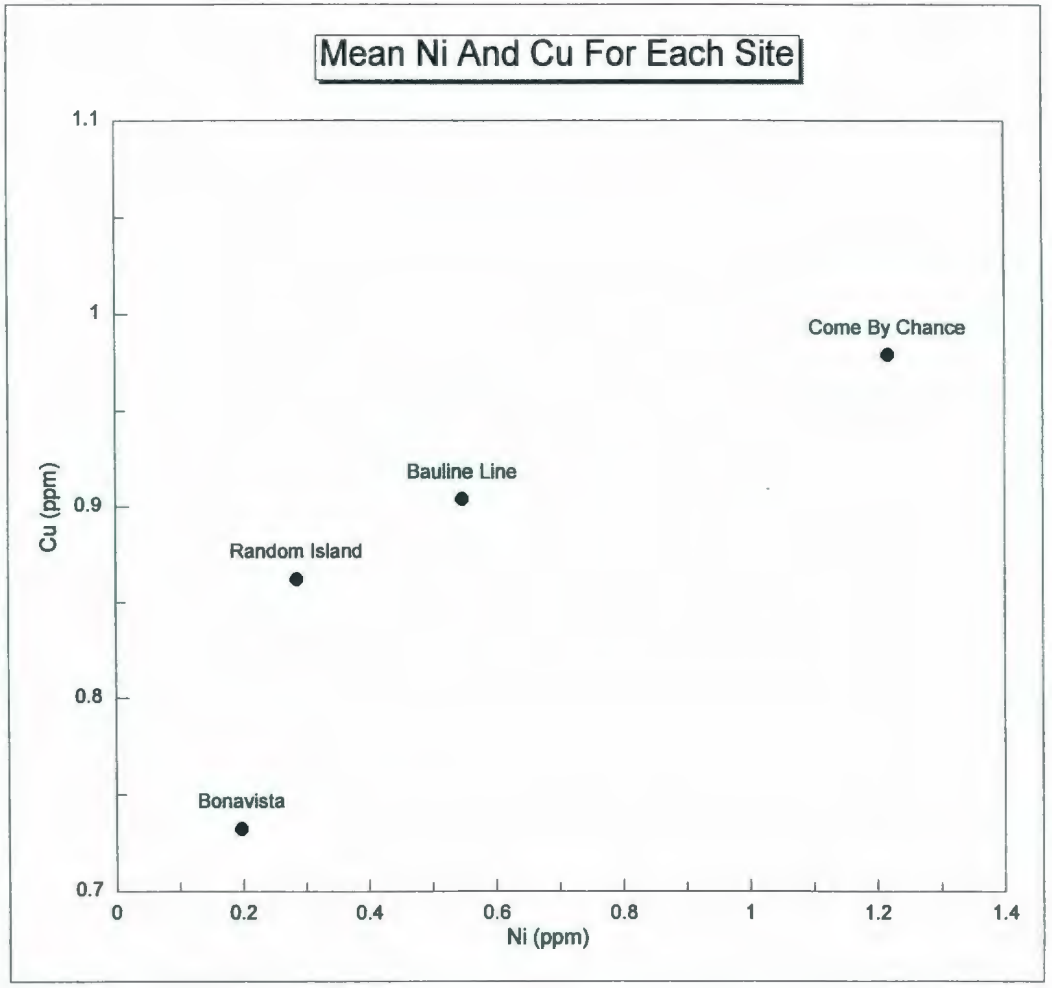

Figure 3.21: X-Y plot of Cu versus Ni concentrations (in ppm) for *Alectoria sarmentosa* from each lichen sampling site.

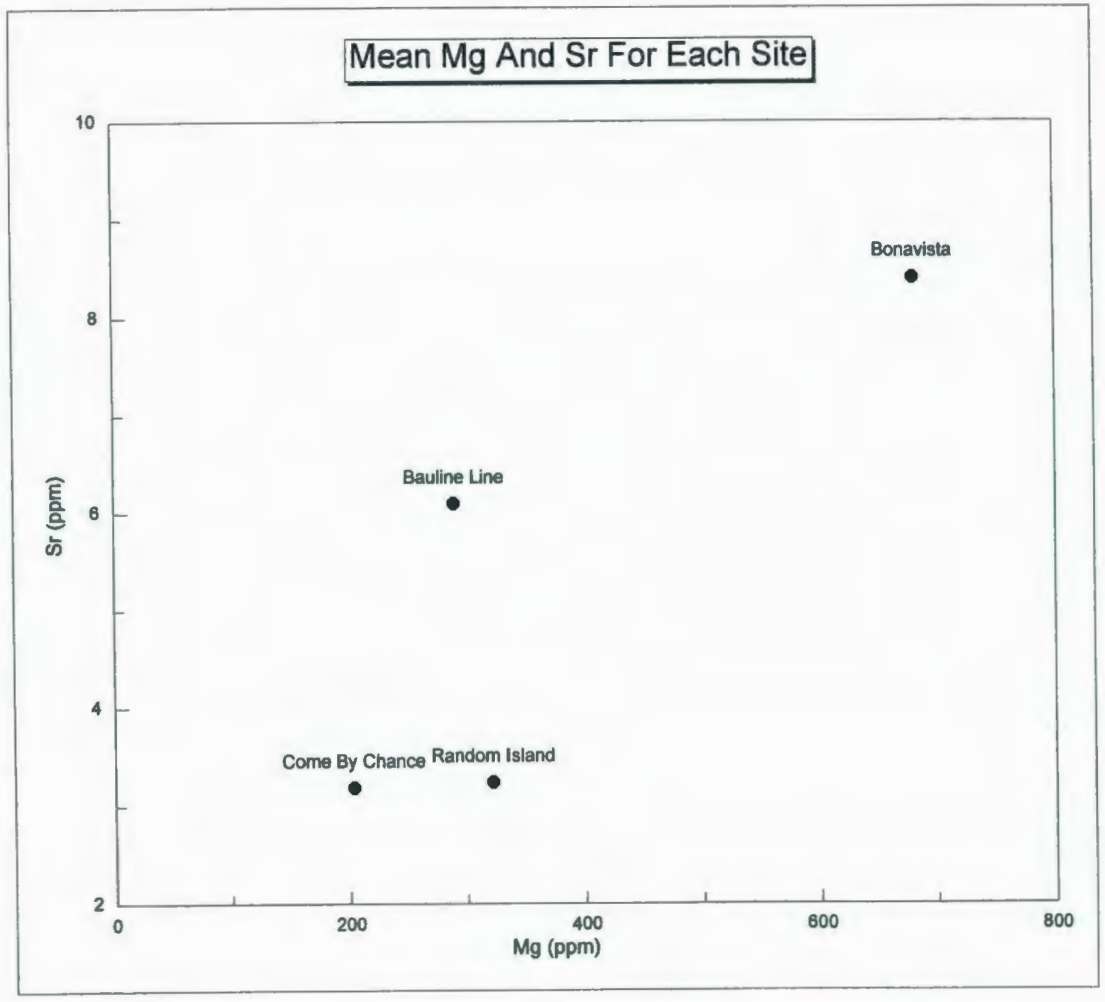

Figure 3.22: X-Y plot of Sr versus Mg concentrations (in ppm) for *Alectoria sarmentosa* from each lichen sampling site.

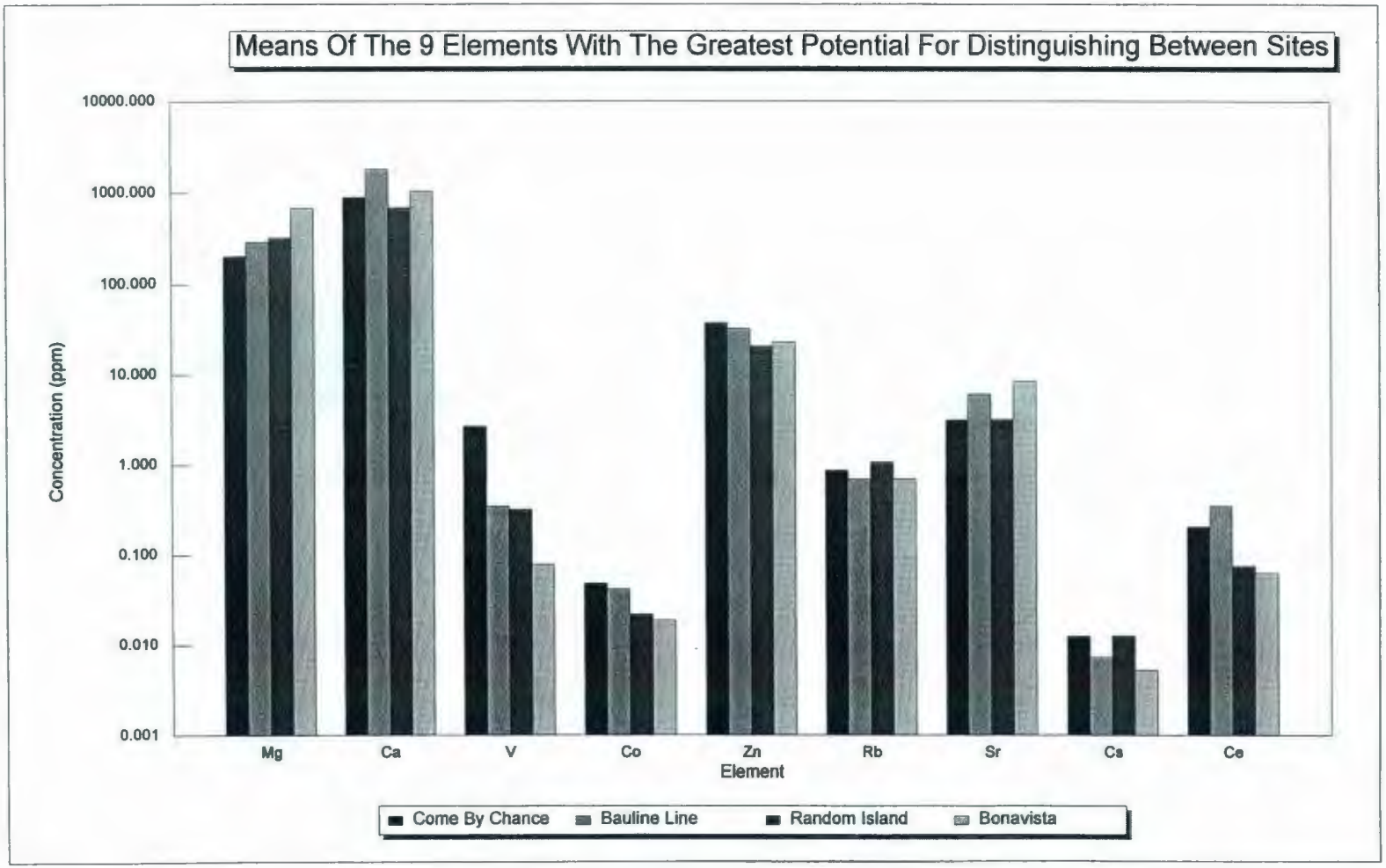

Figure 3.23: Concentrations (in ppm) shown as bar graphs for nine selected elements for *Alectoria sarmentosa* from each of the four lichen sampling sites. These are the elements which have the greatest potential for utilization in distinguishing between sites of varying pollution exposure. Plotted with a logarithmic scale.

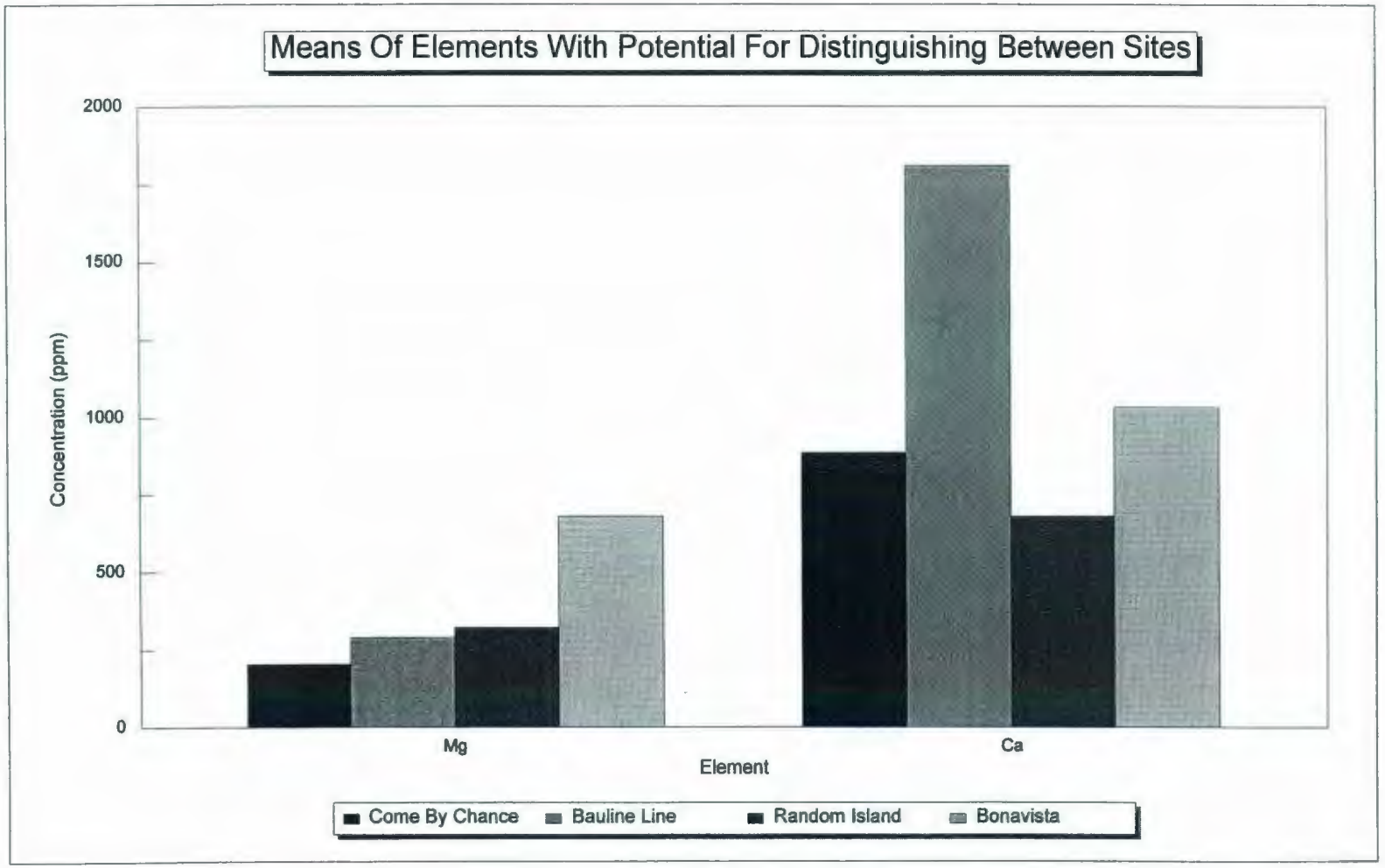

Figure 3.24: Concentrations (in ppm) shown as bar graphs for Mg and Ca for *Alectoria sarmentosa* from each of the four lichen sampling sites. Plotted with a linear scale to show more detail than in Figure 3.23.

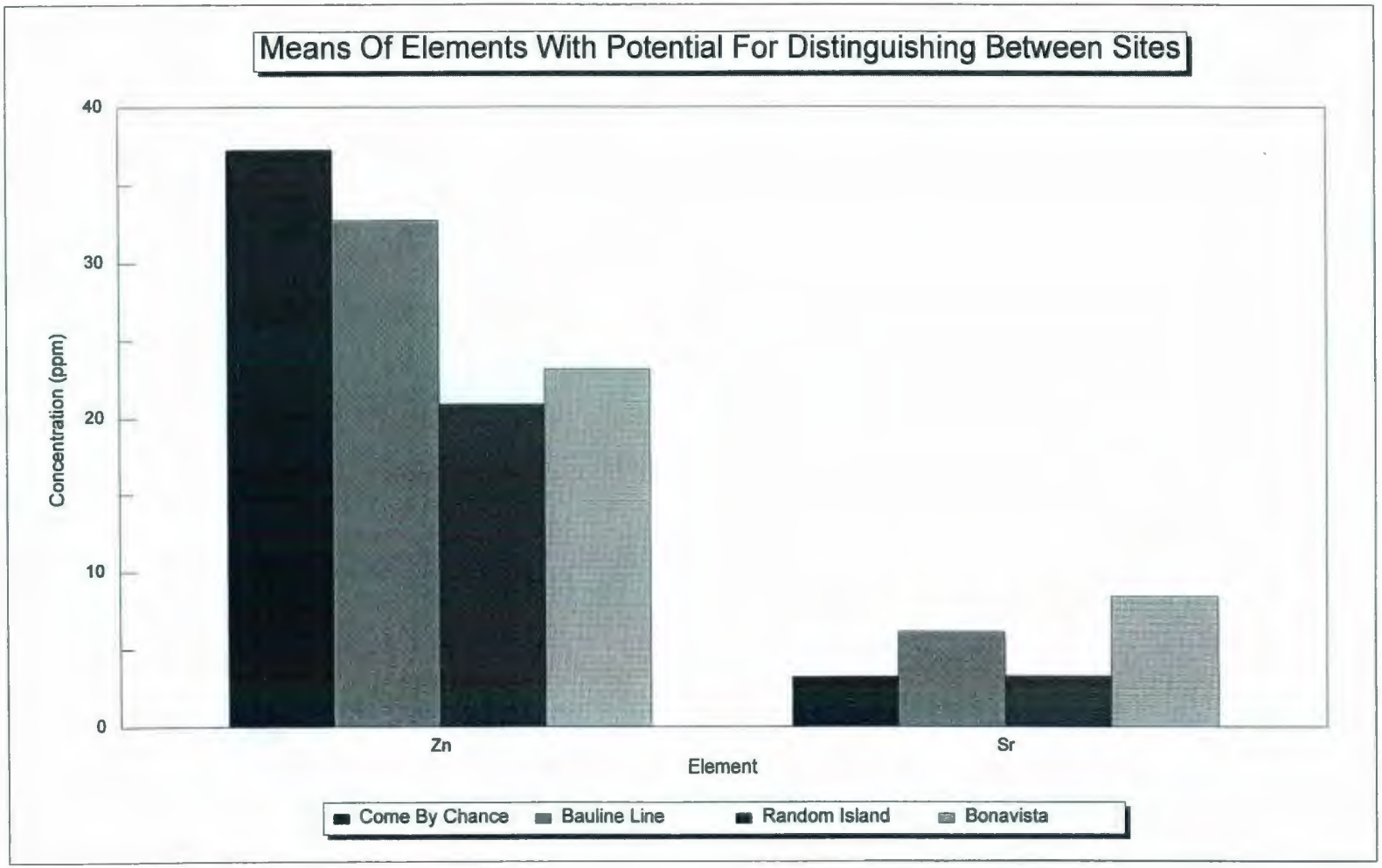

Figure 3.25: Concentrations (in ppm) shown as bar graphs for Zn and Sr for *Alectoria sarmentosa* from each of the four lichen sampling sites. Plotted with a linear scale to show more detail than in Figure 3.23.

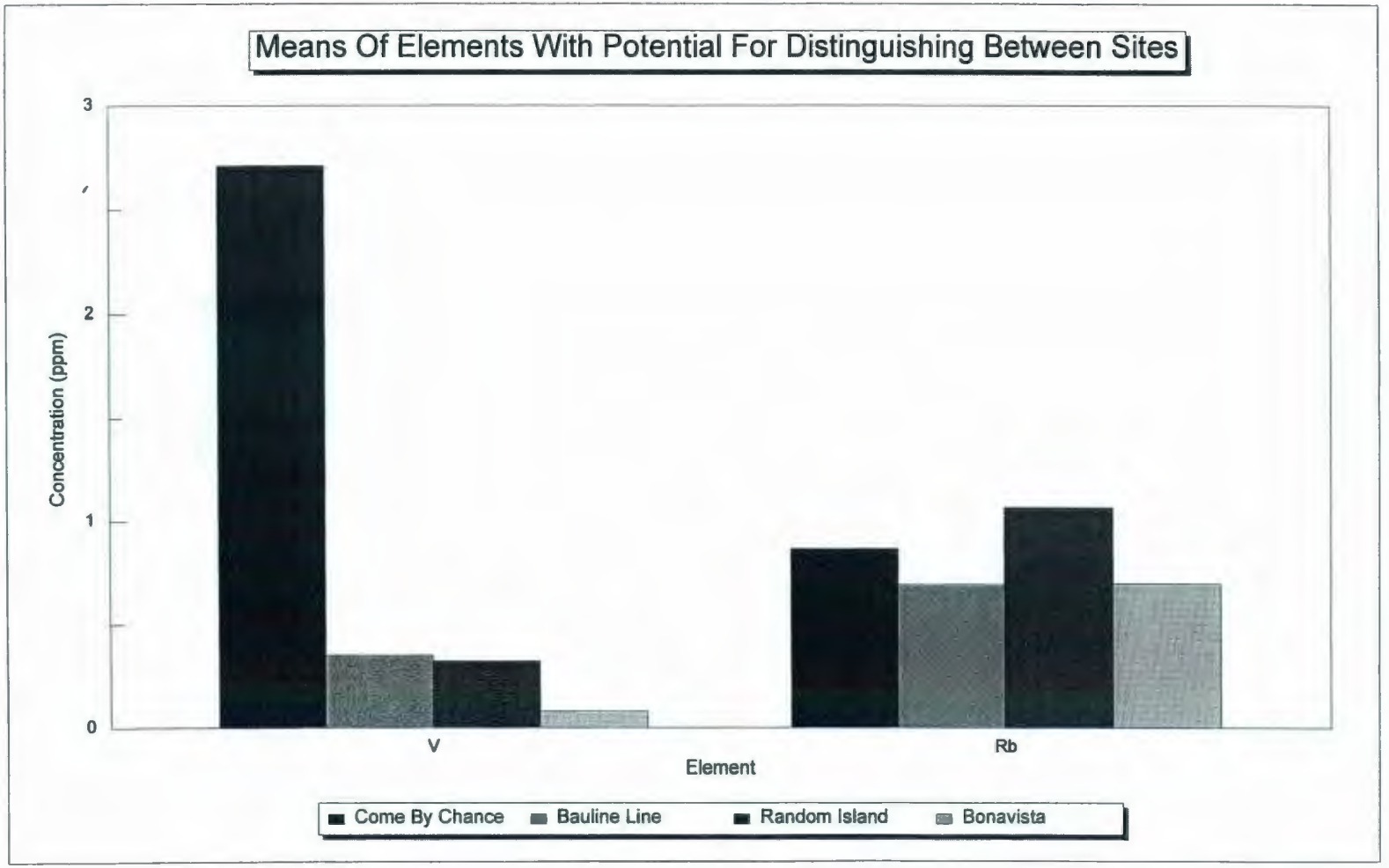

Figure 3.26: Concentrations (in ppm) shown as bar graphs for V and Rb for *Alectoria sarmentosa* from each of the four lichen sampling sites. Plotted with a linear scale to show more detail than in Figure 3.23.

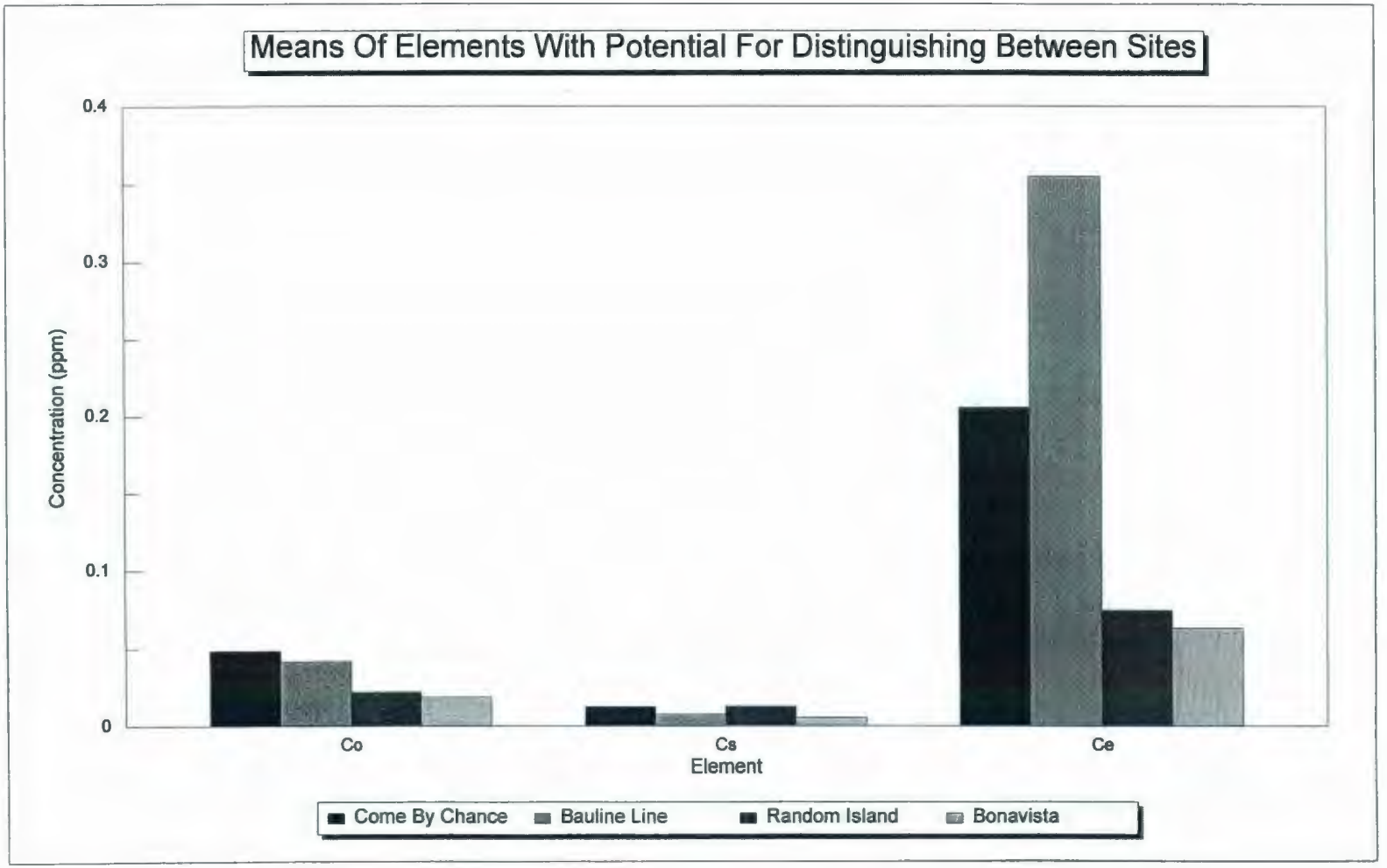

Figure 3.27: Concentrations (in ppm) shown as bar graphs for Co, Cs, and Ce for *Alectoria sarmentosa* from each of the four lichen sampling sites. Plotted with a linear scale to show more detail than in Figure 3.23.

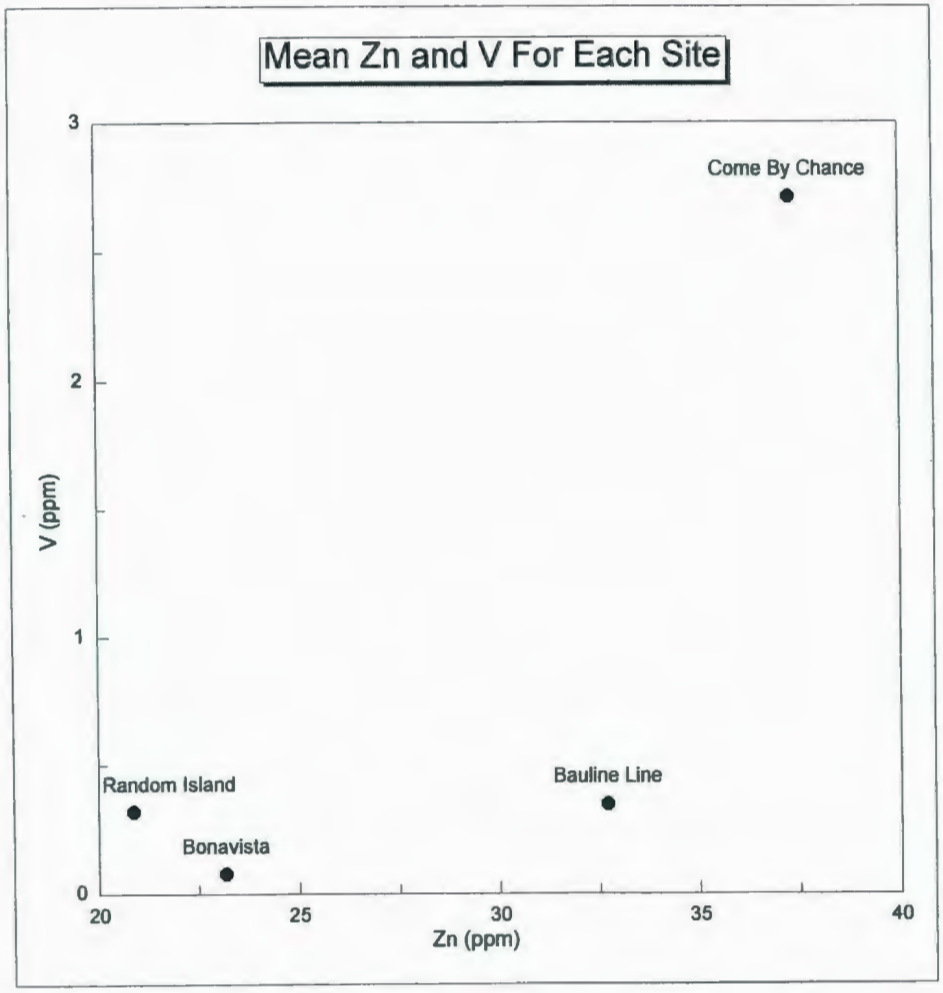

Figure 3.28: X-Y plot of V versus Zn concentrations (in ppm) for *Alectoria sarmentosa* from each sampling site.

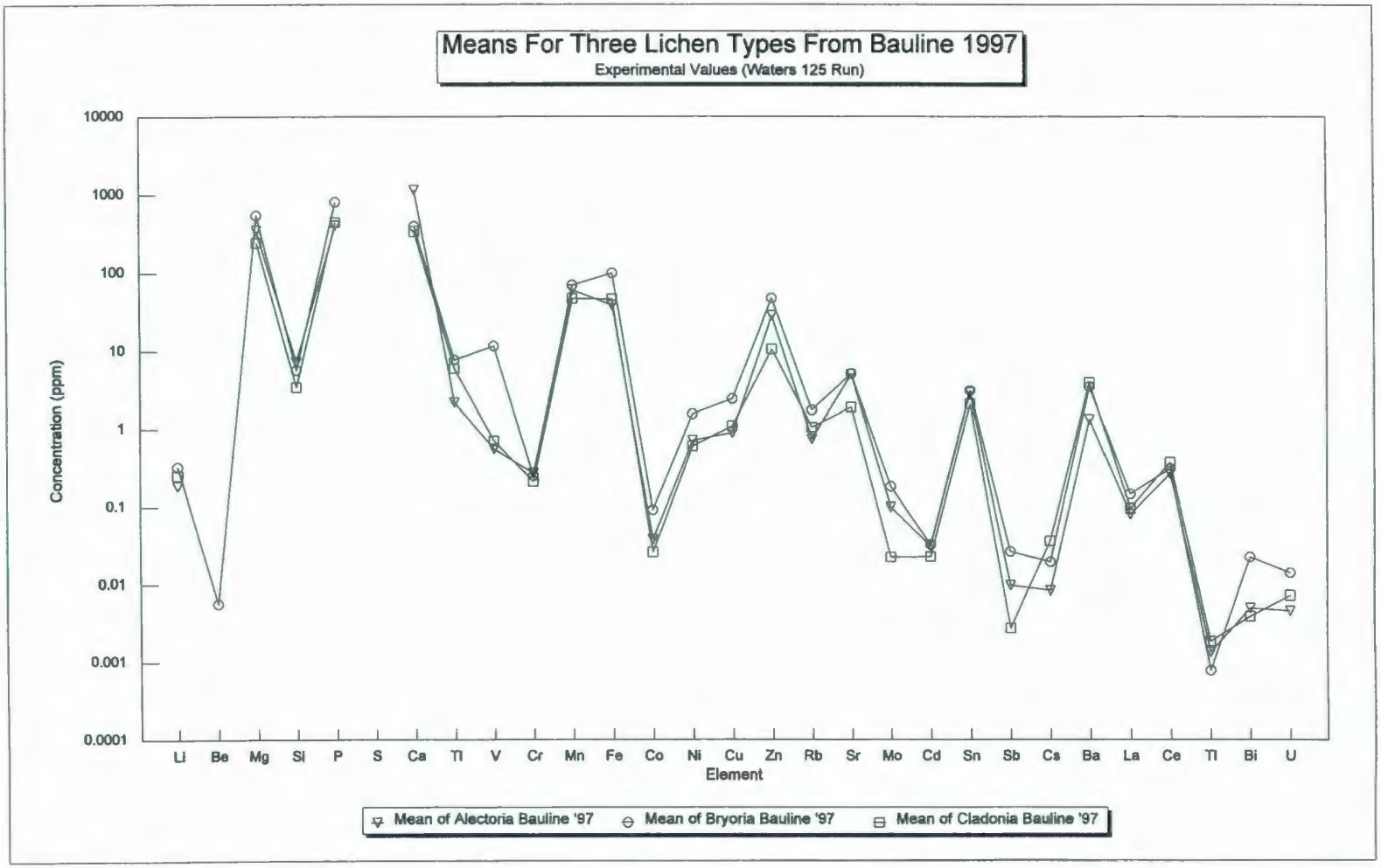

Figure 3.29: Mean concentrations (in ppm) of the three lichen species from Bauline Line (1997). These data are from the ICP-MS Waters 125 Run. The three species are *Alectoria sarmentosa*, *Bryoria sp.*, and *Cladonia alpestris*.

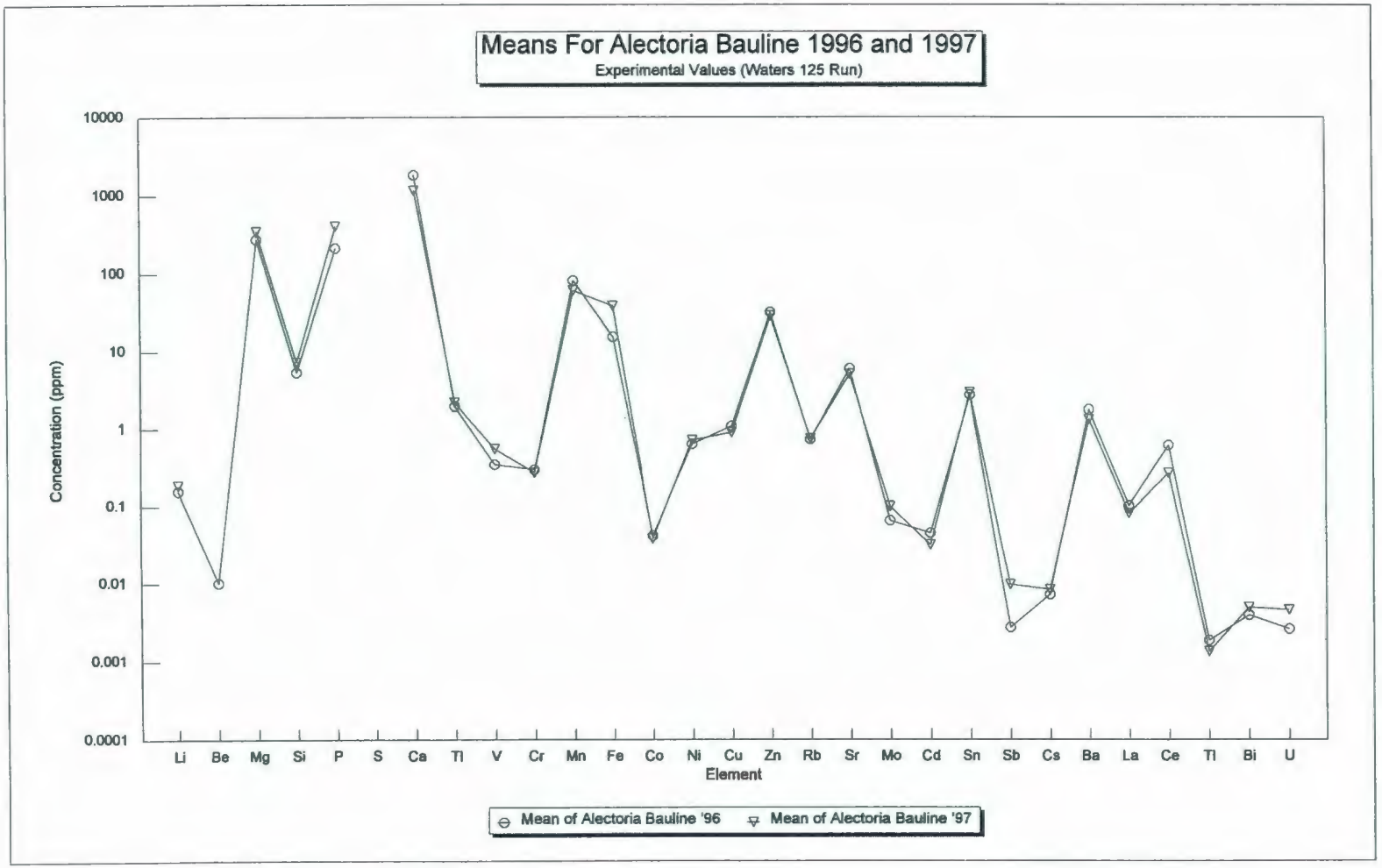

Figure 3.30: Mean concentrations (in ppm) of the *Alectoria sarmentosa* samples collected from Bauline Line in 1996 and 1997.

APPENDIX I : THE DIGESTION PROCEDURE

THE DIGESTION PROCEDURE

Before weighing samples, the certified reference material bottles were rolled on a mechanical roller for approximately 10 minutes; the collected lichen samples were rolled by hand for about 2 minutes. Approximately one gram of sample (crushed or powdered) was transferred into acid-washed quartz test tubes using a teflon-coated spatula, and weighed using an analytical balance. The tubes were covered with parafilm until the samples were dry ashed.

A dry ashing was then carried out. Acid-washed quartz caps were placed on the test tubes containing the samples. The tubes were placed in a 2000 ml beaker, covered by a watchglass, and placed into a muffle furnace at 100 °C. The temperature was increased at 35 °C per hour to a final temperature of 375 °C. The temperature was held at 375 °C until all visible soot cleared from the glassware (approximately one week).

A wet ashing was then done. The samples were cooled, the caps removed, then 0.4 ml of distilled 8 N HNO_3 was added to each. Test tubes were placed in heating blocks on a hot plate and heated to 90 °C. Aliquots of 0.2 ml of 30 % H_2O_2 were added to each test tube every 10-15 minutes; this was repeated for a total of 10 additions. The samples were evaporated to dryness and cooled. Wet ashings were carried out in a fumehood; samples were also under a plastic shield to avoid contamination.

Another dry ashing was carried out. The quartz caps were placed on the test tubes. The test tubes were placed in a 2000 ml beaker, covered by a watchglass, and placed in a muffle furnace at 100 °C. The furnace was set to 375 °C so that the temperature was raised from 100 °C to 375 °C in approximately 20 minutes. The samples were left at 375 °C for about 24 hours.

A second wet ashing was done in a manner similar to the first wet ashing. The samples were cooled, the caps removed, and 0.4 ml of distilled 8 N HNO_3 was added to each. The test tubes were heated to 90 °C in heating blocks on a hot plate. Aliquots of 0.2 ml of 30 % H_2O_2 were added to each sample every 10-15 minutes; this was repeated for a total of 10 additions. The samples were evaporated to dryness and cooled.

Then 2.5 ml of distilled 8 N HNO_3 was added to each sample. The samples were heated in heating blocks on a hot plate to 90 °C and held at that temperature for about 15 minutes in order to redissolve the residues. If all of the residues did not completely redissolve (as was generally the case), between 3-8 drops of 30 % H_2O_2 were added to aid in the dissolution. The samples were cooled.

Most of the samples were filtered by gravity using Whatman #1 (125 mm diameter) filter papers. The samples which were to have the residual particulates examined by SEM-EDX were filtered by suction filtration using acid-washed glassware and Millipore filter papers (25 mm in diameter). Immediately after filtration, these Millipore filter papers were affixed to glass microscope slides using double-sided tape and placed in a covered container until examination by SEM-EDX. The filtrates of all samples were made up to about 100 g with deionized water on a top-loading balance. A portion of the sample was then poured into a 15 ml plastic test tube and capped until analysis by ICP-MS. If a dilution was required as for the second and third sets of samples, 1 ml of sample was pipetted into a plastic test tube, the weight of sample was recorded, and then approximately 9 ml of 0.2 N HNO_3 was added, and the weight of acid recorded to yield a 1:10 sample dilution. (For those samples which required dilution, a calculation to account for this was included in the ICP-MS data reduction.)

Throughout the digestion procedure, the utmost care was taken to avoid contamination. Anytime the samples were not in use, they were covered by parafilm. Detailed notes were kept at all stages of the digestion procedure. To avoid potential contaminants, no soap was used in the laboratory where the work was carried out. The digestion procedure is summarized in Figure 2.3.

APPENDIX II: APPARATUS CLEANING PROCEDURES

APPARATUS CLEANING PROCEDURES

For procedures used in the analysis of trace metals, particular attention must be given to the methods of cleaning glassware and other equipment. Many of the cleaning procedures involve the use of acid to dissolve any metals present. For glassware utilized in sample digestion, quartz glass is preferable as it has less contaminants as compared to other types of glass, and, as well, quartz can be heated to temperatures greater than 1000°C (Potts, 1987).

The quartz test tubes, quartz test tube caps, and the three pieces of suction filtration glassware were cleaned by a 6 day cleaning process. The dirty glassware was placed in a 2 L beaker of 6 N HCl (covered by a watchglass) on a hot plate at approximately 90 °C for 2 days. The glassware was then cooled and rinsed with deionized water. The glassware was placed in a 2 L beaker of 4 N HNO_3 (covered by a watchglass) on a hot plate at approximately 90 °C for 2 days. The glassware was cooled and rinsed with deionized water. The glassware was then placed in a 2 L beaker of deionized water (covered by a watchglass) on a hot plate at approximately 90 °C for 2 days. The glassware was cooled and rinsed with deionized water and then dried in a HEPA-filtered drying hood.

The beakers used to hold cleaned lichen (before crushing) were placed in 4 N HNO_3 for 2 days. Then the beakers were rinsed in deionized water and placed to dry in a HEPA-filtered drying hood.

The plastic funnels used for filtration by gravity were cleaned by placing them in a 2 L beaker of 2 N HNO_3 for approximately 45 minutes to 1 hour. They were then rinsed in deionized water and dried in a HEPA-filtered drying hood.

The teflon-coated spatula, the teflon-coated tweezers, the agate mortar and pestle, and the plastic sieve and pan were cleaned by rinsing with deionized water, wiping with

kimwipes and ethanol, and rinsing with deionized water. These materials were then dried with kimwipes.

Before use, new glassware was placed for approximately 24 hours in 2 N HNO_3. (This nitric acid was used only for new glassware.) After removal from the nitric acid, the glassware was rinsed in deionized water and dried in a HEPA-filtered drying hood.

Before a lichen sample was crushed in the puck mill, quartz sand was crushed for a total of 30 seconds (15 seconds + 15 seconds). This is intended to remove any contamination from previous samples. The tungsten carbide portions of the puck mill (bowl, cover, ring, and puck) were wiped thoroughly with kimwipes and denatured ethanol before and after sand or a lichen sample was crushed. These materials were allowed to air-dry (the ethanol evaporated quickly).

The plastic test tubes and the plastic test tube caps were placed in 2 N HNO_3 for 24 hours. They were then rinsed with deionized water and dried in a HEPA-filtered drying hood. The plastic snap-cap containers were filled with 2 N HNO_3 and left for 24 hours (they were inverted for approximately the last 4 hours). Then they were rinsed and dried in the same manner as the plastic tubes and caps.

The porcelain crucibles used for moisture content determinations did not require thorough cleaning since they were not used for trace metal analysis. The crucibles were rinsed with deionized water, dried in an incubator oven, and then dried in another oven to 1050 °C.

The rubber stopper and metal clamp of the suction filtration apparatus were rinsed with deionized water and air-dried. Since the stopper and clamp did not contact the sample solutions, they did not need to be rigorously cleaned.

The deionized water used for this research was obtained from Barnstead Nanopure instruments at a resistivity of 17.0 megohm-cm or greater. The inflow into these machines was distilled water.

APPENDIX III: DETAILED SITE DESCRIPTIONS

DETAILED SITE DESCRIPTIONS

All sampling sites were located on the island of Newfoundland, which is on the eastern coast of Canada. Figure 2.1 illustrates the location of each site in Newfoundland. The samples from Come By Chance, Random Island, and Bonavista were collected by others for a related honours project (Evans, 1996). The descriptions of these three sampling sites were taken from Evans (1996). As mentioned in Chapter 2, Come By Chance is relatively polluted, Bauline Line is somewhat polluted, Random Island is intermediate, and Bonavista is relatively pristine. The Come By Chance, Random Island, and Bonavista sites form an approximately SW-NE transect.

Come By Chance

Area 2

Come By Chance is located on the isthmus of the Avalon Peninsula. Samples of *Alectoria sarmentosa* and *Bryoria sp.* were collected at the Area 2 site from Come By Chance (2-CBC) on May 19, 1995 (10:50 am). The samples were collected by Dr. M. Wadleigh, Martin Blake, and Nicholle Evans. The weather was cloudy and cool (about 5 °C). The sampling site is located 1 km from Trans Canada Highway, 100-200 m from houses and a hospital, approximately 50 m from a small pond, and 50 m away from an old railway. The most obvious potential pollution source in this area is the Come By Chance Oil Refinery.

The trees in this area are mature balsam fir and black spruce. The average tree diameter is about 10 cm and the tree spacing is 0.5 m. Lichen was sampled from balsam fir trees. The species of lichen present include: *Alectoria sarmentosa*, *Bryoria sp.*, *Platismatia*

sp., and *Usnea sp.* The *Alectoria sarmentosa* has thick stalks. *Alectoria sarmentosa* is slightly more abundant than *Bryoria sp.* in this area. *Platismatia sp.* is abundant on the trunks and branches of the trees.

Area 3

Samples of *Alectoria sarmentosa* and *Bryoria sp.* were collected at the Area 3 site from Come By Chance (3-CBC) on May 19, 1995 (11:25 am). The samples were collected by Dr. M. Wadleigh, Martin Blake, and Nicholle Evans. The weather was overcast and warmer than about 5°C. The site is located near the town of Come By Chance, at the point where the pavement ends. The site is on the edge of a bog on top of a hill, 200 m from the point where the pavement ends. The most obvious potential source of pollution in this area is the Come By Chance Oil Refinery.

This site was sampled previously by Martin Blake (site #022). The trees are mature with a spacing of 2 m and an average diameter of 15-25 cm. The stand has a well-developed canopy. Both black spruce and balsam fir trees are present in this area. Sampled from balsam fir trees. *Alectoria sarmentosa*, *Bryoria sp.*, and *Usnea sp.* are present. *Alectoria sarmentosa* and *Usnea sp.* are abundant on all trees.

Bauline Line

In General

The Bauline Line sampling site is located off of Bauline Line, which is a road running roughly SSW-NNE. Bauline Line is northeast of St. John's, on the Avalon Peninsula of Newfoundland. This portion of Bauline Line is a dirt road. The sampling site

is to the west of Bauline Line. There is a path that leading downhill from the dirt road to the sampling site. The site is in a wooded area between the road and a marshy area surrounding Piccos Pond. Across from the sampling site on the east side of Bauline Line is an area where peat is being excavated (approximately 50 m from road). The path leading to the sampling site is approximately across from the driveway leading to the peat excavation.

The potential sources of atmospheric pollution in this area include: dust from the dirt road, dust from the peat excavation, car exhaust, and pollution transported from other areas (such as from the nearby city of St. John's). The dirt road is in use, but the traffic is not heavy. The only house nearby is on the opposite side of Bauline Line and it is set back from the road. This house is approximately 150 m north of the driveway leading into the peat bog.

Samples were collected from Bauline Line in 1996 and 1997, but not at the exact locations each year. Below are the specific sampling details from both years.

1996 Sampling

Samples were collected with Gary Bugden on June 6, 1996 (Thursday), from about noon to 1:15 pm. *Alectoria sarmentosa* was collected for digestion in each of the 3 sets of samples analyzed. The weather was overcast at the start of sampling, and it rained slightly before sampling was completed.

The sampling area is approximately 100 m from road. This location is a small open area surrounded by trees. These trees are closely spaced, about 1 m apart (or less). The trees sampled from are approximately 10-15 cm in diameter, and about 3-4.5 m in height. Samples were collected from approximately 5-8 balsam fir trees. *Alectoria sarmentosa* and *Bryoria sp.* are both abundant, but *Bryoria sp.* is more abundant than *Alectoria sarmentosa.*

Usnea longissima is present on a couple of trees, but it is not abundant here.

Two paper sampling bags of *Alectoria sarmentosa* were collected for this work.

1997 Sampling

Samples were collected with Karen Wade on June 24, 1997 (Tuesday), from about 1-5 pm. *Alectoria sarmentosa, Bryoria sp.*, and *Cladonia alpestris* were collected from Bauline Line to compare the elemental composition of different species for the second set of samples digested and analyzed. The weather was overcast and cold during sampling. About 15 minutes after sampling was completed it rained slightly.

This specific location was about 100 m from the dirt road. This is not the exact spot where samples were collected in 1996, but it is the same general area. There were no patches of *Cladonia alpestris* growing in the place where the 1996 samples were collected, so that was not a suitable spot to collect the 3 required species. It was difficult to find a location where the 3 species were in close proximity to each other because patches of *Cladonia alpestris* tend to be in more open areas away from trees. The patch selected to collect the *Cladonia alpestris* was approximately 10-12 m from the trees where the *Alectoria sarmentosa* and *Bryoria sp.* were collected. This specific sampling location was close to a path.

Each of the 3 species collected are abundant in this area. *Bryoria sp.* seems more abundant than *Alectoria sarmentosa* in this location (at least on the living trees). In general, *Alectoria sarmentosa* and *Bryoria sp.* seem to be more abundant on dead trees than on living trees in this location. *Usnea longissima* was also present in several places.

Samples were collected from balsam fir trees. A twig was collected as an example

of balsam fir needles (the needles are flat). The trees sampled from are approximately 4.5 m in height and most trees were about 10-15 cm in diameter (two trees were slightly smaller than this).

Two paper sampling bags of *Alectoria sarmentosa*, two bags of *Bryoria sp.*, and two bags of *Cladonia alpestris* were collected for this research.

Random Island

Area 1

Samples of *Alectoria sarmentosa* and *Bryoria sp.* were collected at the Area 1 site from Random Island (1-RI) on May 18, 1995 (3:00 pm). The sampling was carried out by Dr. M. Wadleigh, Martin Blake, and Nicholle Evans. The weather was cool and overcast, with a slight breeze. This site is next to the mouth of a brook near a cabin. The brook is flowing from Nine Island Pond which flows into Hickmans Harbour Big Pond to the southeast. The site is 3 km from the main highway (231). The site is located next to a gravel road; logging activity occurs in this area. The most obvious potential pollution sources in this area are wood-cutting and dust from the road.

Balsam fir is the main type of tree present, with some black spruce also present. Lichen was sampled from balsam fir trees. The spacing of the trees is 1.5-2 m, and the average tree diameter is about 15 cm. The species of lichens present include: *Alectoria sarmentosa*, *Bryoria sp.*, and *Platismatia sp.* Both *Alectoria sarmentosa* and *Bryoria sp.* are very abundant here. The abundance of these two species is approximately equal on some trees. Overall, *Alectoria sarmentosa* is more abundant, with minor *Platismatia sp.*

Bonavista

Area 4

Samples of *Alectoria sarmentosa* and *Bryoria sp.* were collected at the Area 4 site from Bonavista (4-B) on May 18, 1995 (9:30 am). The samples were collected by Dr. M. Wadleigh, Martin Blake, and Nicholle Evans. The weather was overcast and cool (approximately 5 °C). The site is located 500 m from the main highway of Bonavista (230) and 50 m from a gravel trail (both to the east), and highway 235 is to the west 700 m. The most obvious potential pollution source in this area is wood smoke.

This site has a more mature forest than the previous sites. The average diameter of the trees is about 25-30 cm. This stand is more widely spaced, with a spacing of 2-4 m. The canopy is particularly open. The age of the stand is 41-60 years, the tree height is 3.6-6.5 m, and the ownership-nature of tenure is private/freehold. Both balsam fir and black spruce trees are present, with black spruce being more abundant. Lichens were sampled from balsam fir trees. The types of lichen present include: *Alectoria sarmentosa*, *Bryoria sp.*, and *Platismatia sp.*

Area 5

Samples of *Alectoria sarmentosa* and *Bryoria sp.* were collected at the Area 5 site from Bonavista (5-B) on May 18, 1995 (11:30 am). The samples were collected by Dr. M. Wadleigh, Martin Blake, and Nicholle Evans. The weather was sunny and about 8-10 °C, with relatively clear skies. The site is located 1.6 km away from the main highway of Bonavista (230) and 1 km from an abandoned railway, both to the east. The site is near a cut area as well as a bog. The most obvious potential pollution source in this area is wood

smoke.

This area has a relatively young dense black spruce and balsam fir forest, with the black spruce trees being more abundant. The tree spacing is 0.5 m, with an average tree diameter of approximately 10-15 cm. The species of lichen present include: *Platismatia sp.* (less abundant here), *Alectoria sarmentosa*, and *Bryoria sp.* *Alectoria sarmentosa* may be somewhat more abundant than *Bryoria sp.* here. *Alectoria sarmentosa* and *Bryoria sp.* were collected from both types of trees, and the samples were separated according to tree type.

APPENDIX IV: ICP-MS DATA (IN PPM)

ICP-MS DATA

The following pages present the ICP-MS data for this research. The concentration data are given in parts per million (ppm). The complete sample names are given in Tables 2.2-2.4.

Abbreviations used in the tables of data are as follows:

@avg = mean concentration

@stds = sample standard deviation

RSD = relative standard deviation

The RSD is expressed as a percentage and is calculated as follows:

RSD = mean concentration / sample standard deviation

Table IV.1a: ICP-MS data from the Waters 120 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt-1	Peach Leaves CRM	-0.06	-0.02	15.30	3621.21	193.28	16.78	1268.16	1077.66	285.44	14038.50	4.04	0.28
jt-2	Peach Leaves CRM	-0.12	-0.02	13.76	3529.67	190.22	20.24	1314.07	1317.95	350.91	14004.73	3.94	0.25
jt-3	Peach Leaves CRM	-0.11	-0.02	13.29	3550.15	186.66	25.40	1346.53	1502.07	382.62	13821.87	3.66	0.25
	@avg	-0.10	-0.02	14.12	3567.01	190.05	20.81	1309.59	1299.23	339.66	13955.03	3.88	0.26
	@stds	0.04	0.00	1.05	48.04	3.31	4.34	39.38	212.82	49.56	116.56	0.20	0.02
	RSD = stds / avg	-0.36	-0.06	0.07	0.01	0.02	0.21	0.03	0.16	0.15	0.01	0.05	0.07
jt-4	IAEA lichen CRM	0.29	-0.01	-1.34	555.26	430.79	27.30	541.72	861.57	336.58	2084.82	6.47	1.03
jt-5	IAEA lichen CRM	0.31	-0.01	-1.35	523.51	417.79	27.28	507.80	690.53	291.84	1957.74	6.20	0.99
jt-6	IAEA lichen CRM	0.43	-0.00	-1.17	581.98	466.89	28.90	560.45	740.67	285.99	2175.28	7.13	1.08
jt-7	IAEA lichen CRM	0.48	0.00	-0.72	588.78	486.09	21.88	555.16	642.08	203.64	2044.95	7.21	1.11
	@avg	0.38	-0.00	-1.15	562.38	450.39	26.34	541.29	733.71	279.51	2065.70	6.75	1.05
	@stds	0.09	0.01	0.29	29.68	31.59	3.07	23.68	94.26	55.40	90.30	0.49	0.05
	RSD = stds / avg	0.24	-1.81	-0.26	0.05	0.07	0.12	0.04	0.13	0.20	0.04	0.07	0.05
jt-8	BCR lichen CRM	0.67	0.03	1.29	471.60	589.35	13.66	611.54	645.92	23.51	1936.49	10.95	2.82
jt-9	BCR lichen CRM	0.68	0.03	1.12	503.16	642.12	8.29	617.76	524.25	15.22	2000.33	12.44	2.92
jt-10	BCR lichen CRM	0.67	0.03	1.24	508.78	655.73	9.39	633.91	582.64	9.52	2008.31	12.59	2.97
jt-11	BCR lichen CRM	0.63	0.03	1.27	487.20	613.47	8.58	604.45	499.98	-6.83	1890.52	11.74	2.88
	@avg	0.66	0.03	1.23	492.69	625.17	9.98	616.91	563.20	10.35	1958.91	11.93	2.89
	@stds	0.02	0.00	0.08	16.77	29.67	2.50	12.57	65.15	12.81	55.78	0.75	0.06
	RSD	0.04	0.08	0.06	0.03	0.05	0.25	0.02	0.12	1.24	0.03	0.06	0.02
jt-12	Alect. CBC(2) m&p	0.11	0.00	0.41	188.87	17.21	-1.04	220.02	-58.19	-24.79	1119.21	1.02	1.99
jt-13	Alect. CBC(2) m&p	0.13	-0.00	0.50	195.38	17.19	0.89	220.05	218.90	21.02	1243.14	1.01	2.02
	@avg	0.12	0.00	0.46	192.12	17.20	-0.08	220.04	80.36	-1.88	1181.17	1.02	2.00
	@stds	0.02	0.00	0.06	4.60	0.01	1.37	0.02	195.93	32.39	87.63	0.01	0.02
	RSD	0.12	-9.97	0.14	0.02	0.00	-17.93	0.00	2.44	-17.20	0.07	0.01	0.01
jt-14	Alect. CBC(2) p.m.	0.41	0.00	0.62	201.36	19.00	6.11	245.60	309.57	48.98	1116.48	1.17	1.73
jt-15	Alect. CBC(2) p.m.	0.36	-0.00	0.36	200.43	18.79	7.47	283.67	230.95	48.76	1088.76	1.26	1.76
	@avg	0.38	-0.00	0.49	200.89	18.89	6.79	264.64	270.26	48.87	1102.62	1.22	1.74
	@stds	0.04	0.00	0.18	0.66	0.15	0.96	26.92	55.60	0.16	19.60	0.06	0.02
	RSD	0.09	-1.05	0.38	0.00	0.01	0.14	0.10	0.21	0.00	0.02	0.05	0.01

Table IV.1a continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt-1	Peach Leaves CRM	0.81	84.09	194.95	0.10	0.55	3.46	16.70	0.09	4.14	0.13	19.28	51.66
jt-2	Peach Leaves CRM	0.85	82.17	152.97	0.09	0.41	3.43	16.39	0.08	6.37	0.09	19.10	49.78
jt-3	Peach Leaves CRM	0.84	82.21	194.19	0.09	0.59	3.53	16.55	0.08	7.24	0.15	18.52	48.52
	@avg	0.83	82.82	180.71	0.09	0.52	3.47	16.54	0.09	5.92	0.12	18.97	49.99
	@stds	0.02	1.09	24.02	0.00	0.09	0.05	0.15	0.01	1.60	0.03	0.40	1.58
	RSD = stds / avg	0.03	0.01	0.13	0.03	0.18	0.01	0.01	0.06	0.27	0.24	0.02	0.03
jt-4	IAEA lichen CRM	0.83	56.38	337.69	0.25	0.85	3.16	28.18	0.28	3.57	-0.08	1.24	7.59
jt-5	IAEA lichen CRM	0.76	52.67	323.23	0.25	0.86	3.07	27.31	0.28	2.38	0.05	1.20	7.40
jt-6	IAEA lichen CRM	0.85	58.94	354.24	0.27	0.90	3.26	29.89	0.27	1.82	0.19	1.30	8.06
jt-7	IAEA lichen CRM	0.84	59.17	365.35	0.27	0.91	3.95	34.56	0.31	1.32	0.19	1.34	8.03
	@avg	0.82	56.79	345.13	0.26	0.88	3.36	29.98	0.29	2.27	0.09	1.27	7.77
	@stds	0.04	3.02	18.50	0.01	0.03	0.40	3.23	0.02	0.97	0.13	0.06	0.33
	RSD = stds / avg	0.05	0.05	0.05	0.05	0.04	0.12	0.11	0.06	0.43	1.42	0.05	0.04
jt-8	BCR lichen CRM	2.87	25.31	629.67	0.26	2.10	5.79	89.73	0.27	0.67	0.40	7.79	8.72
jt-9	BCR lichen CRM	3.17	26.03	668.90	0.27	2.56	6.08	92.92	0.24	1.40	0.19	8.21	9.04
jt-10	BCR lichen CRM	3.14	26.48	678.14	0.27	2.13	6.06	95.05	0.28	1.30	0.28	8.35	9.11
jt-11	BCR lichen CRM	2.95	25.67	657.91	0.26	2.41	5.87	98.25	0.25	1.19	0.23	8.00	8.66
	@avg	3.03	25.87	658.65	0.27	2.30	5.95	93.99	0.26	1.14	0.27	8.09	8.88
	@stds	0.15	0.50	21.02	0.01	0.22	0.14	3.59	0.02	0.32	0.09	0.25	0.23
	RSD	0.05	0.02	0.03	0.02	0.10	0.02	0.04	0.06	0.28	0.34	0.03	0.03
jt-12	Alect. CBC(2) m&p	0.21	37.79	14.28	0.05	1.27	1.17	42.01	0.03	0.01	-0.01	0.93	3.64
jt-13	Alect. CBC(2) m&p	0.25	37.76	14.19	0.05	1.22	0.97	43.19	0.03	-0.13	0.11	0.93	3.84
	@avg	0.23	37.78	14.23	0.05	1.24	1.07	42.60	0.03	-0.07	0.05	0.93	3.74
	@stds	0.03	0.02	0.07	0.00	0.04	0.14	0.83	0.00	0.10	0.09	0.00	0.14
	RSD	0.12	0.00	0.00	0.01	0.03	0.13	0.02	0.11	-1.52	1.57	0.00	0.04
jt-14	Alect. CBC(2) p.m.	0.21	43.86	15.26	9.77	1.17	1.03	41.86	0.03	-0.10	0.04	0.97	3.43
jt-15	Alect. CBC(2) p.m.	0.20	43.54	14.47	9.62	2.46	2.44	45.24	0.04	-0.17	0.21	0.96	3.43
	@avg	0.21	43.70	14.87	9.69	1.82	1.74	43.55	0.03	-0.13	0.13	0.96	3.43
	@stds	0.01	0.23	0.56	0.11	0.91	0.99	2.40	0.01	0.05	0.12	0.00	0.00
	RSD	0.04	0.01	0.04	0.01	0.50	0.57	0.06	0.16	-0.33	0.95	0.00	0.00

Table IV.1a continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt-1	Peach Leaves CRM	0.06	0.00	0.03	4.18	0.01	-0.02	0.10	115.84	9.12	10.16	0.01	0.02
jt-2	Peach Leaves CRM	0.07	0.00	0.02	3.81	0.00	-0.03	0.08	115.02	8.91	10.30	0.05	0.02
jt-3	Peach Leaves CRM	0.08	0.00	0.03	3.83	0.01	-0.03	0.07	114.06	8.96	10.22	0.02	0.02
	@avg	0.07	0.00	0.03	3.94	0.01	-0.02	0.09	114.97	9.00	10.23	0.02	0.02
	@stds	0.01	0.00	0.00	0.21	0.00	0.01	0.02	0.89	0.11	0.07	0.02	0.00
	RSD = stds / avg	0.11	0.58	0.11	0.05	0.19	-0.32	0.17	0.01	0.01	0.01	0.75	0.08
jt-4	IAEA lichen CRM	0.05	0.01	0.11	4.14	0.02	-0.04	0.09	4.60	0.44	0.93	0.01	0.01
jt-5	IAEA lichen CRM	0.05	0.01	0.10	3.95	0.01	-0.04	0.08	4.25	0.40	0.86	0.01	0.01
jt-6	IAEA lichen CRM	0.05	0.01	0.10	4.39	0.01	-0.04	0.09	4.89	0.46	0.98	-0.00	0.00
jt-7	IAEA lichen CRM	0.05	0.01	0.10	4.40	0.02	-0.04	0.09	5.15	0.48	1.04	0.01	0.01
	@avg	0.05	0.01	0.10	4.22	0.02	-0.04	0.09	4.72	0.44	0.95	0.01	0.01
	@stds	0.00	0.00	0.01	0.22	0.00	0.00	0.01	0.39	0.04	0.08	0.01	0.00
	RSD = stds / avg	0.05	0.16	0.06	0.05	0.14	-0.08	0.05	0.08	0.08	0.08	0.83	0.29
jt-8	BCR lichen CRM	0.33	0.01	0.50	7.33	0.03	-0.01	0.17	9.31	0.52	1.08	-0.02	0.03
jt-9	BCR lichen CRM	0.33	0.03	0.50	6.86	0.04	-0.02	0.19	10.13	0.58	1.20	-0.02	0.03
jt-10	BCR lichen CRM	0.32	0.02	0.50	7.37	0.04	-0.02	0.19	10.17	0.57	1.18	-0.03	0.03
jt-11	BCR lichen CRM	0.30	0.02	0.49	6.50	0.04	-0.03	0.18	9.44	0.51	1.09	-0.03	0.03
	@avg	0.32	0.02	0.50	7.01	0.04	-0.02	0.18	9.76	0.54	1.14	-0.02	0.03
	@stds	0.01	0.01	0.00	0.42	0.00	0.01	0.01	0.45	0.03	0.06	0.01	0.00
	RSD	0.04	0.33	0.01	0.06	0.11	-0.38	0.04	0.05	0.06	0.05	-0.30	0.05
jt-12	Alect. CBC(2) m&p	0.07	0.00	0.06	4.24	0.07	-0.03	0.01	1.93	0.06	0.16	0.00	-0.00
jt-13	Alect. CBC(2) m&p	0.08	0.00	0.06	4.51	0.06	-0.03	0.01	1.89	0.07	0.18	-0.02	0.00
	@avg	0.08	0.00	0.06	4.38	0.06	-0.03	0.01	1.91	0.06	0.17	-0.01	0.00
	@stds	0.00	0.00	0.00	0.19	0.01	0.00	0.00	0.03	0.00	0.01	0.02	0.00
	RSD	0.03	3.04	0.07	0.04	0.08	-0.02	0.06	0.02	0.04	0.07	-1.48	3.04
jt-14	Alect. CBC(2) p.m.	0.08	0.01	0.06	3.93	0.13	-0.03	0.02	2.29	0.06	0.15	0.02	0.00
jt-15	Alect. CBC(2) p.m.	0.09	0.01	0.06	4.05	0.14	-0.04	0.01	2.34	0.07	0.15	0.02	0.00
	@avg	0.08	0.01	0.06	3.99	0.14	-0.03	0.02	2.32	0.06	0.15	0.02	0.00
	@stds	0.00	0.00	0.00	0.09	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.00
	RSD	0.03	0.12	0.02	0.02	0.05	-0.06	0.07	0.01	0.04	0.01	0.07	0.89

Table IV.1a continued

Sample #	Sample Name	Pb	Bi	U
jt-1	Peach Leaves CRM	0.85	0.00	0.01
jt-2	Peach Leaves CRM	0.85	0.00	0.01
jt-3	Peach Leaves CRM	0.82	0.00	0.01
	@avg	0.84	0.00	0.01
	@stds	0.02	0.00	0.00
	RSD = stds / avg	0.02	0.24	0.17
jt-4	IAEA lichen CRM	4.17	0.01	0.03
jt-5	IAEA lichen CRM	4.15	0.01	0.03
jt-6	IAEA lichen CRM	4.26	0.01	0.03
jt-7	IAEA lichen CRM	4.51	0.02	0.03
	@avg	4.27	0.01	0.03
	@stds	0.17	0.00	0.00
	RSD = stds / avg	0.04	0.08	0.07
jt-8	BCR lichen CRM	32.23	0.09	0.03
jt-9	BCR lichen CRM	33.57	0.10	0.03
jt-10	BCR lichen CRM	33.74	0.09	0.04
jt-11	BCR lichen CRM	32.73	0.09	0.03
	@avg	33.07	0.09	0.03
	@stds	0.71	0.00	0.00
	RSD	0.02	0.02	0.06
jt-12	Alect. CBC(2) m&p	3.12	0.01	0.00
jt-13	Alect. CBC(2) m&p	3.43	0.01	0.01
	@avg	3.28	0.01	0.00
	@stds	0.22	0.00	0.00
	RSD	0.07	0.09	0.26
jt-14	Alect. CBC(2) p.m.	3.19	0.01	0.00
jt-15	Alect. CBC(2) p.m.	3.37	0.01	0.01
	@avg	3.28	0.01	0.01
	@stds	0.13	0.00	0.00
	RSD	0.04	0.25	0.34

Table IV.1b: ICP-MS data from the Waters 120 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt-16	Alect. Baul. '96 m&p	0.18	-0.01	0.00	312.33	23.19	1.14	226.38	268.32	32.61	1647.78	1.51	0.40
jt-17	Alect. Baul. '96 m&p	0.20	-0.01	-0.31	336.64	25.44	-1.62	236.70	276.49	53.52	1708.84	1.65	0.42
	@avg	0.19	-0.01	-0.15	324.49	24.31	-0.24	231.54	272.40	43.06	1678.31	1.58	0.41
	@stds	0.01	0.00	0.22	17.19	1.60	1.95	7.30	5.77	14.79	43.18	0.10	0.01
	RSD	0.07	-0.05	-1.44	0.05	0.07	-8.13	0.03	0.02	0.34	0.03	0.06	0.03
jt-18	Alect. Baul. '96 p.m.	0.22	-0.01	-0.38	344.31	20.71	-2.66	275.45	383.48	58.25	2843.39	1.59	0.30
jt-19	Alect. Baul. '96 p.m.	0.22	-0.01	-0.27	351.11	20.87	5.47	274.22	535.62	71.28	2806.32	1.70	0.31
	@avg	0.22	-0.01	-0.32	347.71	20.79	1.40	274.84	459.55	64.76	2824.86	1.64	0.30
	@stds	0.00	0.00	0.08	4.81	0.11	5.75	0.88	107.58	9.22	26.21	0.07	0.01
	RSD	0.01	-0.30	-0.24	0.01	0.01	4.09	0.00	0.23	0.14	0.01	0.04	0.03
jt-20	Alect. Bon.(4) m&p	0.19	-0.01	-0.30	755.51	14.92	2.54	523.85	410.72	89.06	1029.79	1.07	0.06
jt-21	Alect. Bon.(4) m&p	0.28	-0.01	-0.00	781.49	15.07	9.18	530.99	595.70	132.79	1048.26	0.98	0.06
	@avg	0.24	-0.01	-0.15	768.50	15.00	5.86	527.42	503.21	110.92	1039.03	1.03	0.06
	@stds	0.06	0.00	0.21	18.37	0.11	4.69	5.05	130.80	30.92	13.06	0.06	0.00
	RSD	0.26	-0.10	-1.38	0.02	0.01	0.80	0.01	0.26	0.28	0.01	0.06	0.00
jt-22	Alect. Bon.(4) p.m.	0.37	-0.00	-0.00	779.41	17.80	8.51	571.86	524.49	105.54	1208.44	1.01	0.05
jt-23	Alect. Bon.(4) p.m.	0.41	-0.00	0.28	751.59	17.35	-0.29	577.04	419.18	44.06	1216.56	1.10	0.08
	@avg	0.39	-0.00	0.14	765.50	17.57	4.11	574.45	471.84	74.80	1212.50	1.05	0.06
	@stds	0.03	0.00	0.20	19.67	0.32	6.23	3.66	74.47	43.48	5.74	0.07	0.02
	RSD	0.08	-0.31	1.44	0.03	0.02	1.51	0.01	0.16	0.58	0.00	0.06	0.26
jt-24	Reagent Blank	-0.00	-0.00	0.17	6.12	0.40	-1.31	3.31	239.94	59.47	54.33	0.01	-0.02
jt-25	Reagent Blank	-0.01	-0.01	0.10	5.12	0.31	-0.90	3.76	179.54	68.20	45.36	0.03	-0.03
jt-26	Reagent Blank	-0.01	-0.00	-0.14	7.04	0.51	-6.99	2.53	61.95	79.59	60.71	0.01	-0.04
	@avg	-0.01	-0.00	0.04	6.10	0.41	-3.07	3.20	160.48	69.09	53.47	0.02	-0.03
	@stds	0.00	0.00	0.16	0.96	0.10	3.40	0.62	90.51	10.09	7.72	0.01	0.01
	RSD	-0.80	-0.16	3.86	0.16	0.24	-1.11	0.19	0.56	0.15	0.14	0.67	-0.25
jt-27*	Duplicate of jt-7 (IAEA)	0.63	0.02	0.98	573.84	499.39	4.99	556.00	330.92	58.65	2300.45	7.25	1.07
jt-28*	Dup. of jt-10 (BCR)	0.61	0.04	0.81	486.00	648.41	12.06	660.07	889.10	77.97	2051.32	12.44	2.88
jt-30*	Dup. of jt-22 (Alect. Bon.)	0.38	0.00	0.10	764.59	17.67	1.53	579.20	618.31	101.08	1242.00	1.06	0.03

Table IV.1b continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt-16	Alect. Baul. '96 m&p	0.39	88.37	13.02	0.05	0.46	0.87	33.36	0.01	-0.15	0.07	0.66	5.94
jt-17	Alect. Baul. '96 m&p	0.42	92.21	16.10	0.05	0.46	0.85	35.44	0.01	-0.34	0.11	0.72	6.37
	@avg	0.41	90.29	14.56	0.05	0.46	0.86	34.40	0.01	-0.25	0.09	0.69	6.16
	@stds	0.03	2.72	2.18	0.00	0.00	0.02	1.47	0.00	0.13	0.02	0.04	0.31
	RSD	0.06	0.03	0.15	0.07	0.01	0.02	0.04	0.09	-0.55	0.26	0.06	0.05
jt-18	Alect. Baul. '96 p.m.	0.26	100.95	8.78	10.10	0.52	0.77	37.22	0.01	-0.44	0.15	0.70	9.29
jt-19	Alect. Baul. '96 p.m.	0.28	101.04	14.51	10.03	0.97	1.34	39.41	0.01	-0.43	0.13	0.71	9.38
	@avg	0.27	101.00	11.65	10.07	0.74	1.06	38.31	0.01	-0.43	0.14	0.71	9.34
	@stds	0.01	0.06	4.05	0.05	0.32	0.41	1.55	0.01	0.01	0.01	0.00	0.07
	RSD	0.05	0.00	0.35	0.00	0.43	0.38	0.04	0.61	-0.03	0.10	0.00	0.01
jt-20	Alect. Bon.(4) m&p	0.26	130.01	15.79	0.03	0.15	0.78	28.76	0.01	-0.28	0.16	0.79	8.82
jt-21	Alect. Bon.(4) m&p	0.26	135.60	14.58	0.03	0.14	0.85	28.85	0.01	-0.16	0.22	0.80	8.79
	@avg	0.26	132.80	15.18	0.03	0.14	0.81	28.81	0.01	-0.22	0.19	0.79	8.81
	@stds	0.00	3.95	0.86	0.00	0.01	0.05	0.07	0.00	0.09	0.05	0.01	0.02
	RSD	0.01	0.03	0.06	0.03	0.04	0.06	0.00	0.03	-0.41	0.25	0.01	0.00
jt-22	Alect. Bon.(4) p.m.	0.24	125.09	14.47	9.16	0.16	0.80	29.26	0.02	-0.24	0.24	0.78	9.18
jt-23	Alect. Bon.(4) p.m.	0.22	121.91	15.53	9.19	0.14	0.71	27.94	0.02	-0.22	0.08	0.78	9.26
	@avg	0.23	123.50	15.00	9.18	0.15	0.75	28.60	0.02	-0.23	0.16	0.78	9.22
	@stds	0.02	2.24	0.75	0.02	0.01	0.07	0.94	0.00	0.02	0.12	0.00	0.06
	RSD	0.08	0.02	0.05	0.00	0.07	0.09	0.03	0.10	-0.07	0.74	0.00	0.01
jt-24	Reagent Blank	0.03	0.05	-0.18	-0.00	0.02	0.18	0.33	-0.01	-0.49	-0.11	0.00	0.16
jt-25	Reagent Blank	0.01	0.04	-0.38	0.00	0.02	0.09	0.18	-0.01	-0.48	-0.05	0.00	0.14
jt-26	Reagent Blank	-0.00	0.05	-1.20	-0.00	0.02	0.18	0.28	-0.01	-0.54	-0.04	0.00	0.19
	@avg	0.01	0.05	-0.58	-0.00	0.02	0.15	0.26	-0.01	-0.50	-0.07	0.00	0.16
	@stds	0.02	0.01	0.54	0.00	0.00	0.05	0.07	0.00	0.03	0.04	0.00	0.03
	RSD	1.29	0.15	-0.93	-1.25	0.15	0.32	0.28	-0.07	-0.07	-0.57	0.40	0.17
jt-27*	Duplicate of jt-7 (IAEA)	0.82	59.70	373.00	0.26	0.89	3.80	33.68	0.30	-0.38	0.08	1.34	8.00
jt-28*	Dup. of jt-10 (BCR)	3.10	26.23	661.70	0.28	2.18	6.09	90.90	0.30	0.52	0.17	8.03	8.91
jt-30*	Dup. of jt-22 (Alect. Bon.)	0.20	123.01	13.88	9.20	0.15	0.77	28.85	0.01	-0.19	-0.01	0.78	9.16

Table IV.1b continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt-16	Alect. Baul. '96 m&p	0.08	0.00	0.05	4.16	0.00	-0.02	0.01	1.80	0.07	0.15	0.00	0.00
jt-17	Alect. Baul. '96 m&p	0.09	0.00	0.05	4.15	0.00	-0.02	0.01	1.92	0.08	0.15	0.01	0.00
	@avg	0.08	0.00	0.05	4.16	0.00	-0.02	0.01	1.86	0.08	0.15	0.01	0.00
	@stds	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.00	0.00	0.01	0.00
	RSD	0.07	2.27	0.03	0.00	0.03	-0.15	0.03	0.05	0.03	0.01	0.74	1.20
jt-18	Alect. Baul. '96 p.m.	0.09	0.01	0.08	3.64	0.00	-0.03	0.01	2.17	0.11	0.24	0.03	0.00
jt-19	Alect. Baul. '96 p.m.	0.09	0.01	0.08	3.63	0.00	-0.03	0.01	2.24	0.11	0.24	0.03	0.00
	@avg	0.09	0.01	0.08	3.64	0.00	-0.03	0.01	2.20	0.11	0.24	0.03	0.00
	@stds	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00
	RSD	0.04	0.30	0.05	0.00	0.00	-0.03	0.07	0.02	0.01	0.01	0.07	0.61
jt-20	Alect. Bon.(4) m&p	0.05	0.00	0.05	4.36	0.00	-0.03	0.01	2.28	0.02	0.08	0.02	0.00
jt-21	Alect. Bon.(4) m&p	0.04	0.00	0.05	4.31	0.01	-0.03	0.01	2.31	0.02	0.08	0.01	0.00
	@avg	0.04	0.00	0.05	4.33	0.01	-0.03	0.01	2.29	0.02	0.08	0.01	0.00
	@stds	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00
	RSD	0.08	2.24	0.05	0.01	0.14	-0.01	0.03	0.01	0.02	0.04	0.80	-0.30
jt-22	Alect. Bon.(4) p.m.	0.06	0.01	0.06	3.36	0.00	-0.04	0.01	2.26	0.03	0.07	0.03	0.00
jt-23	Alect. Bon.(4) p.m.	0.06	0.01	0.06	3.33	0.00	-0.01	0.01	2.25	0.03	0.07	0.02	0.00
	@avg	0.06	0.01	0.06	3.35	0.00	-0.02	0.01	2.25	0.03	0.07	0.03	0.00
	@stds	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.01	0.00
	RSD	0.04	0.13	0.02	0.01	0.01	-0.78	0.22	0.00	0.02	0.02	0.32	0.26
jt-24	Reagent Blank	0.00	-0.00	-0.00	3.21	0.00	-0.02	-0.00	0.12	0.00	0.00	0.02	0.00
jt-25	Reagent Blank	0.00	-0.00	0.00	3.02	0.00	-0.03	0.00	0.12	0.00	0.00	0.03	0.00
jt-26	Reagent Blank	-0.00	-0.00	0.00	3.27	0.00	-0.03	0.00	0.61	0.00	0.00	0.01	-0.00
	@avg	0.00	-0.00	0.00	3.17	0.00	-0.03	0.00	0.29	0.00	0.00	0.02	0.00
	@stds	0.00	0.00	0.00	0.13	0.00	0.01	0.00	0.28	0.00	0.00	0.01	0.00
	RSD	13.75	-0.01	2.79	0.04	-18.08	-0.20	-0.57	0.99	1.07	0.39	0.37	-0.38
jt-27*	Duplicate of jt-7 (IAEA)	0.05	0.01	0.09	4.56	0.02	-0.04	0.09	5.15	0.47	1.04	0.01	0.01
jt-28*	Dup. of jt-10 (BCR)	0.33	0.02	0.48	6.98	0.04	-0.04	0.19	9.72	0.56	1.20	-0.01	0.03
jt-30*	Dup. of jt-22 (Alect. Bon.)	0.06	0.01	0.05	3.29	0.00	-0.04	0.01	2.19	0.03	0.07	0.03	0.00

Table IV.1b continued

Sample #	Sample Name	Pb	Bi	U
jt-16	Alect. Baul. '96 m&p	2.78	0.01	0.00
jt-17	Alect. Baul. '96 m&p	2.92	0.00	0.00
	@avg	2.85	0.00	0.00
	@stds	0.10	0.00	0.00
	RSD	0.03	0.33	0.21
jt-18	Alect. Baul. '96 p.m.	4.74	0.01	0.00
jt-19	Alect. Baul. '96 p.m.	4.71	0.01	0.00
	@avg	4.72	0.01	0.00
	@stds	0.02	0.00	0.00
	RSD	0.00	0.09	0.12
jt-20	Alect. Bon.(4) m&p	1.34	0.00	0.00
jt-21	Alect. Bon.(4) m&p	1.27	0.00	0.01
	@avg	1.31	0.00	0.01
	@stds	0.05	0.00	0.00
	RSD	0.04	0.32	0.32
jt-22	Alect. Bon.(4) p.m.	1.61	0.00	0.01
jt-23	Alect. Bon.(4) p.m.	1.62	0.00	0.00
	@avg	1.62	0.00	0.00
	@stds	0.01	0.00	0.00
	RSD	0.00	0.26	0.26
jt-24	Reagent Blank	0.01	-0.00	0.00
jt-25	Reagent Blank	0.01	0.00	0.00
jt-26	Reagent Blank	0.01	0.00	0.00
	@avg	0.01	0.00	0.00
	@stds	0.00	0.00	0.00
	RSD	0.22	-1.35	3.04
jt-27*	Duplicate of jt-7 (IAEA)	4.41	0.01	0.03
jt-28*	Dup. of jt-10 (BCR)	33.83	0.10	0.04
jt-30*	Dup. of jt-22 (Alect. Bon.)	1.58	0.00	0.00

Table IV.2a: ICP-MS data from the Waters 125 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt-24-bk	reagent blank	0.11	0.01	5.40	6.15	0.51	6.52	-11.88	—	45.26	57.49	0.00	0.01
jt-25-bk	reagent blank	0.07	-0.00	-0.34	6.68	0.38	4.47	9.64	—	37.43	64.63	0.04	0.01
jt26-bk	reagent blank	0.10	0.00	2.94	7.08	0.44	2.70	-24.08	—	68.31	62.10	0.07	0.02
	@avg	0.09	0.00	2.67	6.64	0.44	4.56	-8.77	—	50.34	61.41	0.04	0.02
	@stds	0.02	0.00	2.88	0.47	0.07	1.91	17.07	—	16.05	3.62	0.03	0.01
	RSD = stds / avg	0.21	1.33	1.08	0.07	0.15	0.42	-1.95	—	0.32	0.06	0.91	0.43
jt1	peach CRM	-0.18	0.01	17.68	3632.24	201.53	11.05	1373.77	—	91.55	13307.48	5.25	0.28
jt2	peach CRM	-0.19	0.02	16.74	3685.64	204.63	14.43	1416.91	—	91.08	13395.14	5.27	0.29
jt3	peach CRM	-0.19	0.01	18.34	3726.20	207.02	15.65	1445.78	—	165.06	13572.99	5.61	0.28
	@avg	-0.19	0.01	17.58	3681.36	204.40	13.71	1412.15	—	115.89	13425.21	5.38	0.28
	@stds	0.01	0.00	0.81	47.12	2.75	2.39	36.24	—	42.58	135.29	0.20	0.01
	RSD = stds / avg	-0.03	0.22	0.05	0.01	0.01	0.17	0.03	—	0.37	0.01	0.04	0.02
jt4	IAEA CRM	0.18	0.03	3.95	524.06	481.84	16.05	576.28	—	117.62	1264.97	8.23	1.14
jt5	IAEA CRM	0.53	0.02	9.26	558.49	493.91	5.50	561.53	—	45.77	1542.63	8.15	1.18
jt6	IAEA CRM	0.52	0.03	2.17	558.27	499.17	6.34	564.65	—	-4.16	1538.54	8.53	1.19
	@avg	0.41	0.03	5.12	546.94	491.64	9.30	567.49	—	53.08	1448.71	8.30	1.17
	@stds	0.20	0.01	3.69	19.82	8.89	5.87	7.77	—	61.22	159.14	0.20	0.03
	RSD = stds / avg	0.49	0.23	0.72	0.04	0.02	0.63	0.01	—	1.15	0.11	0.02	0.02
jt7	BCR CRM	0.53	0.02	5.16	477.13	82.33	-2.03	672.09	—	-11.72	2217.35	14.36	3.07
jt8	BCR CRM	0.53	0.02	22.33	482.19	73.16	0.20	673.16	—	4.10	2256.39	14.06	3.06
jt9	BCR CRM	0.55	0.02	2.47	484.97	96.04	-1.99	678.71	—	-38.29	2204.74	15.06	3.11
	@avg	0.54	0.02	9.99	481.43	83.85	-1.27	674.65	—	-15.30	2226.16	14.49	3.08
	@stds	0.01	0.00	10.78	3.97	11.51	1.28	3.55	—	21.42	26.93	0.51	0.03
	RSD = stds / avg	0.02	0.05	1.08	0.01	0.14	-1.00	0.01	—	-1.40	0.01	0.04	0.01

Table IV.2a continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt-24-bk	reagent blank	0.27	0.06	-3.10	-0.00	0.19	0.26	1.06	-0.00	0.03	0.49	0.23	0.16
jt-25-bk	reagent blank	0.01	0.07	-4.92	0.00	0.05	0.21	0.64	-0.00	-0.13	-0.25	0.18	0.18
jt26-bk	reagent blank	0.16	0.02	-5.96	0.00	0.16	0.36	0.97	-0.01	0.03	0.17	0.11	0.18
	@avg	0.15	0.05	-4.66	0.00	0.13	0.28	0.89	-0.00	-0.02	0.14	0.17	0.18
	@stds	0.13	0.03	1.44	0.00	0.07	0.08	0.22	0.00	0.10	0.37	0.06	0.01
	RSD = stds / avg	0.88	0.53	-0.31	4.43	0.53	0.29	0.25	-0.74	-4.01	2.66	0.35	0.07
jt1	peach CRM	0.97	83.20	52.98	0.05	1.03	3.38	19.15	0.84	2.99	2.92	17.64	49.97
jt2	peach CRM	0.94	85.05	102.84	0.07	1.31	3.16	18.66	0.91	3.10	2.41	17.50	50.43
jt3	peach CRM	0.91	86.08	100.82	0.07	1.08	2.90	18.59	0.90	3.30	2.67	17.41	50.81
	@avg	0.94	84.78	85.55	0.06	1.14	3.14	18.80	0.88	3.13	2.67	17.51	50.40
	@stds	0.03	1.46	28.22	0.01	0.15	0.24	0.31	0.04	0.16	0.25	0.12	0.42
	RSD = stds / avg	0.03	0.02	0.33	0.14	0.13	0.08	0.02	0.05	0.05	0.09	0.01	0.01
jt4	IAEA CRM	1.03	58.65	351.53	0.30	1.39	3.33	31.33	0.33	0.64	0.24	0.90	8.18
jt5	IAEA CRM	1.87	58.16	387.32	0.27	1.50	3.47	31.59	0.28	-0.53	0.36	1.51	8.19
jt6	IAEA CRM	1.54	58.62	382.99	0.28	1.56	3.22	31.56	0.30	-0.09	-0.43	1.51	8.35
	@avg	1.48	58.48	373.95	0.28	1.49	3.34	31.49	0.30	0.01	0.06	1.31	8.24
	@stds	0.42	0.28	19.53	0.01	0.09	0.13	0.15	0.03	0.59	0.42	0.35	0.09
	RSD = stds / avg	0.29	0.00	0.05	0.05	0.06	0.04	0.00	0.09	92.33	7.44	0.27	0.01
jt7	BCR CRM	2.87	25.56	584.62	0.26	2.21	6.15	92.65	0.29	-0.16	1.37	8.30	9.06
jt8	BCR CRM	3.50	25.44	573.96	0.27	2.31	6.44	93.51	0.30	-0.05	0.57	8.16	8.94
jt9	BCR CRM	3.27	25.45	585.86	0.27	2.36	6.33	92.95	0.34	-0.26	1.00	8.29	8.98
	@avg	3.21	25.48	581.48	0.27	2.29	6.31	93.04	0.31	-0.16	0.98	8.25	9.00
	@stds	0.32	0.07	6.54	0.00	0.08	0.15	0.44	0.03	0.10	0.40	0.08	0.06
	RSD = stds / avg	0.10	0.00	0.01	0.01	0.03	0.02	0.00	0.09	-0.65	0.41	0.01	0.01

Table IV.2a continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt-24-bk	reagent blank	0.01	0.00	0.00	2.54	0.00	0.11	0.03	0.13	0.00	0.00	0.00	0.00
jt-25-bk	reagent blank	0.00	-0.00	0.00	2.63	0.00	0.12	0.02	0.16	0.00	0.00	-0.02	0.00
jt26-bk	reagent blank	0.00	-0.00	-0.01	2.38	-0.00	0.09	0.01	0.15	0.00	0.00	-0.02	0.00
	@avg	0.00	0.00	-0.00	2.52	0.00	0.11	0.02	0.15	0.00	0.00	-0.01	0.00
	@stds	0.00	0.00	0.01	0.12	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.00
	RSD = stds / avg	0.68	2.22	-8.61	0.05	1.90	0.12	0.36	0.12	0.87	0.65	-1.10	0.85
jt1	peach CRM	0.05	0.00	0.01	2.82	0.01	0.13	0.05	108.51	8.71	10.12	-0.00	0.02
jt2	peach CRM	0.05	0.00	0.04	2.67	0.01	0.12	0.03	110.69	8.82	10.27	-0.01	0.02
jt3	peach CRM	0.05	0.00	0.04	2.71	0.01	0.13	0.03	111.85	8.90	10.38	0.00	0.02
	@avg	0.05	0.00	0.03	2.73	0.01	0.13	0.03	110.35	8.81	10.26	-0.00	0.02
	@stds	0.00	0.00	0.01	0.08	0.00	0.01	0.01	1.70	0.09	0.13	0.00	0.00
	RSD = stds / avg	0.04	0.35	0.43	0.03	0.05	0.05	0.29	0.02	0.01	0.01	-1.17	0.10
jt4	IAEA CRM	0.05	0.02	0.09	3.02	0.01	0.10	0.04	5.07	0.47	1.01	0.02	0.00
jt5	IAEA CRM	0.04	0.02	0.07	3.04	0.01	0.11	0.09	4.85	0.47	1.02	0.01	0.01
jt6	IAEA CRM	0.05	0.02	0.11	3.19	0.01	0.11	0.10	4.84	0.48	1.04	0.02	0.01
	@avg	0.05	0.02	0.09	3.08	0.01	0.10	0.08	4.92	0.47	1.03	0.02	0.01
	@stds	0.01	0.00	0.02	0.09	0.00	0.00	0.03	0.13	0.01	0.02	0.01	0.00
	RSD = stds / avg	0.15	0.03	0.20	0.03	0.36	0.04	0.41	0.03	0.02	0.02	0.58	0.23
jt7	BCR CRM	0.32	0.04	0.48	5.43	0.02	0.11	0.17	9.67	0.56	1.19	0.01	0.03
jt8	BCR CRM	0.31	0.04	0.56	5.19	0.03	0.11	0.18	9.50	0.55	1.16	-0.00	0.02
jt9	BCR CRM	0.34	0.06	0.47	4.70	0.02	0.10	0.19	9.95	0.59	1.21	0.03	0.02
	@avg	0.32	0.05	0.50	5.11	0.03	0.10	0.18	9.71	0.57	1.19	0.01	0.02
	@stds	0.01	0.01	0.05	0.37	0.00	0.01	0.01	0.23	0.02	0.03	0.02	0.00
	RSD = stds / avg	0.03	0.15	0.10	0.07	0.13	0.07	0.05	0.02	0.04	0.02	1.33	0.13

Table IV.2a continued

Sample #	Sample Name	Pb	Bi	U
jt-24-bk	reagent blank	0.01	-0.00	-0.00
jt-25-bk	reagent blank	0.01	-0.00	0.00
jt26-bk	reagent blank	0.01	0.00	-0.00
	@avg	0.01	-0.00	0.00
	@stds	0.00	0.00	0.00
	RSD = stds / avg	0.12	-2.59	24.23
jt1	peach CRM	0.35	0.00	0.01
jt2	peach CRM	0.34	0.00	0.01
jt3	peach CRM	0.33	0.00	0.00
	@avg	0.34	0.00	0.01
	@stds	0.01	0.00	0.00
	RSD = stds / avg	0.03	0.77	0.32
jt4	IAEA CRM	1.70	0.01	0.03
jt5	IAEA CRM	1.93	0.01	0.03
jt6	IAEA CRM	1.82	0.01	0.03
	@avg	1.82	0.01	0.03
	@stds	0.11	0.00	0.00
	RSD = stds / avg	0.06	0.07	0.07
jt7	BCR CRM	13.48	0.10	0.03
jt8	BCR CRM	13.47	0.10	0.04
jt9	BCR CRM	13.63	0.10	0.03
	@avg	13.53	0.10	0.03
	@stds	0.09	0.00	0.00
	RSD = stds / avg	0.01	0.01	0.07

Table IV.2b: ICP-MS data from the Waters 125 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt10	Alectoria Bauline '96	0.23	0.01	1.17	286.92	20.92	3.13	220.66	—	78.29	1849.82	2.04	0.37
jt11	Alectoria Bauline '96	0.14	0.01	5.61	276.11	19.80	4.36	216.38	—	-46.01	1844.49	2.02	0.34
jt12	Alectoria Bauline '96	0.10	0.01	4.52	269.45	19.53	8.55	206.02	—	73.25	1835.11	1.78	0.31
	@avg	0.16	0.01	3.77	277.50	20.08	5.35	214.36	—	35.18	1843.14	1.95	0.34
	@stds	0.07	0.00	2.31	8.81	0.74	2.84	7.53	—	70.36	7.44	0.14	0.03
	RSD = stds / avg	0.42	0.46	0.61	0.03	0.04	0.53	0.04	—	2.00	0.00	0.07	0.09
jt13	Alectoria Bauline '97	0.15	-0.00	10.29	347.71	20.43	7.61	402.03	—	62.27	1155.51	2.11	0.55
jt14	Alectoria Bauline '97	0.30	0.00	1.61	345.01	20.26	7.71	386.26	—	14.57	1163.77	2.27	0.53
jt15	Alectoria Bauline '97	0.10	-0.00	23.96	348.95	20.34	6.50	416.14	—	9.15	1145.82	2.16	0.58
	@avg	0.18	-0.00	11.95	347.22	20.34	7.27	401.48	—	28.66	1155.03	2.18	0.56
	@stds	0.11	0.00	11.27	2.02	0.09	0.67	14.95	—	29.23	8.99	0.08	0.03
	RSD = stds / avg	0.58	-12.79	0.94	0.01	0.00	0.09	0.04	—	1.02	0.01	0.04	0.05
jt16	Bryoria Bauline '97	0.19	0.01	3.44	519.89	90.82	5.02	783.37	—	-52.95	339.08	7.13	11.29
jt18	Bryoria Bauline '97	0.35	0.01	2.71	546.48	94.07	5.82	812.12	—	-1.15	412.73	7.89	11.59
jt19	Bryoria Bauline '97	0.44	-0.00	18.50	564.39	100.26	6.44	828.12	—	12.15	428.81	8.03	12.05
	@avg	0.33	0.01	8.22	543.58	95.05	5.76	807.87	—	-13.98	393.54	7.68	11.64
	@stds	0.13	0.01	8.91	22.39	4.79	0.71	22.67	—	34.40	47.84	0.49	0.39
	RSD = stds / avg	0.38	1.04	1.09	0.04	0.05	0.12	0.03	—	-2.46	0.12	0.06	0.03
jt20	Cladonia Bauline '97	0.33	-0.00	0.60	246.74	79.67	6.05	450.08	—	-8.87	272.50	5.98	0.78
jt21	Cladonia Bauline '97	0.30	-0.00	0.60	248.28	76.61	5.32	444.85	—	18.45	355.56	5.52	0.72
jt22	Cladonia Bauline '97	0.18	-0.00	0.57	243.45	64.89	3.11	445.61	—	35.14	363.84	6.41	0.68
jt23	Cladonia Bauline '97	0.19	-0.00	0.79	239.11	61.37	-0.58	431.25	—	13.12	364.23	5.93	0.65
	@avg	0.25	-0.00	0.64	244.39	70.64	3.48	442.95	—	14.46	339.03	5.96	0.71
	@stds	0.08	0.00	0.10	4.06	8.87	2.98	8.13	—	18.17	44.54	0.36	0.06
	RSD = stds / avg	0.30	-0.51	0.16	0.02	0.13	0.86	0.02	—	1.26	0.13	0.06	0.08

Table IV.2b continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt10	Alectoria Bauline '96	0.18	83.48	0.02	0.04	0.79	1.57	32.96	0.01	-0.42	-1.16	0.85	6.13
jt11	Alectoria Bauline '96	0.34	80.65	4.56	0.04	0.47	0.71	31.12	0.02	-0.41	-0.45	0.71	5.98
jt12	Alectoria Bauline '96	0.36	78.05	41.51	0.04	0.65	0.99	31.05	0.01	-0.21	-0.20	0.63	5.84
	@avg	0.29	80.73	15.37	0.04	0.64	1.09	31.71	0.01	-0.35	-0.60	0.73	5.99
	@stds	0.10	2.72	22.76	0.00	0.16	0.44	1.08	0.01	0.12	0.50	0.11	0.15
	RSD = stds / avg	0.33	0.03	1.48	0.05	0.25	0.40	0.03	0.44	-0.35	-0.83	0.15	0.02
jt13	Alectoria Bauline '97	0.33	60.25	47.04	0.04	0.69	0.88	28.30	0.01	0.03	0.26	0.77	5.04
jt14	Alectoria Bauline '97	0.20	59.57	42.30	0.03	0.67	0.90	27.80	0.01	-0.43	1.08	0.71	5.04
jt15	Alectoria Bauline '97	0.29	60.65	25.88	0.04	0.78	0.91	28.70	0.01	-0.46	0.31	0.76	5.11
	@avg	0.27	60.15	38.40	0.04	0.71	0.90	28.26	0.01	-0.28	0.55	0.75	5.06
	@stds	0.07	0.55	11.11	0.00	0.06	0.01	0.45	0.00	0.28	0.46	0.03	0.04
	RSD = stds / avg	0.26	0.01	0.29	0.08	0.08	0.01	0.02	0.11	-0.97	0.84	0.04	0.01
jt16	Bryoria Bauline '97	0.15	66.93	92.73	0.08	1.46	2.63	46.78	0.06	-0.83	0.13	1.74	4.91
jt18	Bryoria Bauline '97	0.19	69.20	93.20	0.09	1.74	2.39	47.99	0.05	-0.16	0.18	1.78	5.20
jt19	Bryoria Bauline '97	0.41	71.91	110.21	0.10	1.49	2.35	47.88	0.04	-0.16	-0.69	1.77	5.33
	@avg	0.25	69.35	98.71	0.09	1.56	2.46	47.55	0.05	-0.38	-0.13	1.76	5.15
	@stds	0.14	2.49	9.96	0.01	0.15	0.15	0.67	0.01	0.39	0.49	0.02	0.22
	RSD = stds / avg	0.55	0.04	0.10	0.07	0.10	0.06	0.01	0.27	-1.01	-3.89	0.01	0.04
jt20	Cladonia Bauline '97	0.35	47.89	52.25	0.03	0.69	1.27	12.10	0.02	-0.23	-0.23	1.09	1.84
jt21	Cladonia Bauline '97	0.17	47.19	52.48	0.02	0.74	1.06	10.43	0.02	-0.38	-0.82	1.03	2.00
jt22	Cladonia Bauline '97	0.16	46.30	38.67	0.02	0.47	1.01	10.23	0.03	-0.44	0.09	1.07	1.95
jt23	Cladonia Bauline '97	0.16	45.48	40.06	0.02	0.49	1.06	9.85	0.03	0.02	0.39	1.01	1.89
	@avg	0.21	46.72	45.86	0.03	0.60	1.10	10.65	0.03	-0.26	-0.14	1.05	1.92
	@stds	0.09	1.05	7.53	0.00	0.14	0.11	1.00	0.00	0.21	0.52	0.03	0.07
	RSD = stds / avg	0.44	0.02	0.16	0.11	0.23	0.10	0.09	0.14	-0.80	-3.60	0.03	0.03

Table IV.2b continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt10	Alectoria Bauline '96	0.07	0.01	0.05	2.83	0.01	0.11	0.01	1.82	0.10	0.63	-0.01	0.00
jt11	Alectoria Bauline '96	0.06	0.01	0.04	2.73	-0.00	0.09	0.01	1.82	0.10	0.62	0.02	0.00
jt12	Alectoria Bauline '96	0.07	0.01	0.05	2.63	0.00	0.09	0.01	1.66	0.10	0.59	0.02	0.00
	@avg	0.06	0.01	0.04	2.73	0.00	0.10	0.01	1.77	0.10	0.61	0.01	0.00
	@stds	0.00	0.00	0.01	0.10	0.00	0.02	0.00	0.09	0.00	0.02	0.02	0.00
	RSD = stds / avg	0.08	0.13	0.18	0.04	1.31	0.16	0.15	0.05	0.04	0.03	1.67	0.58
jt13	Alectoria Bauline '97	0.09	0.01	0.02	2.76	0.01	0.18	0.01	1.28	0.08	0.27	0.00	0.00
jt14	Alectoria Bauline '97	0.10	0.01	0.02	2.93	0.01	0.15	0.01	1.41	0.08	0.26	0.00	0.00
jt15	Alectoria Bauline '97	0.10	0.01	0.05	3.10	0.01	0.11	0.01	1.33	0.08	0.27	-0.00	-0.00
	@avg	0.10	0.01	0.03	2.93	0.01	0.15	0.01	1.34	0.08	0.27	0.00	0.00
	@stds	0.01	0.00	0.02	0.17	0.00	0.03	0.00	0.06	0.00	0.01	0.00	0.00
	RSD = stds / avg	0.06	0.23	0.51	0.06	0.15	0.22	0.32	0.05	0.05	0.03	1.95	1.08
jt16	Bryoria Bauline '97	0.17	0.01	0.02	2.70	0.02	0.13	0.02	5.16	0.14	0.29	0.02	0.00
jt18	Bryoria Bauline '97	0.19	0.00	0.03	3.42	0.02	0.17	0.02	2.55	0.14	0.31	0.03	0.00
jt19	Bryoria Bauline '97	0.18	0.01	0.04	3.22	0.03	0.19	0.02	2.76	0.15	0.32	0.04	0.00
	@avg	0.18	0.01	0.03	3.11	0.03	0.16	0.02	3.49	0.15	0.31	0.03	0.00
	@stds	0.01	0.00	0.01	0.37	0.01	0.03	0.00	1.45	0.01	0.01	0.01	0.00
	RSD = stds / avg	0.04	0.52	0.26	0.12	0.28	0.18	0.14	0.41	0.05	0.05	0.46	0.82
jt20	Cladonia Bauline '97	0.02	0.02	0.02	1.68	0.00	0.12	0.04	4.72	0.10	0.39	0.02	0.00
jt21	Cladonia Bauline '97	0.02	0.03	0.02	1.21	-0.00	0.08	0.04	3.82	0.09	0.39	0.03	0.00
jt22	Cladonia Bauline '97	0.02	0.02	0.02	2.96	0.00	0.12	0.04	3.69	0.09	0.37	0.01	0.00
jt23	Cladonia Bauline '97	0.02	0.02	0.03	2.62	0.01	0.11	0.03	3.75	0.09	0.36	-0.01	0.00
	@avg	0.02	0.02	0.02	2.12	0.00	0.11	0.04	4.00	0.10	0.38	0.01	0.00
	@stds	0.00	0.00	0.00	0.81	0.00	0.02	0.00	0.49	0.00	0.02	0.02	0.00
	RSD = stds / avg	0.10	0.06	0.19	0.38	1.20	0.17	0.06	0.12	0.05	0.04	1.13	0.30

Table IV.2b continued

Sample #	Sample Name	Pb	Bi	U
jt10	Alectoria Bauline '96	1.30	0.00	0.00
jt11	Alectoria Bauline '96	1.26	0.00	0.00
jt12	Alectoria Bauline '96	1.25	0.00	0.00
	@avg	1.27	0.00	0.00
	@stds	0.03	0.00	0.00
	RSD = stds / avg	0.02	0.31	0.78
jt13	Alectoria Bauline '97	0.94	0.00	0.00
jt14	Alectoria Bauline '97	0.92	0.01	0.01
jt15	Alectoria Bauline '97	0.93	0.00	0.00
	@avg	0.93	0.00	0.00
	@stds	0.01	0.00	0.00
	RSD = stds / avg	0.01	0.13	0.11
jt16	Bryoria Bauline '97	1.70	0.02	0.01
jt18	Bryoria Bauline '97	1.73	0.02	0.01
jt19	Bryoria Bauline '97	1.75	0.02	0.02
	@avg	1.73	0.02	0.01
	@stds	0.02	0.00	0.00
	RSD = stds / avg	0.01	0.07	0.12
jt20	Cladonia Bauline '97	0.23	0.00	0.01
jt21	Cladonia Bauline '97	0.21	0.00	0.02
jt22	Cladonia Bauline '97	0.21	0.00	0.00
jt23	Cladonia Bauline '97	0.21	0.00	0.00
	@avg	0.21	0.00	0.01
	@stds	0.01	0.00	0.01
	RSD = stds / avg	0.05	0.23	0.81

Table IV.3a: ICP-MS data from the Waters 903 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt1	IAEA lichen CRM	0.70	0.02	1.36	564.76	400.18	15.35	536.74	370.62	-5.61	2086.40	6.51	1.15
jt3	BCR lichen CRM	0.56	0.02	0.88	486.28	354.88	8.04	635.59	910.56	-36.80	1852.10	12.67	2.92
jt4	BCR lichen CRM	0.58	0.03	-0.56	468.11	366.94	4.32	641.65	883.11	-71.98	1836.88	12.61	2.97
	@avg	0.57	0.03	0.16	477.20	360.91	6.18	638.62	896.84	-54.39	1844.49	12.64	2.94
	@stds	0.01	0.00	1.02	12.85	8.53	2.63	4.29	19.41	24.87	10.76	0.04	0.03
	RSD = stds / avg	0.02	0.07	6.41	0.03	0.02	0.43	0.01	0.02	-0.46	0.01	0.00	0.01
jt5	Alect. Baul. '96	0.11	0.00	-1.61	267.30	19.79	8.66	199.98	134.26	-88.87	1868.84	1.38	0.30
jt6	Alect. Baul. '96	0.08	0.00	0.45	282.03	19.68	9.14	202.88	193.77	-40.43	1920.56	1.38	0.30
	@avg	0.09	0.00	-0.58	274.66	19.74	8.90	201.43	164.01	-64.65	1894.70	1.38	0.30
	@stds	0.02	0.00	1.46	10.42	0.08	0.34	2.05	42.08	34.25	36.58	0.00	0.00
	RSD	0.20	0.08	-2.50	0.04	0.00	0.04	0.01	0.26	-0.53	0.02	0.00	0.01
jt7	Alect. CBC(3)	0.08	0.00	-0.04	219.58	40.46	6.07	188.51	179.51	-58.01	590.56	1.66	3.49
jt8	Alect. CBC(3)	0.05	0.00	-1.40	209.68	38.81	4.95	180.24	146.46	-83.57	591.43	1.63	3.35
	@avg	0.06	0.00	-0.72	214.63	39.64	5.51	184.37	162.99	-70.79	590.99	1.64	3.42
	@stds	0.02	0.00	0.96	7.00	1.16	0.80	5.85	23.37	18.08	0.61	0.02	0.10
	RSD	0.37	0.03	-1.34	0.03	0.03	0.14	0.03	0.14	-0.26	0.00	0.01	0.03

Table IV.3a continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt1	IAEA lichen CRM	1.05	57.11	357.15	0.25	1.00	3.11	33.75	0.31	1.00	-0.25	1.37	8.20
jt3	BCR lichen CRM	3.08	24.88	631.08	0.26	2.09	5.18	86.83	0.25	2.36	-0.46	7.57	8.66
jt4	BCR lichen CRM	3.20	24.98	631.45	0.26	2.34	5.32	89.50	0.26	2.17	-0.67	7.75	8.79
	@avg	3.14	24.93	631.26	0.26	2.21	5.25	88.16	0.25	2.27	-0.57	7.66	8.73
	@stds	0.09	0.07	0.26	0.00	0.18	0.10	1.89	0.00	0.13	0.15	0.13	0.09
	RSD = stds / avg	0.03	0.00	0.00	0.00	0.08	0.02	0.02	0.01	0.06	-0.26	0.02	0.01
jt5	Alect. Baul. '96	0.03	76.79	9.44	0.03	0.42	0.61	31.38	0.02	-0.08	-1.19	0.63	6.14
jt6	Alect. Baul. '96	0.10	76.84	13.36	0.03	0.58	0.73	33.59	0.02	-0.13	-1.33	0.63	6.29
	@avg	0.07	76.82	11.40	0.03	0.50	0.67	32.49	0.02	-0.11	-1.26	0.63	6.21
	@stds	0.05	0.04	2.77	0.00	0.11	0.09	1.56	0.00	0.04	0.10	0.00	0.11
	RSD	0.74	0.00	0.24	0.01	0.22	0.13	0.05	0.18	-0.33	-0.08	0.00	0.02
jt7	Alect. CBC(3)	0.11	23.65	25.11	0.05	1.19	0.92	32.68	0.03	-0.09	-1.54	0.80	2.62
jt8	Alect. CBC(3)	0.04	22.90	23.55	0.05	1.19	0.85	31.24	0.03	-0.38	-1.91	0.80	2.64
	@avg	0.08	23.27	24.33	0.05	1.19	0.89	31.96	0.03	-0.23	-1.73	0.80	2.63
	@stds	0.05	0.54	1.10	0.00	0.00	0.05	1.01	0.00	0.20	0.26	0.00	0.02
	RSD	0.68	0.02	0.05	0.02	0.00	0.06	0.03	0.09	-0.88	-0.15	0.00	0.01

Table IV.3a continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt1	IAEA lichen CRM	0.05	0.02	0.10	3.47	0.02	0.01	0.09	5.12	0.49	1.07	-0.00	0.01
jt3	BCR lichen CRM	0.31	0.03	0.47	6.76	0.04	0.00	0.18	9.56	0.56	1.16	-0.00	0.03
jt4	BCR lichen CRM	0.34	0.03	0.47	6.37	0.05	0.00	0.18	11.73	0.56	1.18	-0.00	0.02
	@avg	0.32	0.03	0.47	6.56	0.04	0.00	0.18	10.64	0.56	1.17	-0.00	0.02
	@stds	0.02	0.00	0.00	0.27	0.00	0.00	0.01	1.53	0.01	0.01	0.00	0.00
	RSD = stds / avg	0.05	0.03	0.00	0.04	0.10	0.37	0.03	0.14	0.01	0.01	-0.33	0.10
jt5	Alect. Baul. '96	0.08	0.00	0.05	4.31	0.00	-0.01	0.01	2.12	0.08	0.17	-0.00	-0.00
jt6	Alect. Baul. '96	0.07	0.00	0.05	4.37	0.00	-0.04	0.01	1.71	0.08	0.17	0.00	-0.00
	@avg	0.07	0.00	0.05	4.34	0.00	-0.02	0.01	1.91	0.08	0.17	-0.00	-0.00
	@stds	0.00	0.00	0.00	0.05	0.00	0.02	0.00	0.29	0.00	0.00	0.00	0.00
	RSD	0.04	0.06	0.03	0.01	0.11	-0.83	0.01	0.15	0.00	0.00	-7.58	-1.01
jt7	Alect. CBC(3)	0.06	0.00	0.03	4.37	0.00	-0.01	0.01	0.85	0.10	0.24	0.00	-0.00
jt8	Alect. CBC(3)	0.06	0.00	0.02	4.48	0.00	-0.05	0.01	0.88	0.10	0.24	0.00	-0.00
	@avg	0.06	0.00	0.02	4.43	0.00	-0.03	0.01	0.86	0.10	0.24	0.00	-0.00
	@stds	0.00	0.00	0.00	0.08	0.00	0.03	0.00	0.02	0.00	0.00	0.00	0.00
	RSD	0.00	0.01	0.10	0.02	0.25	-0.98	0.03	0.02	0.00	0.00	0.76	-0.08

Table IV.3a continued

Sample #	Sample Name	Pb	Bi	U
jt1	IAEA lichen CRM	4.14	0.01	0.03
jt3	BCR lichen CRM	31.55	0.09	0.03
jt4	BCR lichen CRM	32.14	0.09	0.03
	@avg	31.84	0.09	0.03
	@stds	0.42	0.00	0.00
	RSD = stds / avg	0.01	0.03	0.01
jt5	Alect. Baul. '96	3.41	0.01	0.00
jt6	Alect. Baul. '96	3.37	0.01	0.00
	@avg	3.39	0.01	0.00
	@stds	0.03	0.00	0.00
	RSD	0.01	0.17	0.20
jt7	Alect. CBC(3)	1.96	0.00	0.01
jt8	Alect. CBC(3)	1.99	0.00	0.01
	@avg	1.98	0.00	0.01
	@stds	0.02	0.00	0.00
	RSD	0.01	0.06	0.10

Table IV.3b; ICP-MS data from the Waters 903 Run (ppm).

Sample #	Sample Name	Li	Be	B	Mg	Al	Si	P	S	Cl	Ca	Ti	V
jt9	Alect. RI(1)	0.27	0.00	0.17	341.89	19.16	7.36	179.02	162.94	-48.03	695.18	1.06	0.34
jt10	Alect. RI(1)	0.16	0.00	0.16	301.48	16.38	7.14	166.46	210.88	-64.94	668.41	0.96	0.31
	@avg	0.22	0.00	0.16	321.68	17.77	7.25	172.74	186.91	-56.48	681.80	1.01	0.32
	@stds	0.08	0.00	0.01	28.58	1.96	0.15	8.88	33.90	11.95	18.93	0.07	0.02
	RSD	0.36	0.51	0.06	0.09	0.11	0.02	0.05	0.18	-0.21	0.03	0.07	0.07
jt11	Alect. Bon.(5)	0.10	0.00	-0.30	586.53	9.10	5.31	263.99	90.23	-81.04	1008.65	0.55	0.09
jt12	Alect. Bon.(5)	0.12	0.00	0.00	599.64	9.46	5.74	273.20	69.70	-82.34	1043.49	0.55	0.10
	@avg	0.11	0.00	-0.15	593.08	9.28	5.53	268.59	79.96	-81.69	1026.07	0.55	0.10
	@stds	0.01	0.00	0.21	9.27	0.26	0.30	6.51	14.52	0.92	24.63	0.00	0.00
	RSD	0.11	0.22	-1.45	0.02	0.03	0.05	0.02	0.18	-0.01	0.02	0.01	0.04
jt13	Reagent Blank	0.03	-0.00	0.16	6.24	0.37	7.20	-0.76	-45.62	-71.91	56.32	0.00	0.01
jt14	Reagent Blank	0.01	0.00	0.78	5.70	0.34	5.67	1.20	-29.80	-77.46	50.84	0.00	0.01
	@avg	0.02	-0.00	0.47	5.97	0.35	6.44	0.22	-37.71	-74.69	53.58	0.00	0.01
	@stds	0.01	0.00	0.44	0.38	0.02	1.08	1.39	11.19	3.93	3.87	0.00	0.00
	RSD	0.47	-2.36	0.93	0.06	0.04	0.17	6.35	-0.30	-0.05	0.07	0.22	0.47
jt15*	Dup. of jt-1 (IAEA)	0.49	0.02	0.35	564.17	405.19	6.92	566.04	350.58	-95.66	2120.61	6.56	1.14
jt16*	Dup. of jt-10 (Alect. R.I.)	0.17	0.00	-1.37	339.78	17.69	10.26	163.06	207.27	-21.93	661.08	0.98	0.33

Table IV.3b continued

Sample #	Sample Name	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Br	Se	Rb	Sr
jt9	Alect. RI(1)	0.06	41.15	14.51	0.02	0.34	0.95	22.66	0.02	0.10	12.14	1.10	3.35
jt10	Alect. RI(1)	0.03	37.54	11.80	0.02	0.24	0.78	19.09	0.02	-0.27	11.48	1.03	3.13
	@avg	0.05	39.35	13.16	0.02	0.29	0.86	20.88	0.02	-0.08	11.81	1.07	3.24
	@stds	0.02	2.55	1.92	0.00	0.07	0.12	2.52	0.00	0.26	0.47	0.05	0.15
	RSD	0.44	0.06	0.15	0.04	0.25	0.14	0.12	0.03	-3.17	0.04	0.05	0.05
jt11	Alect. Bon.(5)	-0.07	55.87	5.17	0.01	0.35	0.65	17.38	0.01	-0.28	10.13	0.59	7.89
jt12	Alect. Bon.(5)	-0.04	56.39	3.85	0.01	0.15	0.65	17.68	0.02	-0.32	9.07	0.61	8.11
	@avg	-0.05	56.13	4.51	0.01	0.25	0.65	17.53	0.01	-0.30	9.60	0.60	8.00
	@stds	0.02	0.37	0.93	0.00	0.15	0.00	0.21	0.00	0.02	0.75	0.02	0.16
	RSD	-0.40	0.01	0.21	0.01	0.58	0.00	0.01	0.11	-0.08	0.08	0.03	0.02
jt13	Reagent Blank	-0.06	0.08	-2.51	0.00	0.04	0.27	2.03	0.00	-1.34	7.71	0.00	0.16
jt14	Reagent Blank	-0.01	0.05	-2.06	0.00	0.04	0.16	0.33	0.00	-1.42	6.75	0.00	0.14
	@avg	-0.03	0.07	-2.29	0.00	0.04	0.22	1.18	0.00	-1.38	7.23	0.00	0.15
	@stds	0.04	0.02	0.32	0.00	0.00	0.08	1.20	0.00	0.06	0.69	0.00	0.01
	RSD	-1.09	0.31	-0.14	0.71	0.12	0.37	1.02	0.88	-0.04	0.09	0.53	0.09
jt15*	Dup. of jt-1 (IAEA)	0.76	58.27	359.37	0.26	1.00	3.20	33.58	0.30	-0.31	6.06	1.37	8.21
jt16*	Dup. of jt-10 (Alect. R.I.)	0.06	41.18	14.84	0.02	0.25	0.79	19.10	0.02	-0.00	-0.98	1.02	3.09

Table IV.3b continued

Sample #	Sample Name	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba	La	Ce	Hg	Tl
jt9	Alect. RI(1)	0.06	0.01	0.03	4.87	0.01	-0.01	0.01	2.09	0.03	0.08	0.00	-0.00
jt10	Alect. RI(1)	0.06	0.01	0.03	4.58	0.00	-0.02	0.01	1.91	0.03	0.07	-0.00	-0.00
	@avg	0.06	0.01	0.03	4.72	0.00	-0.02	0.01	2.00	0.03	0.07	-0.00	-0.00
	@stds	0.00	0.00	0.00	0.21	0.00	0.01	0.00	0.13	0.00	0.00	0.00	0.00
	RSD	0.08	0.20	0.09	0.04	0.16	-0.55	0.00	0.06	0.05	0.03	-12.74	-0.09
jt11	Alect. Bon.(5)	0.04	0.00	0.03	3.98	0.00	-0.04	0.00	1.23	0.02	0.05	0.00	-0.00
jt12	Alect. Bon.(5)	0.04	0.00	0.03	4.50	0.00	-0.02	0.00	1.26	0.02	0.05	0.00	-0.00
	@avg	0.04	0.00	0.03	4.24	0.00	-0.03	0.00	1.25	0.02	0.05	0.00	-0.00
	@stds	0.00	0.00	0.00	0.37	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00
	RSD	0.03	0.06	0.01	0.09	0.12	-0.59	0.06	0.01	0.00	0.02	0.91	-0.19
jt13	Reagent Blank	0.00	0.00	-0.00	2.57	0.00	0.00	-0.00	0.15	-0.00	0.00	-0.00	-0.00
jt14	Reagent Blank	0.00	0.00	-0.00	2.78	-0.00	-0.04	-0.00	0.14	-0.00	0.00	0.00	-0.00
	@avg	0.00	0.00	-0.00	2.67	0.00	-0.02	-0.00	0.15	-0.00	0.00	-0.00	-0.00
	@stds	0.00	0.00	0.00	0.15	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00
	RSD	0.47	1.29	-1.40	0.05	1.44	-1.62	-0.11	0.05	-0.47	0.04	-1.98	-0.01
jt15*	Dup. of jt-1 (IAEA)	0.05	0.02	0.10	3.46	0.02	-0.08	0.09	5.08	0.49	1.07	-0.00	0.01
jt16*	Dup. of jt-10 (Alect. R.I.)	0.05	0.01	0.03	4.59	0.00	-0.03	0.01	1.91	0.03	0.08	-0.00	0.00

Table IV.3b continued

Sample #	Sample Name	Pb	Bi	U
jt9	Alect. RI(1)	1.01	0.00	0.00
jt10	Alect. RI(1)	0.97	0.00	0.00
	@avg	0.99	0.00	0.00
	@stds	0.03	0.00	0.00
	RSD	0.03	0.00	0.06
jt11	Alect. Bon.(5)	1.56	0.00	0.00
jt12	Alect. Bon.(5)	1.56	0.00	0.00
	@avg	1.56	0.00	0.00
	@stds	0.00	0.00	0.00
	RSD	0.00	0.17	0.09
jt13	Reagent Blank	0.03	-0.00	-0.00
jt14	Reagent Blank	0.02	-0.00	-0.00
	@avg	0.02	-0.00	-0.00
	@stds	0.01	0.00	0.00
	RSD	0.31	-0.81	-1.13
jt15*	Dup. of jt-1 (IAEA)	4.17	0.01	0.03
jt16*	Dup. of jt-10 (Alect. R.I.)	0.93	0.00	0.00

APPENDIX V: CONCENTRATIONS FOR THE CERTIFIED REFERENCE MATERIALS (CRMS)

Table V.1: Certified concentrations for the three certified reference materials (CRMs) used in this study. Concentrations are in parts per million (ppm). A dash indicates that there was no certified concentration for that element.

Certified Reference Material	Li	Be	B	Mg	Al	Si	P	S
Peach Leaves (NIST SRM 1547)	-	-	29	4320	249	-	1370	-
BCR Lichen (CRM 482)	-	-	-	-	1103	-	-	-
IAEA Lichen (IAEA-336)	-	-	-	-	-	-	-	-

Certified Reference Material	Cl	Ca	Ti	V	Cr	Mn	Fe	Co
Peach Leaves (NIST SRM 1547)	360	15600	-	0.37	-	98	218	-
BCR Lichen (CRM 482)	-	-	-	-	4.12	-	-	-
IAEA Lichen (IAEA-336)	-	-	-	-	-	64.0	426.0	0.287

Certified Reference Material	Ni	Cu	Zn	As	Br	Se	Rb	Sr
Peach Leaves (NIST SRM 1547)	0.69	3.7	17.9	0.060	-	0.120	19.7	53
BCR Lichen (CRM 482)	2.47	7.03	100.6	0.85	-	-	-	-
IAEA Lichen (IAEA-336)	-	3.55	31.6	0.639	12.9	0.216	1.72	-

Certified Reference Material	Mo	Ag	Cd	Sn	Sb	I	Cs	Ba
Peach Leaves (NIST SRM 1547)	0.060	-	0.026	-	-	-	-	124
BCR Lichen (CRM 482)	-	-	0.56	-	-	-	-	-
IAEA Lichen (IAEA-336)	-	-	0.117	-	0.073	-	0.110	-

Certified Reference Material	La	Ce	Hg	Tl	Pb	Bi	U
Peach Leaves (NIST SRM 1547)	-	-	0.031	-	0.87	-	-
BCR Lichen (CRM 482)	-	-	0.48	-	40.9	-	-
IAEA Lichen (IAEA-336)	-	1.27	0.200	-	-	-	-

APPENDIX VI: SEM-EDX OBSERVATIONS

SEM-EDX OBSERVATIONS

Table VI.1: General observations made of the residual particles after digestion of each Certified Reference Material (CRM) sample type using a dissection microscope and SEM-EDX. The observations for the Peach Leaves CRM were made prior to this study (Tucker, 1995).

Sample	Observations
Peach Leaves CRM	- As viewed by the dissection microscope, the residue appears to consist of a continuous crust of fine white particles, with clusters of these particles in some areas (stucco-like in appearance). - Most particles examined by SEM-EDX had a high Si content and little or no other elements. - Some of the high Si particles had lesser amounts of Al, K, Mg, Na, and/or Ca. A few particles contained a low quantity of Fe. One particle had a very low amount of Ti. - Most particles are granular, but some particles had a generally flat appearance, and a few were somewhat spherical. -Some particles had a relatively high amount of Al or K.
BCR Lichen CRM	- As viewed by the dissection microscope, the residue appears to consist of a continuous crust of fine off-white/beige particles, with clusters of these particles in some areas having a stucco-like appearance; some grains appear clear and colourless. There are a noticeable amount of tiny black particles, as well as some tiny deep red particles and tiny orange particles. One yellow particle and one green/blue particle were also observed. It is somewhat difficult to pick out these individual coloured particles using SEM-EDX. Perhaps the deep red particles are garnet. - Most particles were granular in appearance; there are also vitreous grains; there are some flat smooth grains; there are only a few long thin grains which have a lint-like appearance. - Most particles had a high Si content. Some particles also had high Al or K. One particle had high Ca, and a couple of particles had high Ti. Some particles with high Si also had smaller amounts of Al, K, Ca, Mg, and/or Na. Low amounts of Fe and Ti were observed in some particles. P was observed in one particle.
IAEA Lichen CRM	- As viewed by the dissection microscope, the residue appears to consist of a continuous crust of fine white particles, with clusters of these particles in some areas (stucco-like appearance). - Observed some tiny black shiny particles. Observed some tiny particles which are white with a pearly lustre. - Observed a few long thin particles that have a lint-like appearance. - All particles observed had high Si. A lot of these had only high Si. Al was observed in many particles; this Al content ranged from low to high. Some particles observed had low amounts of K, Fe, and/or Ti. Each of the following elements were observed in one particle: Na, Mg, and S.

Table VI.2: General observations made of the residual particles after digestion of each collected lichen sample using a dissection microscope and SEM-EDX. The quantity of residual particles for these lichens is generally much less than for the CRMs.

Sample	Observations
Alectoria sarmentosa	- Most of the particles observed can be classified into three groups based on appearance: 1. clear colourless or white vitreous particles, 2. white granular particles, and 3. long thin particles which have a lint-like appearance. - All particles observed were either clear and colourless or white; no coloured particles were observed. - Many particles were observed that appeared generally flat with tiny brighter particles at the center and a darker appearance around the edge (these particles had high Si and nothing else). - There are a lot of long thin lint-like particles. - There seems to be less granular particles for *Alectoria sarmentosa* than for *Bryoria sp.* - A large number of particles observed had high Si and nothing else. Many particles had low Al, a couple of particles had a high Al content. Many particles had low amounts of K. There was low amounts of Mg, Na, and Cl in a few particles. Low Fe was observed in a couple of particles. A moderate amount of S was observed in a couple of particles. A low Ca concentration was observed in one particle. A low P concentration was observed in one particle. One particle observed had a high Ti content.
Bryoria sp.	- Most of the particles observed can be classified into three groups based on appearance: 1. clear colourless or white vitreous particles, 2. white granular particles, and 3. long thin particles which have a lint-like appearance. - The particles do not cover the entire area of the filter paper (as with the CRMs). - The *Bryoria sp.* slide appears to have less residual particles than the *Cladonia alpestris*. - Most particles observed had a high Si content; some had high Si and little or nothing else. Many particles had high Si, some Al, and a low amount of K. Al was present in many particles in a moderate amount, but some particles had high Al. K was present in many particles in low quantities; one particle had high K. One particle had high Cl. Na, Mg, and/or Ca were present in some particles in low concentrations. Fe was observed in only one grain, in a low amount.
Cladonia alpestris	- As with *Alectoria sarmentosa* and *Bryoria sp.*, most of the *Cladonia alpestris* residual particles can be classified into three groups based on appearance: 1. clear colourless or white vitreous particles, 2. white granular particles, and 3. long thin particles (lint-like appearance). - Most particles are either granular or long and thin (lint-like). Many of the long thin particles appear to be twisted. Observed one spherical particle. - Looking at the visual profile of the slide (unaided), a lot of lint-like particles can be seen extending vertically above the surface of the filter paper. - Most particles appear white or clear and colourless. There are many clear colourless vitreous particles. Most of the lint-like particles observed appear white or clear and colourless. There were several pale orange particles. There was one red vitreous particle. - There was a high Si content in almost every particle observed. There was Al and/or K in a lot of particles observed. Al content ranged from low to high. K content was usually low, but high in one particle. Low Fe was observed in some particles. Low Ti was observed in several particles, but high Ti was observed in a couple of particles. Low Na was observed in many particles. Low Mg was observed in many particles. A moderate to high amount of Ca was observed in some particles. - The *Cladonia alpestris* slide had many more particles than the *Bryoria sp.* slide, and *Cladonia alpestris* has many more long thin particles.

Table VI.3: General description of the six stubs used for the surface examination of *Alectoria sarmentosa* strands.

Stub	Strand Description From Dissection Microscope Observations
Stub A Come By Chance (#018) Not washed	Strand 1 - thin strand Strand 2 - thin greyish strand Strand 3 - thick strand (ends flattened due to cutting with scissors)
Stub B Come By Chance (#019) Not washed	Strand 1 - thick strand with several depressions in surface Strand 2 - thin strand with many tiny "branches" Strand 3 - very thin strand
Stub C Torbay (#037) Not washed	Strand 1 - thick strand; appears to have dark greenish discolouration Strand 2 - thick strand with bumps Strand 3 - thin strand
Stub D Come By Chance (#018) Washed	Strand 1 - thin strand Strand 2 - thin strand; appears to have dark greenish discolouration Strand 3 - medium thickness strand with bumps
Stub E Come By Chance (#019) Washed	Strand 1 - medium thickness strand with tiny projections Strand 2 - thin strand; appears to have brownish discolouration Strand 3 - medium thickness strand with whitish spots; the green colour of this strand seems relatively dark
Stub F Torbay (#037) Washed	Strand 1 - medium thickness strand; discoloured to dark green/brown; with whitish spots/lines Strand 2 - medium thickness strand; green and almost translucent in appearance Strand 3 - relatively thin strand

APPENDIX VII: TABLES OF P-VALUES FROM T-TESTS

P-VALUES FOR THE COMPARISON OF SITES

Table VII.1: P-values from t-tests for the comparison of means for Bonavista and Random Island.

Element	P-Value
Ca	P = 0.0000
Sr	P = 0.0001
V	P = 0.0002
Cs	P = 0.0015
Mo	P = 0.0050
Mg	P = 0.0098
Rb	P = 0.014
La	P = 0.064
Sn	P = 0.081
P	P = 0.12
Bi	P = 0.14
Mn	P = 0.17
Cu	P = 0.22
Be	P = 0.24
Cd	P = 0.34
Ni	P = 0.35
Sb	P = 0.35
Ti	P = 0.35
Ce	P = 0.42
Si	P = 0.49
Fe	P = 0.52
Li	P = 0.56
U	P = 0.57
Tl	P = 0.58
Ba	P = 0.64
Zn	P = 0.67
Cr	P = 0.70
Co	P = 0.70

Table VII.2: P-values from t-tests for the comparison of means for Bonavista and Bauline Line.

Element	P-Value
La	P = 0.0000
Ca	P = 0.0000
Sr	P = 0.0000
V	P = 0.0000
Mo	P = 0.0002
Ti	P = 0.0006
Co	P = 0.0014
Ni	P = 0.0015
Mg	P = 0.0049
Cs	P = 0.0064
Ce	P = 0.019
Bi	P = 0.040
Tl	P = 0.046
Zn	P = 0.064
P	P = 0.093
U	P = 0.095
Cd	P = 0.13
Sn	P = 0.14
Cr	P = 0.15
Be	P = 0.18
Cu	P = 0.33
Sb	P = 0.41
Li	P = 0.50
Fe	P = 0.58
Mn	P = 0.62
Si	P = 0.70
Ba	P = 0.84
Rb	P = 0.92

Table VII.3: P-values from t-tests for the comparison of means for Bonavista and Come By Chance.

Element	P-Value
Ni	P = 0.0000
Sr	P = 0.0000
Cs	P = 0.0004
Ce	P = 0.0010
Mo	P = 0.0012
Mg	P = 0.0026
V	P = 0.0077
Bi	P = 0.0093
La	P = 0.010
Co	P = 0.012
Zn	P = 0.020
Cu	P = 0.027
Rb	P = 0.046
Ti	P = 0.056
Mn	P = 0.066
Fe	P = 0.069
P	P = 0.081
Li	P = 0.12
Be	P = 0.13
Sb	P = 0.19
Si	P = 0.22
U	P = 0.30
Ba	P = 0.40
Sn	P = 0.40
Tl	P = 0.41
Ca	P = 0.46
Cr	P = 0.64
Cd	P = 0.91

Table VII.4: P-values from t-tests for the comparison of means for Random Island and Bauline Line.

Element	P-Value
Ca	P = 0.0000
Cs	P = 0.0000
Mn	P = 0.0000
Sr	P = 0.0000
Zn	P = 0.0001
Rb	P = 0.0004
La	P = 0.0005
Co	P = 0.0037
P	P = 0.0043
Cd	P = 0.010
Ti	P = 0.015
Ce	P = 0.022
Ni	P = 0.036
Bi	P = 0.073
Mo	P = 0.087
Tl	P = 0.096
Cr	P = 0.10
Sn	P = 0.11
Mg	P = 0.17
Li	P = 0.17
Ba	P = 0.20
Sb	P = 0.32
U	P = 0.39
Si	P = 0.45
V	P = 0.48
Be	P = 0.74
Cu	P = 0.87
Fe	P = 0.93

Table VII.5: P-values from t-tests for the comparison of means for Random Island and Come By Chance.

Element	P-Value
Ni	P = 0.0000
Co	P = 0.0001
Mg	P = 0.0019
V	P = 0.010
Ce	P = 0.017
La	P = 0.025
Zn	P = 0.026
Rb	P = 0.027
Li	P = 0.047
Bi	P = 0.050
Sn	P = 0.067
P	P = 0.14
Si	P = 0.15
U	P = 0.16
Sb	P = 0.20
Cr	P = 0.21
Mo	P = 0.23
Fe	P = 0.24
Mn	P = 0.24
Ba	P = 0.25
Ti	P = 0.31
Cu	P = 0.37
Tl	P = 0.40
Ca	P = 0.47
Cd	P = 0.61
Cs	P = 0.82
Be	P = 0.92
Sr	P = 0.92

Table VII.6: P-values for t-tests for the comparison of means for Bauline Line and Come By Chance.

Element	P-Value
Ni	P = 0.0000
Mn	P = 0.0000
Cs	P = 0.0000
Mg	P = 0.0002
Sr	P = 0.0031
Rb	P = 0.0053
U	P = 0.0089
V	P = 0.010
Ca	P = 0.013
Sn	P = 0.043
Co	P = 0.064
Ti	P = 0.10
Li	P = 0.12
Ce	P = 0.16
Sb	P = 0.18
Tl	P = 0.21
P	P = 0.22
Cr	P = 0.23
Zn	P = 0.24
Ba	P = 0.24
Mo	P = 0.39
Si	P = 0.43
Fe	P = 0.48
Bi	P = 0.54
Be	P = 0.60
La	P = 0.62
Cd	P = 0.64
Cu	P = 0.67

P-VALUES FOR THE COMPARISON OF SPECIES

Table VII.7: P-values from the t-test for the comparison of *Alectoria sarmentosa* and *Bryoria sp.*

Element	P-Value
P	0.0000
Ca	0.0000
Ti	0.0000
Zn	0.0000
Rb	0.0000
Mo	0.0001
Bi	0.0001
Co	0.0002
La	0.0002
V	0.0004
U	0.0007
Ni	0.0008
Fe	0.0022
Cu	0.0032
Mn	0.0034
Mg	0.0043
Cs	0.0069
Ce	0.011
Sb	0.017
Si	0.055
Ba	0.12
Li	0.21
Be	0.23
Sn	0.48
Sr	0.54
Tl	0.57
Cr	0.83
Cd	0.91

Table VII.8: P-values from the t-test for the comparison of *Alectoria sarmentosa* and *Cladonia alpestris*.

Element	P-Value
Mg	0.0000
Ca	0.0000
Ti	0.0000
Mn	0.0000
Zn	0.0000
Sr	0.0000
Mo	0.0000
Cs	0.0000
Rb	0.0001
Ce	0.0001
Ba	0.0017
Co	0.0028
P	0.0050
La	0.0053
V	0.0075
Sb	0.020
Cu	0.037
Si	0.088
Bi	0.14
Sn	0.16
Ni	0.24
Fe	0.33
Cd	0.34
Li	0.36
Cr	0.39
U	0.43
Tl	0.54
Be	0.65

Table VII.9: P-values from the t-test for the comparison of *Bryoria sp.* and *Cladonia alpestris*.

Element	P-Value
P	0.0000
Mn	0.0000
Co	0.0000
Cu	0.0000
Zn	0.0000
Rb	0.0000
Sr	0.0000
Mo	0.0000
Bi	0.0000
La	0.0001
Ni	0.0003
Cs	0.0003
V	0.0004
Fe	0.0005
Mg	0.0019
Sb	0.0020
Ce	0.0021
Ti	0.0029
Tl	0.064
Cd	0.095
Sn	0.11
U	0.11
Be	0.16
Ca	0.18
Si	0.26
Li	0.36
Ba	0.53
Cr	0.66

P-VALUES FOR THE COMPARISON OF

ALECTORIA BAULINE LINE 1996 SAMPLES ANALYZED SEPARATELY

Table VII.10: P-values from the t-test for the comparison of the Waters 120 Alectoria Bauline Line 1996 samples and the Waters 125 Alectoria Bauline Line 1996 samples.

Element	p-value
Ce	p = 0.0001
Sn	p = 0.0003
La	p = 0.0037
Ca	p = 0.0059
Be	p = 0.021
Mg	p = 0.025
Mo	p = 0.031
Mn	p = 0.031
Co	p = 0.044
Ti	p = 0.051
V	p = 0.072
P	p = 0.086
Zn	p = 0.096
Si	p = 0.098
Ni	p = 0.19
Cr	p = 0.23
Ba	p = 0.34
Tl	p = 0.40
Sr	p = 0.45
Cd	p = 0.50
Cu	p = 0.53
Li	p = 0.58
Rb	p = 0.66
Bi	p = 0.86
U	p = 0.91
Cs	p = 0.96
Fe	p = 0.97
Sb	p = 0.99

Table VII.11: P-values from the t-test for the comparison of the Waters 120 Alectoria Bauline Line samples and the Waters 903 Alectoria Bauline Line samples.

Element	p-value
Be	p = 0.0010
Ce	p = 0.0054
V	p = 0.0087
Cr	p = 0.013
Si	p = 0.023
P	p = 0.030
Li	p = 0.030
Co	p = 0.030
Sn	p = 0.032
Ca	p = 0.033
Mg	p = 0.073
Mn	p = 0.090
Sb	p = 0.098
Cu	p = 0.10
La	p = 0.12
Rb	p = 0.16
Bi	p = 0.17
Mo	p = 0.19
Ti	p = 0.20
U	p = 0.21
Cd	p = 0.22
Tl	p = 0.23
Fe	p = 0.33
Zn	p = 0.33
Ni	p = 0.66
Ba	p = 0.82
Sr	p = 0.83
Cs	p = 0.98

Table VII.12: P-values from the t-test for the comparison of Waters 125 Alectoria Bauline Line samples and Waters 903 Alectoria Bauline Line samples.

Element	p-value
Sn	p = 0.0003
Ce	p = 0.0007
La	p = 0.011
Ti	p = 0.020
Co	p = 0.025
Cr	p = 0.058
Tl	p = 0.067
Ca	p = 0.082
Be	p = 0.083
Mo	p = 0.099
P	p = 0.11
Mn	p = 0.13
Bi	p = 0.13
V	p = 0.15
Sr	p = 0.16
Si	p = 0.19
Rb	p = 0.26
Cu	p = 0.29
Li	p = 0.31
Ni	p = 0.38
Ba	p = 0.44
Zn	p = 0.55
U	p = 0.57
Cd	p = 0.72
Sb	p = 0.75
Mg	p = 0.76
Fe	p = 0.83
Cs	p = 0.95

P-VALUES FOR THE COMPARISON OF

ALECTORIA BAULINE LINE 1996 AND 1997 SAMPLES

Table VII.13: P-values from the t-test for the comparison of the Alectoria Bauline Line 1996 samples with the Alectoria Bauline Line 1997 samples (from the Waters 125 Run).

Element	p-value
Ce	p = 0.0000
Ca	p = 0.0000
P	p = 0.0000
Mn	p = 0.0002
Mg	p = 0.0002
Sr	p = 0.0004
V	p = 0.0007
Mo	p = 0.0013
Ba	p = 0.0025
La	p = 0.0028
Zn	p = 0.0071
Sb	p = 0.035
Be	p = 0.046
Ti	p = 0.068
Sn	p = 0.16
U	p = 0.17
Fe	p = 0.19
Co	p = 0.21
Cd	p = 0.26
Bi	p = 0.26
Si	p = 0.32
Ni	p = 0.47
Cu	p = 0.52
Cs	p = 0.56
Tl	p = 0.64
Li	p = 0.71
Cr	p = 0.75
Rb	p = 0.81