METHOD DEVELOPMENT FOR THE DETERMINATION OF TRACE ELEMENTS IN BIOLOGICAL SAMPLES AS BIOINDICATORS; APPLICATION TO BLACK SPRUCE TREES

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By

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Abstract

Most analytical methods, including ICP-MS, require sample decomposition for elemental analysis of plant materials. Dry ashing was investigated in this study. Factors studied included ashing temperature, duration of ashing, rate of temperature rise, and type and nature of ashing vessel on the digestion of plant matrices. The reagents used in the subsequent leaching were also investigated. Samples were ashed at 450 °C for 8 hours following a temperature ramp of 18 °C/hr, followed by dissolution with HNO_3/HF + H_2O_2. Recovery of silicate elements (Al, Co, Cr, Ni, V, and U) was satisfactory. The procedure was validated with reference materials including pine needles, peach leaves, and black spruce. The result also agreed with that obtained using the wet digestion protocol used by ICP-MS group at MUN. Losses mainly through volatilization were observed for Hg, Se, Br, Bi, I, and As. The dry ashing procedure was applied to a biomonitoring study using black spruce samples from a study area in Holyrood, Newfoundland. The results suggest that the elemental sources include rock weathering, sea spray, atmospheric deposition, the thermal electric plant, vehicular exhaust, and municipal waste leachate.

Dedication

This thesis is dedicated to my parents Mr. Emmanuel Nyade and Mrs. Rose Nyade.

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Chapter 1: Introduction

Plants have trace element signatures which are related to the composition of the soil, water, and air in which they grow. Determination of elemental concentrations in plants constitutes an important aspect of environmental monitoring and geochemical exploration. Plants including mosses, pine needles, and lichens have been used for monitoring levels of elements in air, soil, and water (Gramatica *et al*, 2006; Yun *et al*, 2002). Plants constitute a major component of ecosystems as they provide a transport pathway from the abiotic to the biotic environment. They make specific demands on the physical and other biological components of their environment, and respond critically to changes (Seaward, 1995). Plants as bioindicators represent a fundamental tool for environmental monitoring and surmount some of the shortcomings associated with the direct measurement of pollution, since importantly they provide lower analytical cost because no sampling equipment has to be installed and protected. Also widely distributed and common bioindicators can be used over large areas to record and evaluate heavy metal inputs (Markert *et al.*, 1999). They make it possible to identify sources of emission and also help to measure overland transportation rates of individual elements.

During the last two decades, significant progress has been made in the development of analytical instruments for the determination of trace metals concentration in all kinds of matrices, including environmental samples. Most of these analytical methods require, or are optimally applied to solid samples, following a digestion prior to analysis. The efficiency of the digestion step is of critical importance in order to obtain accurate results. While analytical instrumentation has received a lot of attention, the

fundamental first step of sampling and sample preparation has, with some notable exceptions, been seriously neglected. Both dry and wet ashing techniques have been employed with success to decompose several matrices, but there are numerous challenges regarding complete digestion for a large number of analytes in a wide variety of samples. Problems such as incomplete matrix digestion, volatilization of analytes, and contamination from solvents and crucibles have especially been noted (Gorsuch 1970).

The use of microwave heating for acid digestion of many types of solid samples has received a significant attention in the recent literature (Hoenig 1995; Lamble and Hill 1998; Oliveira 2003), and is used as an alternative to open vessel hot plate digestion. The main advantages of closed vessel microwave digestion are a shorter digestion time, more complete digestion due to the higher temperature and pressure, and lower volatile loss, compared with open vessel methods. These advantages are, however, accompanied by higher labour costs, much more expensive laboratory equipment, and a decreased sample throughput.

With increasing concerns about environmental pollution, it has become very important to develop accurate and economical methods for the analysis of environmental samples, including those with an organic matrix (*i.e.* plants and animals). The goals of this project are:

1. To develop a more general purpose sample preparation procedure for ICP-MS analysis of trace elements in a wide range of organic samples.

2. To develop a procedure which is economical in time and cost.

3. To develop a method which minimizes the loss of volatile sample components, while documenting the extent of these losses.

The goal is to develop a technique which can become more general purpose; economical for the determination of trace metals in a wide variety of plant samples using ICP-MS. Remembering that "no method is a panacea", the limitations of the method are documented. The method is be tested in a case study that analyzes trace metal concentrations in black spruce twigs (*Picea mariana*) as bioindicators near the Holyrood thermal power plant.

Chapter 2: Literature review

2.1 Background information on sample preparation

Sample preparation constitutes a fundamental aspect in the determination of metals in biological samples. It involves all the steps taken to transfer elements into solution for determination, with the exception of the main structural components of the organic matrix *i.e.* carbon, hydrogen, oxygen (Gorsuch 1970).

Sample preparation steps are of paramount importance in order to ensure a high quality analysis. Complete dissolution/decomposition of a sample is necessary to achieve reproducible and accurate elemental results using instrumental analytical methods (Mader *et al,* 1996; Poykio *et al,* 2000). This is especially true for atomic spectrometric methods such as AAS, ICP-MS, and ICP-OES. Interferences due to incompletely decomposed organic matter also occur, to a certain degree, when using analytical techniques such as polarography and voltammetry (Adeloju, 1989). These procedures require complete conversion of the sample to a form compatible with the measurement technique being used.

2.2 Treatment of solid samples

Collected solid samples are often too heterogeneous to satisfy the needs of the analysis hence the need for preliminary treatments to obtain a more representative sub-sample with a smaller particle size. Preparation of solid samples generally includes several stages such as drying (air or oven), homogenization (mixing, crushing), grinding

(mills, mortars), followed by dissolution of the sample (Hoenig 2001). Lyophilisation (drying by freezing) procedures can be applied to samples to allow preservation of the initial sample texture and to facilitate subsequent grinding, while removing water. Homogenization of plants, animal tissues, and food samples is often processed by using various mixers.

2.3 Solubilisation of biological samples

For many analytical methods for determination of trace metals in environmental materials, complete decomposition and solubilization of the sample prior to the instrumental determination is very critical (Mader *et al,* 1996; Mustafa *et al,* 2004). The essence of this step is to "free" the analytes and make them available for instrumental detection. A broad spectrum of decomposition methods has been applied to determine the concentration of trace elements in biological matrices, and these include different combinations of concentrated acids. Open beakers heated on hot plates, digestion tubes in a block digester, and digestion bombs placed in microwave ovens are the most commonly used equipment for digesting solid/biological matrices (Matusiewicz, http://www.pg.gda.pl/chem/CEEAM/Dokumenty/CEEAM_ksiazka/Chapters/chapter13.pdf). While reagents like sulphuric acid, nitric acid, perchloric acid, hydrofluoric acid, hydrochloric acid, and hydrogen peroxide have been used individually or in combinations to release trace and ultratrace elements into solution. Methods such as dry ashing

(oxidation), fusion, and oxidation using uv-light have also been used for this purpose (Hoenig, 2001).

The low concentration of metals in environmental and geological samples can require preconcentration prior to detection, depending upon the instrumental method being applied. For processes involving very low concentrations, volatilization, solvent extraction, coprecipitation, sorption, and chromatographic methods are applied to separate metals from their associated matrices and then preconcentrated to levels detectable by analytical instruments. The effective combination of a digestion procedure with separation and detection steps is important to ensure the reliability of the results.

The size of the test sample which is treated depends primarily upon the homogeneity of the material to be analyzed and upon sensitivity of the analytical technique being employed. The classical dry ashing of biological material offers the possibility of utilizing relatively large amounts of sample which is required for heterogeneous material, while increasing the concentration of the analytes in the digest (Mader *et al*, 1996). The extent of decomposition required is dependent on the analytical method being used. ICP-MS for example, can tolerate some level of undestroyed but dissolved organic matter during total elemental analysis but this is not the case with methods like voltammetry and other spectrophotometric methods (Adeloju *et al,* 1989).

Unlike dissolution of inorganic matrices such as rocks and metals which may result in clear solutions in which analytes and other elements are present in ionic forms, dissolution of organic or mixed matrices such as plants, animal tissues, and soils does not necessarily result in complete decomposition. The analyte may still be partially

incorporated in an organic molecule and hence be masked from the analytical determination. This can lead to interference if not fully decomposed to achieve complete dissolution especially in cases where ionic species are required for detection. Complete dissolution is required in such cases where the analysis involves the determination of the total content of the element present in the matrices in trace amounts.

2.4 Wet digestion procedures

The task of preparing samples with acid treatment for subsequent analysis is common in many laboratories. A variety of techniques, ranging from ambient-pressure wet digestion in a beaker on a hot plate (or hot block), to high pressure microwave or conventional oven heating have been employed. The open vessel acid digestion technique for instance, is one of the oldest methods of decomposing both organic and inorganic sample materials. The method is inexpensive, readily automated, and all relevant parameters (time, temperature, introduction of digestion reagents) lend themselves to straightforward control (Radojevic and Bashkin 1999). Acid digestions are accomplished in many kinds of vessels, usually in glass or PTFE (beaker, conical flasks, *etc.*) with or without a refluxing condenser and using heat from conventional heating sources such as Bunsen burner, hot plate, sand baths, *etc.* Refluxing is used when loss of volatiles is a critical factor when samples are decomposed by an open wet digestion method.

Open vessel systems differ in their ability to reflux and also to prevent losses by volatilization, and splashing. Examples of such systems are PTFE beakers covered with a

lid (Novozamsky *et al,* 1993); flasks with long neck placed at an angle of about 45°, or with narrowing for the opening; and more complicated constructions with air – or water-cooled refluxes and traps (Bethge 1954 in Novozamsky *et al,* 1993). A significant drawback to these systems is their risk of contamination. These systems are also limited by a low maximum digestion temperature which cannot exceed the ambient-pressure boiling point of the corresponding acid or acid mixture. For instance, the oxidizing power of nitric acid with respect to many matrices is insufficient at such low temperatures (boiling point 122 °C).

When carried out in closed systems (bombs), digestion of the sample is performed under the synergistic effect of elevated temperature and pressure resulting in higher reactivity and oxidizing power. There is reduced contamination as the system is isolated from the laboratory environment. Loss of volatiles is minimised and digestion of difficult samples becomes possible. Closed systems for wet digestion are particularly suitable for trace and ultra trace analysis, especially when the amount of sample is limited.

When a microwave heated oven is used, these advantages are even more pronounced because the heating takes place in the mixture. This result in shorter times needed for the digestion (Coley *et al,* 1977). For organic samples, due to the evolution of a large amount of gas, typically only about 300 to 500 mg of dry sample can be used, compared to several grams in open systems. A notable advantage of closed system is that losses through volatilization can be minimized.

2.5 Reagents frequently used for wet (acid) digestion procedures

2.5.1 Nitric acid

Among the reagents used for the oxidation, nitric acid is the only acid which is often used alone. Advantages of nitric acid include: its availability in high purity (and ease of purification), high solubility of nitrates, and its use over a range of temperatures. Complete digestion is usually achieved when nitric acid is applied at high temperatures and pressure. Boiling organic materials in concentrated nitric acid at atmospheric pressure (b.p. 120 °C) rarely leads to complete dissolution; as about 2 – 20 % of the original carbon compounds remain undestroyed after boiling under total reflux for 3 hours (Novozamsky *et al*, 1995). Wurfels *et al,* (1989), however, demonstrated that near complete digestion can be achieved when the sample is heated in a PTFE closed vessel with 69 % HNO_3 at 180 °C for 3 hours. A study by Kingston and Jassie, (1988) using nitric acid in closed PTFE vessels heated in the microwave oven, reported that carbohydrates decomposed at 140 °C, proteins at 150 °C, and lipids at 160 °C.

2.5.2 Perchloric acid

Perchloric acid is also a very clean wet digestion reagent with very high oxidative power and extremely efficient in the destruction of organic matter. However, it is only applied in a combination with other reagents because of the occurrence of occasional explosions of quite stunning violence (Gorsuch 1970). These spectacular explosions often took place in hoods, which lead to the requirement of special wash down hoods for use

with perchloric acid. Mostly used are mixtures with HNO_3 alone, with HNO_3 and H_2SO_4, or with H_2O_2. Griepink *et al,* (1989) suggested the use of perchloric and chloric acids in the later stage of digestion to avoid the possible explosions. They further contended that the safest and most efficient technique was to evaporate the digest to dryness, followed by treatment with $HClO_4$ untill fuming.

When cold, concentrated $HClO_4$ behaves as a strong acid without appreciable oxidizing power. Increasing temperature leads gradually to an increase in oxidizing power (Gorsuch 1970). Norvozamsky *et al,* (1995) reported a procedure which begins with gentle heating of the mixture of $HClO_4$ and HNO_3 until the boiling point of HNO_3 is reached. The solution is held at this temperature for a prolonged period of time and care is taken not to distil the HNO_3 too quickly; *e.g.* in the case of vegetable; the maintenance of this stage for 45 minutes was recommended. Then the remaining HNO_3 was distilled and the temperature increased rapidly to 203 ^{o}C, the boiling point of the perchloric acid-water azeotropic mixture (72.5 % $HClO_4$). Reaction ceases once this temperature is reached and a clear, colourless solution results.

Use of H_2SO_4 in combination with $HClO_4$ and HNO_3 is considered by some investigators as safer (May *et al,* 1984 in Griepink and Tolg 1989), but not by others. The addition of the strong oxidant to nitric acid increases the power of oxidation. Boiling to dryness is not easily achieved when H_2SO_4 is present, but stronger dehydrating conditions are involved. The efficiency of $HClO_4$, HNO_3, and H_2SO_4 mixture has been reported, but it does not always result in complete oxidation, as was shown by Martini and Schilt (1976) who studied the system with 85 different organic substances. May *et a*

(1984) on the other hand digested up to 4 g of organic matrices in a nitric acid and perchloric acid mixture at a temperature of 200 °C. The typical digestion times were: plant tissue, 1.5 hours; fats 3 hours; and sludges 8 hours. This mixture did not fully dissolve metals in silicates.

2.5.3 Hydrogen peroxide

Hydrogen peroxide (30 % or 50 %) is mostly used as a primary oxidant in combination with H_2SO_4 (Novozamsky *et al,* 1995). This mixture has proven to be very effective, as the only decomposition product is water, and reagent purity is high (Gorsuch, 1970). Finely divided carbon produced by charring organic matter with concentrated H_2SO_4, is readily oxidized by H_2O_2. Hydrogen peroxide may be added dropwise to the acid mixture and sample, in order to minimise dilution and cooling of the solution. Practically all kinds of organic sample may be digested using this procedure. The draw back however, is that the sample size is rather limited (for plant tissue about 300 mg dry matter can be used); larger amounts of sample give rise to excessive foaming from the large gas evolution. Losses of volatile elements such as As, Ge, Se, and Hg may occur during the process due to the high temperatures compared to other wet digestion mixtures (Novozamsky *et al*, 1995).

The other disadvantage of use of acid mixtures has to do with the fact that they are unable to completely break silicate bonds. This may lead to incomplete detection of

many elements depending on the original matrix structure. Secondary precipitates of cations may occur in the matrices when H_2SO_4 is used (Temminghoff *et al,* 1992).

Other combinations with H_2O_2, such as H_2O_2 and HNO_3; HNO_3, H_2O_2, and HCl; H_2O_2 and $HClO_4$ (Hoenig 1995); and H_2O_2, HNO_3, and HF (Novozamsky *et al,* 1993) are also used. In these cases complete oxidation is also not necessarily obtained because of the low boiling points of the reagents. In open systems sequential working can be expected, *e.g.* in H_2O_2 and $HClO_4$ mixture, perchloric acid only starts to oxidize after evaporation of the H_2O_2 and H_2O. The usefulness of these mixtures depends upon the matrix involved.

2.6 Dry ashing procedures

Dry ashing involves processes in which organic matter is oxidized by reaction with gaseous oxygen in combination with heat. The classical dry ashing method leads to complete removal of the organic matrix if performed with care (Mader *et al*, 1998b). Examples of dry ashing procedures include: methods in which the sample is heated to a relatively high temperature in a stream of air or oxygen; the related low-temperature technique where excited oxygen is used; bomb (high pressure) methods using oxygen under pressure; and an oxygen flask technique in which the sample is ignited in a closed system at near atmospheric pressure. Mader *et al* (1997) studied the chemistry and energetics of biological matrix decomposition during classical dry ashing of animal and plant materials and identified at least three phases in the decomposition profile. All dry

ashing methods listed above proceed through the following series of processes, although it can sometimes be difficult to establish sharp borders between the overlapping processes.

- Phase I: Evaporation of moisture (dehydration).
- Phase II: Evaporation of volatile materials including those produced by thermal cracking and partial oxidation.
- Phase III: Progressive oxidation of the non-volatile residue, until all organic matter is destroyed.

The relative significance of each of the steps can vary from one method to the other. Dry ashing at elevated temperature at atmospheric pressure is the most frequently applied method.

Generally, two types of procedures can be distinguished in muffle furnace ashing. Firstly, procedures involving the use of closed systems where extra air is supplied to the sample (Gorsuch 1970). The sample glows and reaches higher temperatures than the air atmosphere in the furnace and works well with small quantities of material. The digestion temperature in such a system will vary with the thickness of the sample layer and the air supply. This method has been used successfully for both non-volatile materials which remain in the residue in the combustion boat, and for volatile elements such as mercury which can be trapped. An important advantage claimed for the extra air supply is a substantial reduction in the ashing time, but the procedure becomes much more difficult, especially for volatile elements such as Cd and Pb (Mader *et al*, 1996).

Another procedure is carried out in open systems in a muffle furnace on the assumption that no significant amount of the analytes to be determined will be lost through volatilization. The temperature of the sample will be closer to the furnace temperature although some temperature gradients can occur in the furnace. This method is commonly used for the decomposition of organo-metallic compounds, and is almost exclusively used when large samples are analysed.

The main advantage of dry oxidation procedures is the ability to process large amounts of samples, compared with wet digestion methods (Gorsuch 1970). On the other hand possible losses caused by volatilization at high temperature (*e.g.* As, Cd, Pb, and Hg; these effects are matrix dependent), and reactions with container materials, are higher than in wet digestion methods.

To some extent, drawbacks associated with dry ashing can be surmounted by performing dry ashing procedures at reduced pressure (70 – 100 Pa). This involves the use of gaseous oxidants where oxygen is activated by a high frequency electromagnetic field and the temperature in these so-called low-temperature ashers reaches only about 100 – 200 °C (Carter and Yeoman 1980). The oxidant is activated in glass ashing chambers in which sample crucibles are placed. The use of pure oxygen as a sole reagent is an added advantage while the closed environment in which the sample is ashed, allows building-in a cold finger, so that volatile components can be trapped. The main disadvantage is the long digestion times due to the formation of crusts on the surface of the sample which reduces the surface area of the particles to react with acids (Fabry *et al,* 1972).

Knapp *et al* (1981) developed a partially mechanized decomposition method where an apparatus called the "Trace-O-Mat" was used to oxidize up to 1.0 g of organic material in approximately one hour. The instrument consists of a small volume (75 ml) combustion chamber on top of which is mounted a cooling unit. The sample is burnt in a stream of oxygen, heated by IR lamps. Volatile products are condensed in the cooling unit at liquid nitrogen temperature. Eleven elements, including Cd, Pb, Hg, As, and Se were determined with success. The drawback of their method is the low sample throughput of one sample at a time and the need for skilled operators to use the equipment. Similarly, Adeloju *et al,* (1989) developed three dry ashing procedures for digesting animal muscle, bovine lever, orchard leaves, and oyster tissue for voltammetric analysis of trace elements. These procedures although somewhat effective are not usually applied to ICP-MS analysis due to the use of hydrochloric acid as a leaching agent, since chlorides cause interferences with some elements.

2.6.1 Oxidation with excited oxygen

This technique involves oxidation of the sample in a stream of activated oxygen at temperatures up to 120 °C. Activated oxygen is produced by subjecting a stream of oxygen gas to a high-frequency electrodeless discharge at low pressures to produce reactive atomic and ionic species in the oxygen which then react with the organic material without raising the temperature above 120 °C (Gorsuch 1970). Little attention is required once the sample is placed in the device; the blanks are low, and there are no

hazards from aggressive liquids and explosions (Griepink 1989). Reactions between container and sample do not occur and the rate of the reaction can be monitored (*e.g.* N-emission line at 675 nm). Volatilization is not a significant problem except when fluorides are formed (B, Si, Ti, and U). The technique however, is expensive, few samples can be processed at a time, and the ashing takes several hours and requires dry samples.

2.6.1.1Oxidizing fusion

Some metals are readily attacked by alkaline hydroxides in the presence of an oxidizing agent, such as alkali metal hydroxide mixed with sodium peroxide or nitrate. Sodium hydroxide mixed with sodium peroxide, or sodium peroxide alone, is frequently used as a flux (Balcerzak Maria, 2002). Fusion is usually performed at 450 °C – 600 °C for 15 – 60 minutes. The melt is dissolved in water and acidified with hydrochloric acid for converting the analytes into chloro-complexes, which can serve as the basis for subsequent separation or determination methods.

Fusion is rarely used in trace element analysis of biological and/or environmental materials because it uses large amounts of reagents that are difficult or costly to obtain in high purity (Kucera *et al,* 2007). However, fusion of biological materials with alkali hydroxides has been recognized as a suitable decomposition method for the determination of halogenides, importantly iodine, which can otherwise be easily lost using some methods of sample decomposition (Dermeli *et al,* 1990).

Fusion can increase blank values and/or cause interferences in analytical methods of many trace elements, such as atomic absorption and emission spectrometry, mass spectrometry, *etc*. Large amounts of salts and other contaminants from the wall of the crucible attacked by the flux may be introduced into the sample. The application of the method is restricted to small sample weights of about 0.5 – 2.0 g (Balcerzak Maria, 2002).

2.7 Analytical methods

Various analytical methods have been applied for biological samples that require elemental determination. Many of these applications are in the field of environmental safety, food, and health. For reliable and accurate determination of trace metals, there is a need for a solid knowledge of the various analytical techniques for determination of elements in the various matrices.

2.7.1 Inductively coupled plasma (ICP)

Inductively coupled plasma (ICP) is an excitation source used with optical (OES) or mass spectrometric detection (MS) for the detection of trace metals in a number of different industries including environmental, food and agriculture, semiconductor, clinical and pharmaceutical, geological, nuclear, and chemical. In ICP-OES the elements emit a characteristic wavelength of light which can then be measured. This technology

was first employed in the early 1960's with the intention of improving crystal growing techniques (Thomas 2001, I). ICP has been refined and used in conjunction with other procedures for quantitative analysis. In the early 80's the ICP was interfaced with mass spectrometers giving nearly a thousand fold lower detection limits. In the last decade around an additional 1000 fold decrease in detection limits has been obtained (Thomas 2001, I).

A plasma is a gas like fluid but one which contains a large number of positive ions and free electrons. This plasma has sufficiently high energy to atomize, ionize, and excite the majority of the elements in the periodic table. Although there are several types of plasmas (direct current, microwave induced, *etc.*), the inductively coupled plasma (ICP) has demonstrated the most useful properties as an ion source for analytical spectrometry. Gases such as argon, nitrogen, helium, neon, and air have been used to sustain plasmas useful for analytical purposes; however, the inert gases offer some advantages because, of their desirable ionization properties, their availability in relatively pure forms (Thomas 2001 II), and because they are monatomic gases. Impurities in the plasma gas can result in spectra interferences and backgrounds leading to difficulties in quantitative measurements. Inert gases, specifically argon, also have advantageous property of lower chemical reactivity with various analyte species, which can also result in undesirable analytical results.

2.7.2 The workings of an ICP

The ICP hardware consists of three concentric tubes, most often made of fused silica or quartz. These tubes, termed outer, intermediate, and inner, make up the torch of the ICP. The torch is situated within a water or argon cooled coil excited by a high power radio frequency (r.f.) generator. The plasma gas is passed through the outer annular region at a usual flow rate of from 12 to 17 L/min. A second gas (auxiliary) flows through the intermediate tubes at a rate of from 1 to 2 L/min. The third gas flow which also flows (nebulizer or sample carrier gas) at approximately 1 L/min carries the sample which is usually in the form of a fine droplet aerosol from the sample introduction system to the plasma.

Inductively coupled plasmas are formed by coupling energy produced by a RF generator to the plasma support gas with an electromagnetic field. First a tangential (spiral) flow of argon gas is directed between the outer and the middle tubes of the ICP torch. A load coil surrounds the top end of the torch and is connected to the RF generator. When an RF power (typically 750 – 1500 W) is applied to the load coil, an alternating current oscillates within the coil at a rate corresponding to the frequency of the generator (usually 27 or 40 MHz). This RF oscillation of the current in the coil causes an intense electromagnetic field to be created in the volume at the top of the torch. With argon gas flowing through the torch, a high voltage spark is applied to the gas causing some electrons to be stripped from the argon atoms. These electrons, which are caught up and accelerated in the magnetic field, then collide with other argon atoms, stripping off still more electrons (Thomas 2001, III). This collision induced ionization of argon continues

in a chain reaction, breaking down the gas into argon atoms, argon ions, and electrons, to form the ICP discharge. The ICP discharge is then sustained with the torch and the load coil as the RF energy is continually transferred to it through the inductive coupling process. The amount of energy required to generate argon ions in this process is approximately 15.8 eV, which is enough to ionize the majority of the elements in the periodic table. The sample aerosol is then introduced into the plasma through the sample injector.

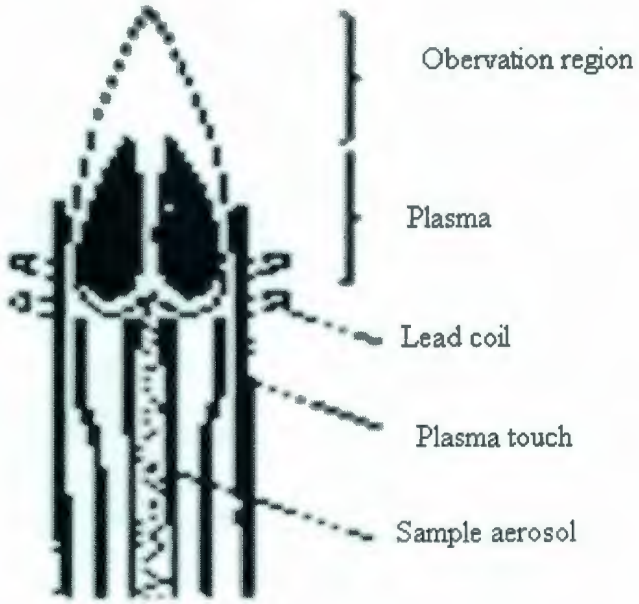

Fig. 2. 1: Schematic diagram of ICP flame (source: Bradford T. and Cook N.M.: www.cee.vt.edu/ewr/environmental/teach/smprimer/icp/icp.html)

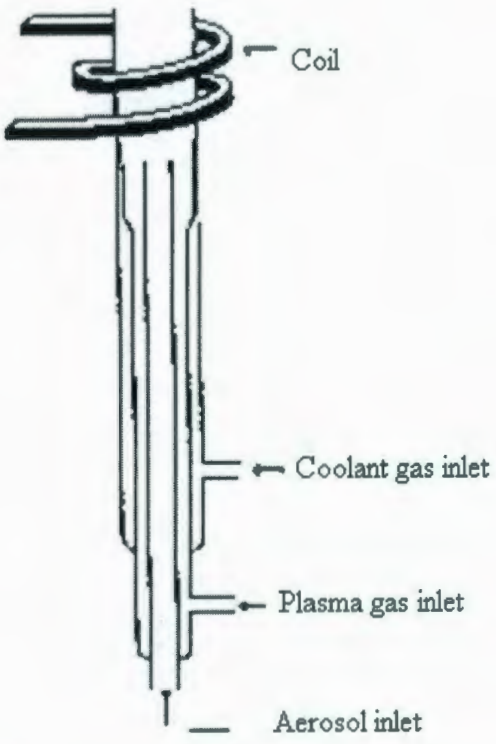

Fig. 2. 2: A typical plasma torch (source: Bradford T. and Cook N.M.: www.cee.vt.edu/ewr/environmental/teach/smprimer/icp/icp.html)

The plasma is insulated from the rest of the instrument by the concurrent flow of gases through the system and this helps to prevent possible short-circuiting and meltdown (Bradford *et al*, 2001). The outer gas (typically argon) has been demonstrated to serve several purposes including maintaining the plasma, stabilizing the position of the plasma,

and thermally isolating the plasma from the outer tube (Jarvis *et al.*, 1992). Argon is commonly used for the intermediate and carrier gas because it is relatively easy to ionize.

2.7.3 ICP combined with mass spectrometry

Introduced in 1983, ICP-MS has gained much popularity within the analytical community as the most promising technique (Vanhaecke *et al*, 1999) for the determination of trace and ultra-trace elements in a variety of matrices. The efficiency of the Inductively Coupled Plasma in producing singly-charged positive ions for most elements makes it an effective ionization source for mass spectrometry (Thomas 2001, I). Inductively coupled plasma-mass spectrometry is unique among the flame and plasma spectroscopy techniques owing to its high speed, excellent detection limits, wide dynamic range, and possibility of accurate multi-element analysis and unique capability of measuring element isotopic ratios (HP 4500 ChemStation Operator's Manual, 1997). The ICP-MS has a wide elemental coverage and measures virtually all elements including alkali and alkaline earth elements, transition, and other metals, metalloids, rare earth elements, most of the halogens and most of the non-metals. Other advantages of ICP-MS include high sample throughput, and the relatively simple spectra, which permit immediate qualitative to semi-quantitative conclusions to be drawn (Jarvis *et al.*, 1992). These features make ICP-MS attractive for applications ranging from ultra-trace analysis in semi-conductor industries and clinical applications through environmental monitoring

of polluted soils, water and air to the determination of elemental species in the life sciences.

However, ICP-MS signals can suffer from interferences of various forms. These interferences can be categorized as spectroscopic and non-spectroscopic in nature (Jarvis *et al.*, 1992). The spectroscopic interferences result from signals of oxides (MO^+) and hydroxides (MOH^+) which occur abundantly under wet plasma conditions, doubly charged ions (M^{2+}), argides, isobaric overlaps, and other polyatomic ions with the same ratio of mass to charge as the elements of interest. Non-spectroscopic interferences are either physical effects which result from the solids present in a solution or analyte suppression and enhancement effects which result from influences of matrix elements in the sample on the yield of ions (Jarvis *et al.*, 1992; Falkner *et al.*, 1995). For nebulization, samples must be free of particles that can cause nebulizer blockage. A high concentration of dissolved solids can result in the build up of material on the sampler and skimmer cone orifices. The worst culprits are elements that can deposit refractory oxides, such as Al, Si, and Zr (Falkner *et al.,* 1995). Residual organic material can also be deposited on the nebulizer, spray chamber, and torch walls, leading to memory effects as well as affecting sample flow rates through altered viscosity. At high levels of organics, carbon can destabilize and even extinguish the plasma as it builds up on the sampling cone. The other limitations of the ICP-MS technique arise from the conversion of solid samples into solution prior to analysis, and the inability to detect the chemical form in which the elements occur (Vanhaecke *et al.*, 1999).

An ICP-MS can be broken down into four main processes, including: sample introduction and aerosol generation, ionization in the argon plasma, mass selection, and the detection (Thomas 2001, I). The conventional method of sample introduction for ICP-MS is by aspiration, via a nebulizer, into a spray chamber (Thomas 2001, II). The sample introduction system produces an aerosol of liquid droplets or solid particles and vapour. An ideal aerosol has: a) constant density, b) a composition that represents the original sample and c) small particles with a narrow distribution that allows complete atomization and ionization in the ICP-MS interface (Nuttall and Gordon, 1995). This is not completely achieved with any sample introduction system available. Calibration for ICP-MS is usually based on external calibration standards, using internal standardization to compensate for changes in the sample introduction and the ionization efficiency. Other approaches include standard additions or isotope dilution methods where the sample introduction has more identical influence on a given element in calibration solutions and the samples (Jiang and Houk, 1986).

Nebulization of solutions is most widely used for sample introduction in ICP-MS measurements. This is because calibration solutions can be prepared in almost any concentration and matrix composition. Fig. 2.3 shows a schematic drawing of a concentric nebulizer. The major disadvantage of solution nebulization, however, is that the majority of real samples are solids, which require digestion before being introduced into the ICP using solution nebulization. This dissolution step requires reagents, increases sample preparation time and is accompanied by the dilution of the original sample. Especially at ultra-trace concentrations in the sub μg/g range, the purity of reagents,

laboratory environment and sample preparation equipment are important factors in cost and labour.

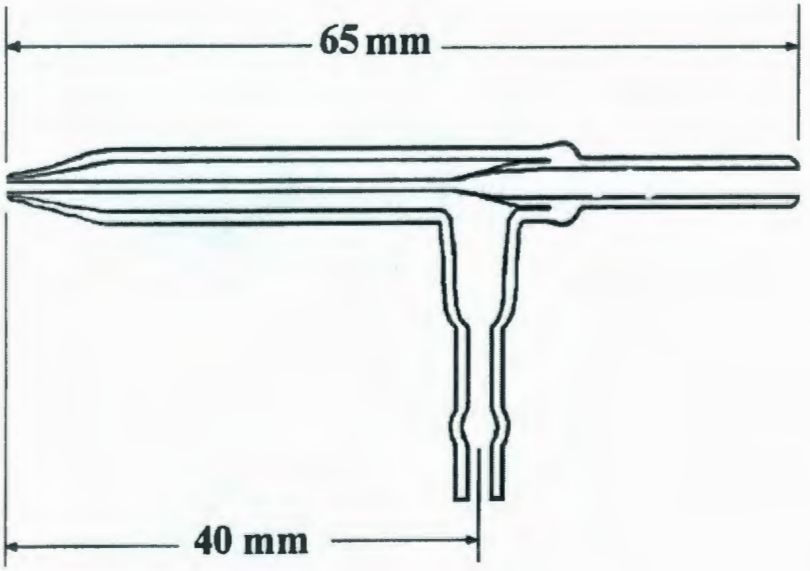

Fig. 2. 3: A schematic drawing of a concentric nebulizer (source: Chemstation Operator's manual, 1997).

These limitations have long triggered the search for direct solid sample introduction systems, including spark ablation (Vanhoven *et al*, 1995) and laser ablation (Mochizuki *et al*, 1991). Spark ablation is restricted to electrically conductive samples and while providing comparably low spatial resolution, whereas laser ablation is

potentially applicable to any solid sample for quantitative analysis at high spatial resolution. Restrictions due to sample heterogeneity are an important limitation to the use of Laser Ablation (LA) for bulk analysis. Note that solutions have the very desirable property of being homogeneous at the molecular level.

The function of the spray chamber is to remove droplets produced by the nebulizer that are greater than approximately 8 μm in diameter allowing only small droplets to enter the plasma and to smooth out pulses that occur during the nebulization process due to the peristaltic pump if used. This allows only small droplets into the plasma for dissociation, atomization, and ionization of the elemental component in a sample. A small fraction of the resulting aerosol is swept by argon into the torch. Approximately 1 mL of sample is required per analytical run, about 99 % of which is wasted (Thomas 2001, II) using conventional nebulizers.

There are basically three designs of spray chambers that are used for commercial ICP-MS instrumentation – double pass, cyclonic, and impact bead spray chambers (Fig. 2.4). The double pass is by far the most common with the cyclonic type rapidly gaining popularity. The impact bead was first used with flame AA, and is also an option for use with ICP-MS. The double pass selects the small droplets by directing the aerosol into a central tube. The larger droplets emerge from the tube and by gravity, exit the spray chamber via a drain tube. The liquid in the drain tube is kept at positive pressure, which forces the small droplets back between the outer wall and the central tube and emerges from the spray chamber into the sample injector of the plasma torch.

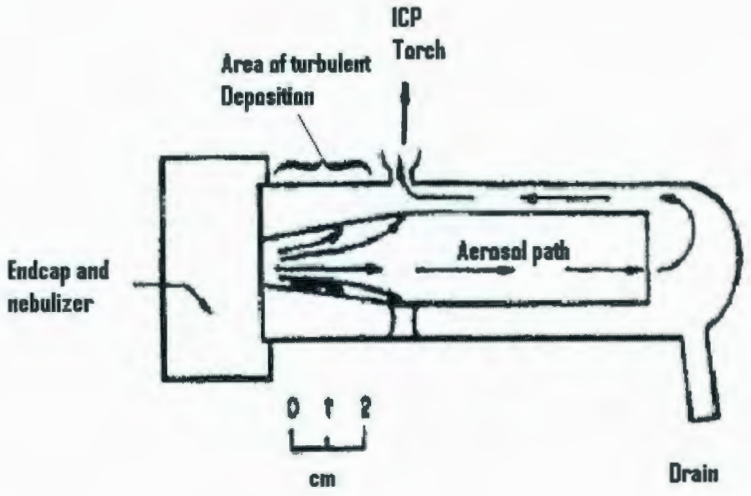

Fig. 2. 4: A schematic diagram of a Scott double pass spray chamber: (source: Chemstation Operator's manual, 1997)

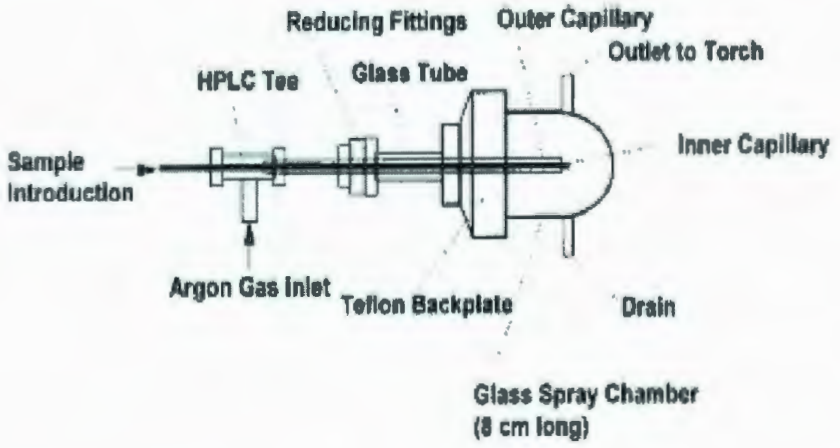

Fig. 2. 5: A schematic diagram of oscillating capillary nebulizer with single pass spray chamber (source: B'Hymer C. et al, 1998)

Fig. 2. 6: Photograph of single spray chamber. (source: Todoli Jose-Luise and Jean-Mermet, 2002).

The cyclonic spray chamber on the other hand operates in a similar manner (Figs. 2.5 and 2.6). Droplets are discriminated according to their size by means of a vortex produced by a tangential flow of the sample aerosol and argon gas inside the chamber. Small droplets are carried with the gas stream into the ICP-MS, while the larger droplets impinge on the outside walls and fall out through the drain. The cyclonic spray chamber has a higher sampling efficiency, which for clean samples, translates to high sensitivity and lower detection limits (Howard 2000). However, the droplet distribution appears to

be different from the double pass design, and for certain type of samples can give slightly inferior precision.

Fig. 2. 7: Photograph of a cyclonic type chamber. (source: Tololi Jose-Luise and Jean Mermet, 2002).

Ion Extraction

After the analyte ions are formed at atmospheric pressure, they are analyzed in a mass spectrometer, which must operate in a vacuum. Extracting ions from the plasma into the vacuum system is the critical step. A diagram of an extraction interface is shown in Fig 2.8. The ions enter a region evacuated by a mechanical pump through the orifice

(approximately 1mm) of a cooled cone (sampler cone). Then the ions pass through a second orifice, called the skimmer (Fig. 2.8). At the back of the skimmer cone a lower vacuum pressure is usually maintained by a turbo molecular pump backed by a rotary pump. In most modern units a third larger orifice separates an additional region which is maintained by a second turbo pump at approximately 100 fold lower pressure. Ion lenses focus the ions into the entrance of the mass spectrometer, while limiting the passage of high energy photons which could be detected depending upon the detector system being used.

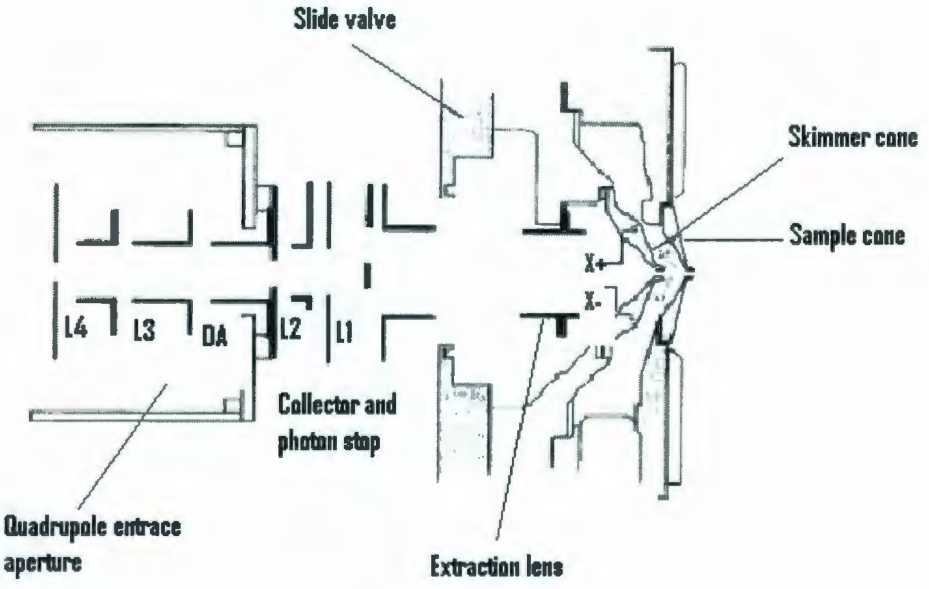

Fig. 2. 8: Schematic diagram of the standard ion extraction interface and ion optics. (source: Carter *et al*, 2003)

The gas expands behind the first orifice, and approximately one percent passes through the second orifice in the skimmer cone. A series of ion lenses, maintained at appropriate voltages, are used to direct the ions into the mass analyzer, which is most commonly a quadrupole, although magnetic sector and time of flight analysers are also commercially available. In the case of the quadrupole, the ion is transmitted through the quadrupole on the basis of the selected mass to charge ratios and then to a detector which is commonly an electron multiplier.

The quadrupole mass analyzer is usually set to give slightly better than unit mass resolution over mass range up to m/z = 300. The quadrupole based ICP-MS system is a sequential multielement analyzer that can complete a full mass scan in less than 20 ms, although times of the order of several hundred ms are more commonly used. The signal intensity is a function of the number of analyte ions in the plasma and the mass-dependent transport through the sample introduction system and the mass spectrometer.

The most important advantages of ICP-MS include multi-element capability, high sensitivity, and the possibility to obtain isotopic information about the elements determined. Disadvantages inherent to the ICP-MS system include the interferences produced by polyatomic species arising from the plasma gas and other atmospheric gases. The isotopes of hydrogen, carbon, nitrogen, oxygen, and argon combine with themselves or with other elements to produce a large set of background ions. ICP-MS is not as useful in the detection of non-metals (Thomas 2002, XII) due to their higher ionisation potential. However all the elements in the periodic table, except He, can be detected

although fluorine, with its very high ionisation potential, can only be determined using high resolution or negative ion detection, and with very high detection limits.

2.8 Use of plants as indicators of environmental pollution

Anthropogenic emissions of pollutants have increased greatly in the last two centuries since the onset of the industrial age. An estimation of the atmospheric inputs of Zn, Pb, and Hg in 1988 amounted to 840,000 t, 400,000 t and 11,000 t respectively (Markert *et al.*, 1997). These continuing anthropogenic emissions and the resulting input into the environment are causing severe damage to plants, animals, and humans. In particular, accumulation in soils, groundwater, and organisms may have incalculable consequences within links in the food chain. This necessitates careful monitoring of deposition and its effects, for which the use of boindicators and biomonitors provide an indirect integrating method for estimating the pollution levels in an area.

A bioinidicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (Markert *et al*, 1997; Figueiredo *et al*, 2007) while biological monitors (biomonitors) have commonly been defined as organisms that provide quantitative information on some aspects of their environment, such as how much of a pollutant is present (Keane *et al*, 2001; De Temmerman *et al*, 2004; Figueiredo *et al*, 2007). Both bioindicators and biomonitors react to changes in their environment caused by one or more pollutant substances by changing their way of life with respect to their morphology and/or metabolism. These

changes being observable or measurable. Monitoring by observation may include examination for changes such as needle or leaf discolouration, changes in population density or distribution, intermodular stretching, *etc.* in an organism or a population of organisms. Monitoring may involve physical or chemical determination of heavy metals, nutrients, or various enzyme activities and biochemical investigation of metabolic reactions and secondary plant constituents.

The responses of plants to a concentration gradient of trace elements in their environment (air, water, and soil) can follow one of three main patterns *i.e.* as accumulators, monitors, or excluders. Accumulators build up pollutants to a level several orders of magnitude higher than in their environment. The uptake of pollutants by such plants varies linearly with increasing environmental input until a threshold where metal uptake becomes constant. They tolerate high concentrations of trace elements in their tissues, and this accumulation can be produced even at low external concentrations in the environment. The excluders on the other hand maintain low concentration of a substance irrespective of the quantities in the environment, and resist any increases in metal uptake until a level in the environment is reached which breaks down the regulatory mechanisms of the plants. Biomonitors have a correlation between the concentration of the pollutant in the environment and that in the organism and therefore reflect the actual trend of pollutant input into the environment.

Bioindicators are useful tools for environmental monitoring due to their high tolerance to substances accumulated in their tissues over an extended period of time. The use of bioindicator plants to monitor environmental pollution has advantages such as ease

of monitoring large areas and the low cost of plant sampling (De Temmerman *et al*, 2004). Thus biological monitoring with plants provides low-cost and effective methods to estimate the amount of pollutants and their impact on biological receptors as compared to direct methods of pollution measurement, especially as no collecting or measuring device has to be installed and protected against vandalism and the weather. Use of bioindicators generally help to detect changes in the natural environment; monitor the presence of pollutant and its effect on the ecosystem in which the organisms live; monitor the progress of environmental clean up; and to test substances such as drinking water for the presence of contaminants.

Use of biomonitors also helps to facilitate analytical measurements and thus helps to detect low concentrations that are not always easy to measure directly using chemical extraction techniques (Market *et al.*, 1999; Madejon *et al.*, 2004). Errors due to analytical measurements are reduced because most accumulators build up substances to be determined to a level several orders of magnitude higher than that of their environment. Finally, use of individual parts of an ecosystem in determining the latter's trace or heavy metal status has the advantage that it permits conclusions going beyond the biomonitor itself (Market *et al*, 1999). By occupying a niche in the ecosystem, biomonitors make it possible to integrate the results of the analysis in an overall biological system and this permits ecologically relevant statements about the whole community of organisms as well as the biomonitors themselves. Tingey (1989) observed that "there is not a better indicator of the status of a species or a system than the species or system itself". Physical and chemical methods do not provide sufficient information on the risk associated with

an exposure (Mulgrew *et al.*, 2000). It is therefore evident that plants play a significant role in the biomonitoring of pollution as analysis of plant tissue provides direct quantitative information on relative concentration load.

Plants as biomonitors have been used since the beginning of the 20th century; for instance, the alterations in the composition of some species in the beginning of the 1920's provided information about the pollution in areas exposed to fumes originating from coal burning industrial plants (Ruston 1921 in Figueiredo *et al.*, 2007). Since then, a variety of organisms and material have been proposed for biomonitoring purposes. These include mosses, lichens, tree bark, tree rings, pine needles, grass, leaves, and ferns (De Temmerman 2004, Figueiredo *et al.*, 2007). Studies in many parts of the world have used tree leaves as bioaccumulators of trace elements, in the surroundings of industrial facilities (Giertych *et al*, 1997; Mieieta and Murin 1998; Rautio *et al*, 1998) and in urban environments (Monaci *et al.*, 2000; Aboal *et al.*, 2004; Figueiredo *et al*, 2007). In most of these studies, the elemental concentrations in the tissues of plants used as pollution bioindicators reflect largely the concentration of the pollutants in the monitored environments.

Spruce needles were used by the German environmental sample bank for the permanent monitoring of known pollutants and retrospective determination of pollutants that were not known or could not be accurately analyzed at the time of accumulation (Market B. *et al*, 1999). De Temmerman *et al.*, (2004) used leafy vegetable crops for biomonitoring Pb and Cd deposition, and they concluded that vegetables are suitable during the growing season if their specific differences in accumulation rates are taken

into account. Their study further revealed that growth rates are important parameters according to the accumulation efficiency.

Bioaccumulative indicators are frequently regarded as biomonitors. Plants act as bioaccumulative indictors by accumulating pollutants from their surroundings without necessarily displaying an obvious response (Sabah *et al.*, 2004). They are useful in determining past pollution exposure and analysis of their tissues provides an estimate of the environmental load of pollutants. For example, high levels of heavy/trace metals in plants often correlate to levels of such metals in soil, air, and water. Bioaccumulation therefore is the result of the equilibrium process of biota compound intake/discharge from and into the surrounding environment (Conti *et al.*, 2001).

Several vascular terrestrial and aquatic plants have been used for both environmental and geochemical studies, especially for trace metal exploration/monitoring (Kabata-Pendias and Dudka 1991). Recent investigations identified black spruce as an effective tool for monitoring trace metal levels in soils. Zayed *et al* (1991) used black as a bioindicator to evaluate aluminum contamination in the Saguenay region while up to 130 μg/g uranium was measured in black spruce twigs obtained from the Midwest uranium deposit area of the Athabasca basin (Northern Saskatchewan) (Gordon 1999 in CCME). This shows that spruce tree is a bioaccumulative indicator and therefore essential for trace metal biomonitoring. A key feature of this conifer is its metal-absorbing capability to reflect the prevailing soil concentrations without significant adverse effects on its survival or growth (Zayed *et al*, 1991). Other advantages of using black spruce as

bioindicator of metal contamination are its long life and its abundance and even distribution over the area studies.

2.9 Black spruce (Picea mariana)

2.9.1 Growth habit

Black Spruce is a small to medium sized coniferous tree with a shallow and wide spreading root system. The branches are short, pendulous, and have a tendency to curve up at the ends. The bark is grayish brown, and the surface is broken into thin scales 6 mm to 12 mm thick. The twigs are light reddish brown and densely covered with short hairs, some of which are tipped with glands (Ryan 1989). The cones are 35 mm long, ovoid, and purplish but turning brown at maturity and usually remain on the trees for many years. The cone scales are stiff, have toothed margins, and dark brown seeds. The needles are 4-sided, dull blue-green in color, 6 mm to 35 mm long, blunt-pointed, flexible, and soft to the touch (Maine Forest Service/Department of Conservation, 1995). Average maximum age is about 200 years. The black spruce grows to about 10 to 13 m tall and the diameter of its stem ranges between 150 to 450 mm. It forms an open, irregular crown and has a limited spread.

2.9.2 Fruit/seed description and dispersal methods

The black spruce is monoecious (separated male and female sexes). The male flowers are produced on the outer branches of the crown below the zone of female flowers. They are ovate, 175 to 200 mm long and dark red to purplish. The female flowers, produced in the upper crown, are usually erect, cylindrical and green or purplish in colour and about 150 mm in length. Black spruce flowers in late May/early June (Maine Forest Service/Department of Conservation, 1995; Ryan 1989). Female conelets develop rapidly and contain mature seeds about 3 months after pollination. A few cones may be produced after 10 years, but maximum production is between 100 and 200 years (Ryan 1989). Black spruce seeds mature 3 months after pollination in late August or early September. Some are produced almost every year, but heavy seed years occur at 2 to 6 year intervals. Seeds are dispersed throughout the year, but dispersal is highest in the spring and seeds are not commonly dispersed over long distances.

2.9.3 Habitat

Black spruce is widespread throughout the boreal region of North America and extensive in area from Newfoundland to Alaska, south of British Colombia, Great Lakes Region and Minnesota (Maine Forest Service/Department of Conservation, 1995). It grows on cool upland soils, but is more commonly found along streams, and on the borders of swamps and sphagnum bogs. It has a tolerance to both shade (however, growth

is fastest in full sunlight) and nutrient-poor soils. It is commonly found on poorly drained acidic peatlands.

Chapter 3: Analytical methodology

3.1 Site description (Holyrood area)

Holyrood is located 20 km SW of St John's, Newfoundland. The study area is located between Gull Pond and Big Pond, and a boundary to the west at longitude 53°03'W; on the east at longitude 52°57'W; on the north at latitude 47°28'N; and on the south at latitude 47°25'N. The vegetation in this area falls within the Avalon Forest Ecoregion (Department of Forest Resource and Agrifood, 2000), and the landscape pattern is dominated by trees of balsam fir with a mixture of black spruce (*Picea mariana*) and pine. The land surface is well forested with white and yellow birch scattered throughout the area while bedrock outcrops are common on the hills. The choice of this site was based on the description of a uranium anomaly recorded in a study conducted by Sherwin (1979); which was in turn a follow up to Davenport's report "Uranium Distribution in the Granitoid Rocks of Eastern Newfoundland" which identified the northern end of the Holyrood Granitic Pluton as being enriched in uranium. Uranium concentrations determined in lake bottom sediment samples from Gull Pond and Big Pond ranged from 12 – 42 ppm while that recorded in stream silt samples ranged from 12 – 22 ppm. A goal of this study is to demonstrate whether this anomaly is recorded by the black spruce twigs obtained from the area.

3.2 Geology of the Holyrood area

The sampling site is underlain by late Precambrian granitoid rock of the Holyrood Plutonic series which have intruded volcanic rocks of the Habour Main Group. The Holyrood Granite is unconformably overlain by Eo-Cambrain sediments of the Conception Bay Group near the east boundary of the area and by Cambrain and Ordovician marine sediments of the Manuels River and Chamberlains formations to the north. Volcanic and gabbroic dykes found cutting the granitic rocks are pre Conception Bay in age.

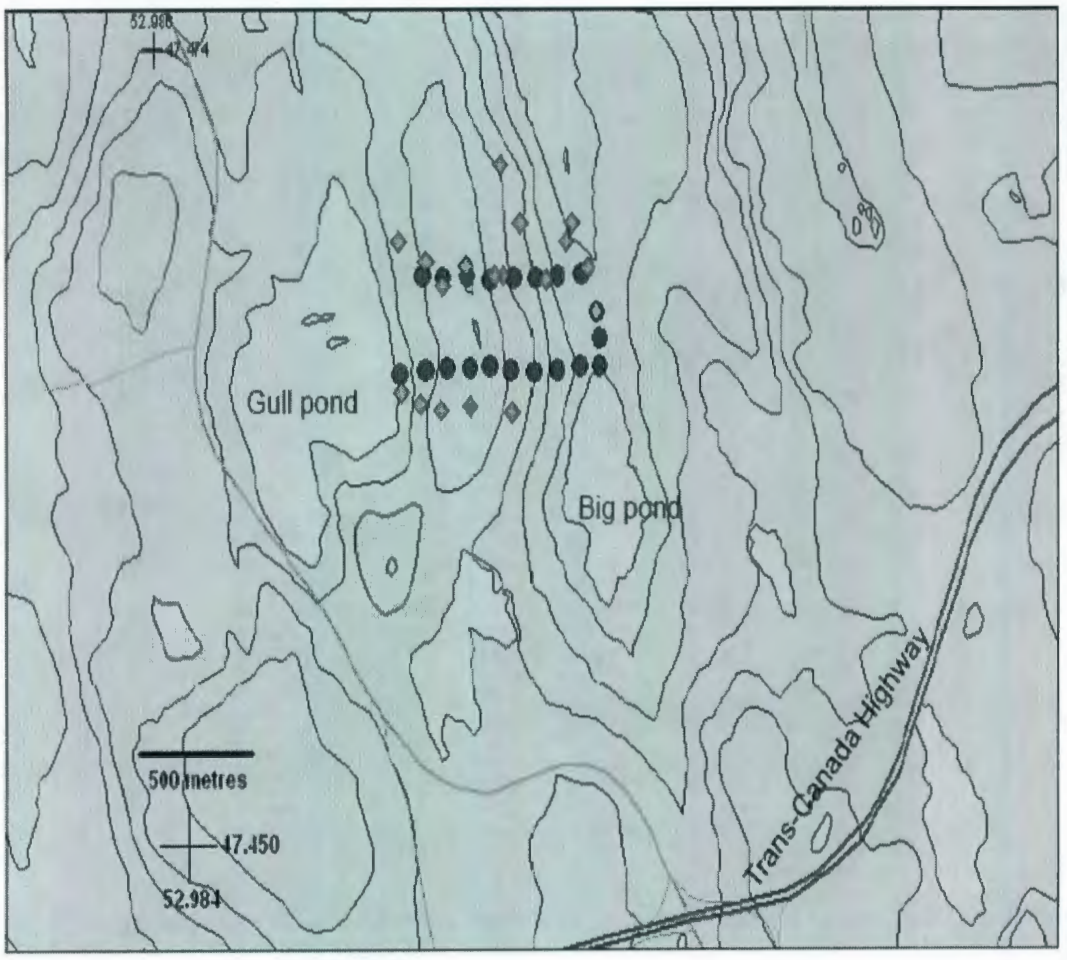

Fig 3. 1: Geology map showing the bedrock of the Holyrood study area (Holyrood granitic intrusion). Dots = winter sample points, Diamonds = spring sample points. The latitude and longitude are given for a point in NW corner of map and scale bar is shown for reference.

3.3 Sample collection

For the purpose of this study, black spruce twigs, about 20 cm long were collected from trees located in the area between the Gull Pond and a stream originating from the north tip of Big Pond (Fig 3.1). At each sample point, the twigs were snipped from trees using a pair of shears labelled clean plastic bags and tied to avoid cross contamination. The UTM coordinates of each sample point were recorded using a GPS. The height of trees on which the samples were collected ranged from 1 to 3 m. The samples were collected in January and May, 2008 and stored at -4 ^{o}C prior to analysis.

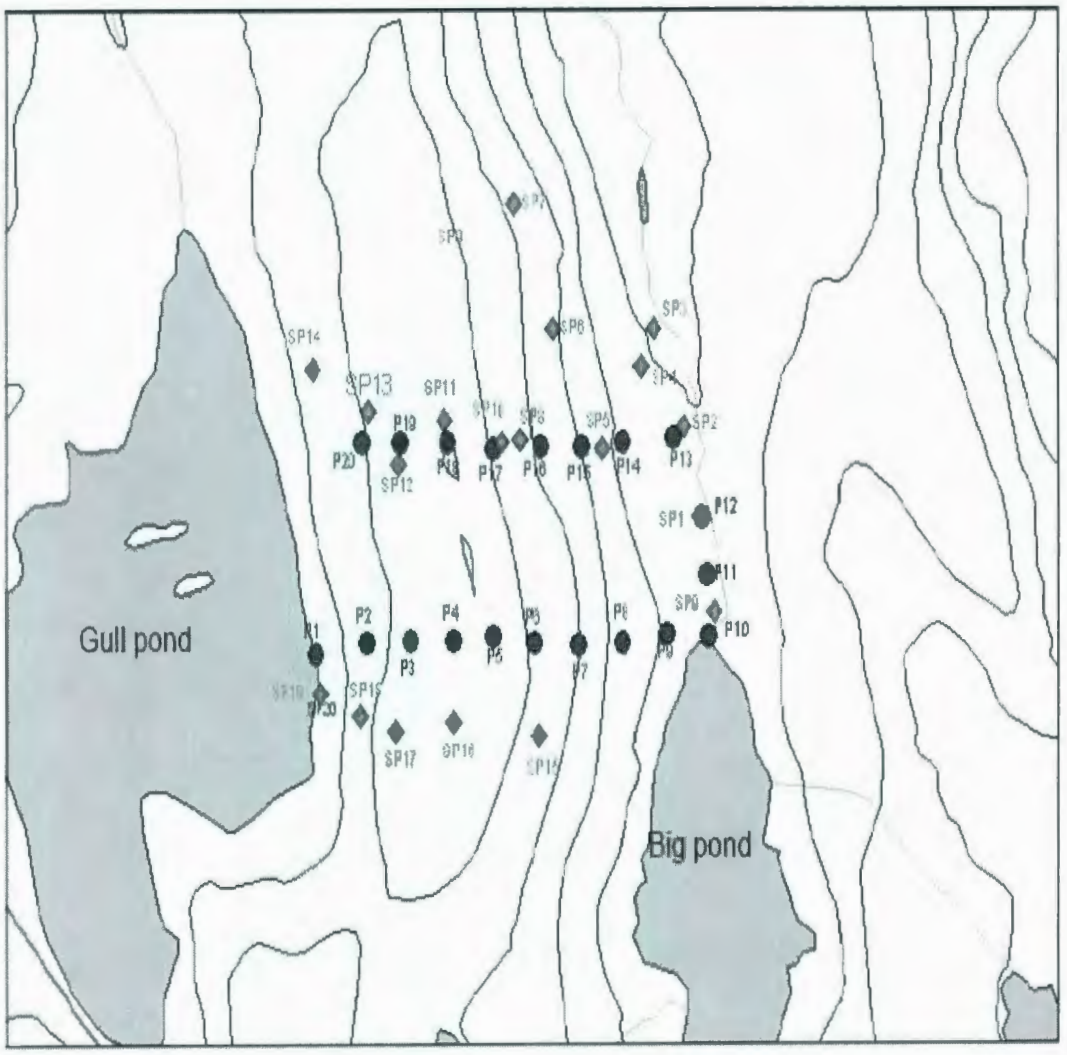

Fig 3. 2: Location map Holyrood field area showing the 40 sample points (P = winter sample points; SP = spring sample point)

3.3 Sample treatment/processing

The samples were physically examined, and all foreign matter and lichen-infested twigs removed. Each sample was washed in dilute solution of non-ionic, phosphorus-free detergent to remove traces of atmospheric deposits, rinsed twice with nanopure water and then swirled in air for about 15 seconds to remove excess water. They were placed on labelled plastic trays lined with brown paper and oven dried at 35 °C in a Fisher Isotemp Incubator (model 503) for two weeks. Partially dried needles were removed from the twigs after drying for a week. The dried twigs were shredded into smaller pieces by hand and milled in a clean cup mill (model TE 100/250, Angsrom Inc, Chicago). The resulting fine powder was stored in labelled clean air-tight plastic containers.

3.4 Certified standard and in-house reference material

Trial digestions were done using coffee, orange pekoe tea, and spruce twig samples (obtained from the forest near the MUN Health Centre) as in-house reference materials. The tea and coffee samples were each separately dried in a Fisher Isotemp Incubator (model 503) at 35 °C for seven days, ground into a fine powder in a cup mill (model TE 100/250, Angstrom Inc, Chicago) and then stored in clean, labelled, air-tight plastic containers. The black spruce in-house material was treated as described in section 3.3 above.

The certified standard reference materials used for this study included National Institute of Standards and Technology pine needles (1575) and peach leaves (1547), and

black spruce vegetative radionucliede CLV-1 and CLV-2 made from black spruce twigs and black spruce needles respectively. The black spruce material was obtained from the Cluff lake uranium mining area in Northern Saskatchewan, Canada and then prepared as an analytical reference material.

3.5 Instrumentation

The inductively coupled plasma-mass spectrometer (ICP-MS) used in this study was a Hewlett Packard 4500 Series ICP-MS which has a quadrupole mass analyzer, an argon inductively coupled plasma source, and a concentric nebulizer. The samples are nebulized into a Scott double pass spray chamber, where larger droplets (>10 um) are deposited on the walls of the spray chamber and then fall into a drain, the finer droplets, as well as the gas phase, are transported to the plasma by the sample carrier gas (Montaser *et al.*, 1998). The instrument laboratory is supplied with two air conditioners to minimize fluctuations resulting from temperature variations. In order to stabilise water loading of the plasma, a Peltier cooling device is supplied to control the spray chamber which was set to 2 °C. Deionized water which cools the Peltier cooling device is supplied by a Neslab CFT-75 refrigerated recirculator. This diminishes water vapour pressure (HP 4500 chemStation Operator's Manual, 1997). It is often thought that this also reduces the formation of molecular oxides and hydroxides which may contribute to spectra overlap (Falkner *et al.*, 1995), however this has been clearly shown not to be the case (Longerich and Diegor 2001), as after optimum sensitivity is re-established after a spray chamber

temperature change, the sensitivity and degree of oxide formation is identical. What happens is that when the spray chamber temperature is reduced, the vapour load of water to the ICP is reduced increasing the effective ICP temperature. To return the ICP to the optimum apparent temperature the nebuliser sample carrier gas must be increased. Water from the cooler is also passed through the ICP load coil, the sample interface, the turbo molecular pump, and the radio frequency (RF) power supply.

3.6 Operating conditions

The tuning parameters for the instrument are given in Table 3.1. The instrument was turned for maximum sensitivity where the thorium oxide formation (ThO^+/Th^+) was less than 5 %. A tuning solution containing 10 ppb Li, Co, Y, Rh, Cs, Tm, Bi, and U in 0.2 M HNO_3 was used to optimize the sensitivity. Optimal operating conditions, particularly the ion lens setting (Longerich *et al.*, 1985), are different for low and high mass element, so tuning for the entire mass range compromises the sensitivity (count rate per unit concentration). The instrument was operated at a radio frequency (RF) power of 1250 - 1275 W.

Once sensitivity was optimized, the conditions for low oxide ion formation, without loss of sensitivity, were found by aspirating the tuning solution and measuring the ion intensities for $^{238}U^+$ and its oxide $^{238}U^{16}O^+$. The oxides decrease with an increase in apparent plasma temperature, as more bonds break (bonds break when heat energy is greater than bond energy), and equilibrium shifts from MO^+ to $M^+ + O$, reducing

polyatomic species formation in the plasma. The operating conditions determined for optimal sensitivity with low oxide ion formation are given in Table 3.2.

Table 3. 1: Element concentration in calibration standards for the waters and biological package

CALIBRATION SOLUTIONS	ELEMENTS	CONCENTRATION
Standard A	Ag, Be V, Mn, Co, Rb, Sr, Mo, Sb, Tl, Pb, U Cd Li, Be, Al, Ti, Cr, Ni, Cu, Zn, As, Sn, Ba B, Mg, Se Fe	5 ppb 10 ppb 30 ppb 20 ppb 50 ppb 120 ppb
Standard B	Ca	4 ppm
Standard C	Cl Br I	25 ppm 150 ppb 10 ppb
Standard D	Si P S	200 ppb 600 ppb 4 ppm
Standard E	C Hg Tl	75 ppm 50 ppb 160 ppb

Table 3. 2: Operating parameters for ICP-MS analysis of plants samples

VARIABLE	VALUE
RF Power	1250 - 1275 W
Carrier Gas (inner)	1.00 L/min
Auxiliary Gas (intermediate)	0.82 L/min
Plasma gas flow (outer)	14 L/min
Peristaltic Pump (liquid sample uptake)	0.4 mL/min
Spray chamber temperature	2 °C
Extract 1	-166 V
Extract 2	-215 V
Einzel 1,3	-50 V
Einzel 2	9.9 V
Quadrupole Focus	-151 V

3.7 Digestion procedures

Several trial sample digestions were done to determine an optimum ashing procedure. The initial digestions involved a combination of dry and wet digestions (acid digestions) over varying time periods. The initial dry ashing procedure applied is as follows: test tubes containing weighed sample (1.0 g) were each capped, transferred to a metal test tube rack, and placed in a muffle furnace (PSG mfg. Kilns & Furnaces, model TE-20M-M2) at a temperature 50 °C. The sample was dry ashed by ramping the temperature at a rate of 18 °C per hour to 500 °C, held at for 16 hours, and then cooled to 50 °C. Details of subsequent trial digestions performed are as follows:

1. The first trial digestion was done using a sample of orange pekoe tea which was dry ashed according to the temperature program stated above. At the end of the first round of ashing, test tubes containing partially ashed sample were transferred into a metal block, 1.0 mL of 8 M HNO_3 added to each, and these were wet ashed at approximately 90 °C for one hour on a hot plate. Hydrogen peroxide (30 %) was added at 2 drops at 10 minutes intervals. A total of ten drops were added to each test tube and then evaporated to dryness. The capped test tubes with the sample were returned to a muffle furnace and dry ashed by ramping the temperature gradually from 50 °C to 500 °C over an eight hour period and held at 500 °C for 4 hours. The sample remained partially ashed after approximately 60 hours of dry and wet ashing (the trial was considered a failure) and so further ashing was aborted. The procedure was repeated for the coffee sample and black spruce sample (collected from the forest near the MUN Health Center).

2. The second trial ashing used 1.0 g of orange pekoe tea in each of 8 test tubes, four of which were capped and four uncapped. Another set of four crucibles each containing 1.0 g sample were used. The samples were transferred into a muffle furnace at 50 °C and ashed according to the initial temperature program described above. The procedure was repeated for coffee and the in-house black spruce sample.

3. The third trial digestion followed the second digestion procedure. A 1 g sample of tea was weighed into each of four labelled test tubes none of which was capped and was ashed according to the initial temperature program. The completely ashed samples in the four tubes were used for further analysis. A set of four different combinations of acids was used to leach the elements. The acid combinations included: 16 M HNO_3, 8 M HNO_3, 8 M HNO_3/6 M HCl, and 8 M HNO_3/29M HF. Tube A was treated with 2.0 ml of 16 M HNO_3, tube B received 2.0 mL 8M HNO_3, tube C received 1.0 mL each of 8 M HNO_3 and 6 M HCl, and tube D received 1.0 ml of 8 M HNO_3. The dissolved ash from each acid combination was transferred to corresponding labelled Teflon containers, after which 1.0 mL of 29 M HF was added to the container labelled D only. Two drops of H_2O_2 were added to each container and the sample-acid mixture evaporated to dryness on a hot plate. The samples were each reconstituted in 1.0 mL of 8 M HNO_3, two drops of H_2O_2 added to each container and these were warmed on a hot plate for two minutes. A sample blank was included as part of the batch. The 1.0 mL solutions were transferred to labelled 120 mL acid cleaned snap seal plastic containers and made up to 60 g with nanopure water for ICP-MS analysis (HP 4500 ICP-MS). This same procedure was repeated for samples of coffee, black spruce (from MUN Botanical Garden), and a NIST Pine needle 1575 certified standard reference material.

4. For the fourth trial digestion, a 1.0 g sample of coffee was weighed into each of four test tubes. These were placed in a metal test tube rack and then transferred into

a muffle furnace at an initial temperature of 50 °C. The samples were ashed by ramping the furnace temperature at a rate of 18 °C per hours up to 500 °C, held at this temperature for 16 hours and then cooled to 50 °C. The completely ashed samples were leached by treating with 8M HNO_3/ 29M HF acid combination as described in trial digestion 3. The procedure was repeated for pekoe tea, in-house black spruce sample, and the certified standard reference materials (NIST 1547 peach leaves, NIST 1575 pine needles, and black spruce CLV-1 and CLV-2).

Optimisation of the ashing procedure

5. For the fifth trial digestion, 1.0 g of in-house black spruce sample was weighed into each of four test tubes and 1.0 g of the NIST 1575 pine needle was weighed into another set of four test tubes. The samples dry were ashed by ramping the temperature at a rate of 18 °C per hour up to 450 °C, held for 8 hours and then cooled to 50 °C. To the ash in each test tube, 1.0 mL of 8M HNO_3 was added and the content transferred to corresponding labelled Teflon container. Each container received 1.0 mL of 29 M HF and 2 drops of 30% H_2O_2. The resulting solutions were evaporated to dryness on a hot plate, the residue reconstituted in 1.0 mL of 8M HNO_3, and then 2 drops of H_2O_2 were added and heated on a hot plate for 2 minutes. The solutions were made up to 60 g in acid clean containers and then analyzed by ICP-MS (HP 4500 ICP-MS). This procedure was repeated for pekoe tea, coffee, and the certified reference materials.

6. For the sixth trial digestion, each of the samples (both in-house and certified reference materials) was ashed by ramping the temperature at a rate of 50 °C per hour and then held at 450 °C for 8 hours. The samples were only charred (partially ashed) and so the procedure was aborted.

7. The seventh trial digestion was done by ramping 1.0 g samples (both in-house and certified reference material) at a rate of 10 °C per hour until 450 °C and then ashed at 450 °C for 8 hours. The samples were cooled to 50 °C and the resultant ash leached by treating with the 8 M HNO_3/29 M HF reagent as described in trial digestion five and then analyzed on HP 4500 series ICP-MS.

8. Successive trial digestions 8 and 9 were similar to the fifth trial digestion where samples were ramped at 18 °C per hour and then ashed at 450 °C but at varying durations of the holding time (4 and 16 hours). The samples ashed for 4 hours were partially ashed and so were each treated with 1.0 mL 8M HNO_3 and transferred into Teflon containers, 1.0 mL of 29 M HF was added to each container, and then diluted with 10 mL of nanopure water. The resulting solutions were filtered by gravity through a 125 mm diameter Whatman #1 filter paper. The filtrates were collected into labelled 120 mL acid cleaned snap seal plastic containers and made up to 60 g with nanopure water for analysis using ICP-MS (HP 4500 ICP-MS). The samples ashed for 16 hours were completely ashed and therefore were treated as in trial digestion five.

3.8 Acid digestion procedure

This acid digestion procedure is used in digesting biological samples by the ICP-MS group at MUN. About 0.2 grams of the plant material was weighed into 15 ml acid clean Teflon containers and 3 mL of 8 M HNO_3 added to each, covered and then warmed on a hot plate at approximately 90 °C for three days. The lids were removed and the solutions were evaporated to dryness. The previous step was repeated and again evaporated to dryness. One mL of 8 M HNO_3 and 1 mL of 30% H_2O_2 were added to each container, covered, warmed at approximately 90 °C for 24 hours and then evaporated to dryness. This step was repeated twice and the residue reconstituted in 2.0 mL of 8 M HNO_3, warmed on a hot plate for 2 minutes and then made up to 60 g with nanopure water in a snap seal container for ICP-MS analysis. The procedure was applied to standard reference materials: NIST 1575 pine needles and 1547 peach leaves, and black spruce vegetative radionuclide CLV-1 and CLV-2.

3.9 Dry ashing procedure of choice

Trial digestion 5 was chosen as it provided the best results for ashing plant materials. Its accuracy and reliability was assessed by analysing standard reference materials: NIST 1575 pine needles and 1547 peach leaves, and black spruce vegetative radionuclide CLV-1 and CLV-2. The mean element recoveries were compared to those obtained by acid digestion.

Chapter 4: Results

4.1 Choice of isotopes

Concentrations of analytes with two or more isotopes were determined by examining the spectra for masses with high analyte isotope abundance and low interferences and backgrounds. Both ^{42}Ca and ^{43}Ca were measured, but the determination of ^{43}Ca was used in the data analysis, because despite the higher isotopic abundance of ^{42}Ca, the occurrence of $^{40}Ar^{2}H$ and $^{40}Ar^{1}H_2$ at 42 amu causes a high background. The other isotopes of calcium were not used for the determination due to the presence of Ar at 40 amu, $^{12}C^{16}O^{16}O$ at 44 amu and the isobaric interferences with Ti at 46 and 48 amu. Three isotopes of Fe were measured, at 54, 56, and 57 amu. Determination of the isotope ^{57}Fe was used as a high background occurred at 56 amu from $^{40}Ar^{16}O$, a high background occurred at 54 amu due to $^{40}Ar^{14}N$, and Cr causes an isobaric interference at 54 amu. The two isotopes of ^{77}Se and ^{82}Se were measured, with high backgrounds occurring due to $(^{40}Ar^{1}H)_2$ and $^{40}Ar_2{}^{1}H_2$, and interferences occurring from $^{40}Ar^{37}Cl$ and $^{81}Br^{1}H$. The determination of ^{82}Se was used for the data analysis although the detection limits of the two isotopes are similar. The determined concentration of these two Se isotopes showed good agreement, which is evidence for good background and interference correction. The isotopes of chromium measured are ^{52}Cr and ^{53}Cr but determination of isotope ^{53}Cr was used because a high background occurred from $^{40}Ar^{12}C$ at 52 amu although the ^{52}Cr isotope has a lower detection limit in the absence of carbon. The ^{54}Cr isotope was not measured due to a high background encountered from $^{40}Ar^{14}N$ and isobaric interference from ^{54}Fe.

4.2 Digestion procedures

The criteria for determining the suitability of a digestion/ashing procedure was based on the completeness of decomposition as determined by the absence of the residual organic matrix (complete combustion/elimination of the sooty substance deposited on the test tubes) and by the extent of recovery of the trace elements (as determined by analysis of the reference materials). Biological samples generally consist of a complex mixture of carbohydrates, proteins, and lipids and so require a digestion method that adequately oxidizes all these components, especially proteins and lipids. Hence any procedure for analysing trace metal content in such matrices must adequately decompose both organic and inorganic matrices (e.g. silicates) to release the metals from the sample matrix into solution.

The partial decomposition observed in the first trial digestion (all test tubes capped) of this study may be attributed to inadequate supply of oxygen to complete the combustion process. The caps on the test tubes obstructed the exchange of gases in an environment where oxygen was already depleted at high ashing temperatures. In addition to an inadequate supply of oxygen, it is suspected that the evaporation step of the wet ashing stage must have induced the formation chemical entities such as oxides which cause the residue to be more difficult to digest. The oxides form crusts on the surface of the sample. This limits the surface area of the particles and hinders the combustion process (Fabry and Nangniot, 1972; Van Paemel *et al,* 2005; Mader *et al,* 1997). Since completeness of a digestion is an essential requirement for obtaining reliable results in ICP-MS analysis, further ashing of samples in Trial digestion 1 was aborted as the ashing

period had exceeded 60 hours and continued ashing would likely lead to further losses of volatile elements.

Losses of trace elements during digestion affect the accuracy of the final results. Gorsuch, (1970) identified possible loss mechanisms associated with all sample decomposition procedures; among others were gaseous evolution (volatilization), absorption or adsorption onto surfaces, precipitation, and the persistence of undissolved material. Loss of sample analytes may also result from their incorporation into a residue which is insoluble in the leaching reagents and therefore lead to the formation of refractory oxides by combinations with other sample constituents hence the need to ensure a complete decomposition.

The second trial digestion procedure was intended to identify the best digestion vessel suited for dry ashing owing to the diversity of the sample matrices involved in the study. The ideal vessel is one that supplies sufficient oxygen to drive the decomposition process to completion while minimizing sample contamination, loss of sample components through volatilization, and reactions with the walls of the vessel. Generally, all four sample types were completely ashed in the uncapped test tubes but the samples in the capped test tubes and crucibles were only partially ashed. The capped test tubes were characterized by the deposition of soot on the walls and a charcoal residue. Similarly, the plant materials in the crucibles were only partially decomposed except for the SRM 1575 (pine needles), which was completely ashed (Table 4.1).

Similarly, Mader *et al*, (1997) encounted considerable difficulty in ashing alfafa leaves at 500 °C in beakers covered with watch glasses. The partial decomposition of the

samples in the crucibles was contrary to expectation and may be attributed to the general depletion of oxygen in the furnace at high temperature. The complete decomposition observed in the uncapped (open) test tubes could be attributed to the utilization of oxygen trapped within the empty space in the test tubes. In addition, the long narrow test tubes reduced the ease of escape of volatile sample components and also limited introduction of external contaminants as compared to the crucibles with wide mouth. The uncapped test tubes were therefore chosen as a suitable vessel for dry ashing.

If performed well, classical dry ashing leads to complete destruction of the organic matrix producing ashes of white, orange, or grey colour. The analytes of interest are completely solubilised and are in a form compatible with the analytical method of choice. The ease and extent of ashing also depends of the composition of sample (carbohydrates, proteins, or fats) as well as its particle size. Powders with finer particles such as SRM 1575 were easier to ash in the crucibles as result of their larger surface to volume ratio. The in-house reference materials were not ground to very fine powders comparable to the reference materials for fear of introducing contaminants during grinding.

Table 4. 1: Effect of nature of vessel on the efficiency of oxidation of plant materials

SAMPLE	TEST TUBE (CAPPED)	TEST TUBE (UNCAPPED)	CRUCIBLE
TEA	–	+	–
COFFEE	–	+	–
BLACK SPRUCE	–	+	–
1575 PINE NEEDLE	–	+	+

Key: + = completely ashed, – = incompletely ashed

Since ICP-MS like many other analytical techniques, requires a sample to be transformed into a solution prior to analysis, a choice of an acid or acid mixture that would efficiently leach the elements into solution is necessary for accurate and reliable results. For instance, factors, such as the fraction of organic matter in a sample, insoluble components of the plant material (*e.g.* silicates) in the leaching acid, or solubilisation of ash may affect the degree of trace element recovery. Also some acids react with trace elements to form volatile compounds or complexes, and therefore result in artificially low concentrations of the elements of interest (Azcue and Mudroch, 1994).

Trial digestion three was effective in decomposing all plant materials tested and produced a whitish to grey ash. The resultant ash was leached with four combinations of acids in order to evaluate their effectiveness in leaching the elements prior to instrumental analysis. The acid combinations included 16 M HNO_3, 8 M HNO_3, 8 M HNO_3/6 M HCl, and 8 M HNO_3/29 M HF. Generally, all four acid combinations tested were satisfactory in leaching the elements into solution, but the dissolution of the ash in 8 M HNO_3/29M HF (2:1) with addition of 2 drops H_2O_2 was observed to be most efficient

in leaching the elements in both in-house reference materials and SRM 1575 (Figs. 4.1-4.5).

Hydrofluoric acid is commonly used to decompose silicate minerals to form volatile SiF_4 and to free the associated elements into solution. Its application in this study is to leach elements bound to any silicious components of the plant matrix. Plant media contain varied concentrations of silica that can attain several percent (Hoenig 2000). As observed by Mader *et al,* (1997), analytes can be quite strongly retained in the siliceous residue with the consequent risk of them not being solubilised during the leaching procedure. The use of HF in combination with nitric acid therefore helps the complete dissolution of both organic matter and inorganic silicates. This is in agreement with the observation by Maurizio *et al*, (2002) that use of HF in the reagent mixture is particularly important for leaching lithogenic elements such as Al, Cr, Fe, U, and Ti which partially associate with silicates.

The relatively low recovery of Al by all other three reagent mixtures (16 M HNO_3, 8 M HNO_3, and 8 M HNO_3/6 M HCl) is an indication of their inefficiency in decomposing silicates as Al is partly associated with silicates. Both 16 M and 8 M HNO_3 were quite efficient in the treatment of the in-house reference samples (coffee, tea, and black spruce) with high recoveries for elements such as Pb, Sn, Br, and Zn (Tables 4.1 to 4.3) but the recoveries were unsatisfactory for Al, Ti, V, and U.

Figure 4.1 shows the element recoveries for the in-house coffee material. The 8N HNO_3/HF reagent combination was more efficient in leaching the majority of the elements. It recovered 4.2 ppb of U while the 8 N HNO_3/HCl combination recovered 3.1

ppb (Table 4.2) when the sample was ashed at 500 °C for 16 hours. Recovery of U in the coffee in-house material with nitric acid alone (both 16 M and 8 M) was generally not satisfactory but its recovery of non-lithogenic elements is impressive.

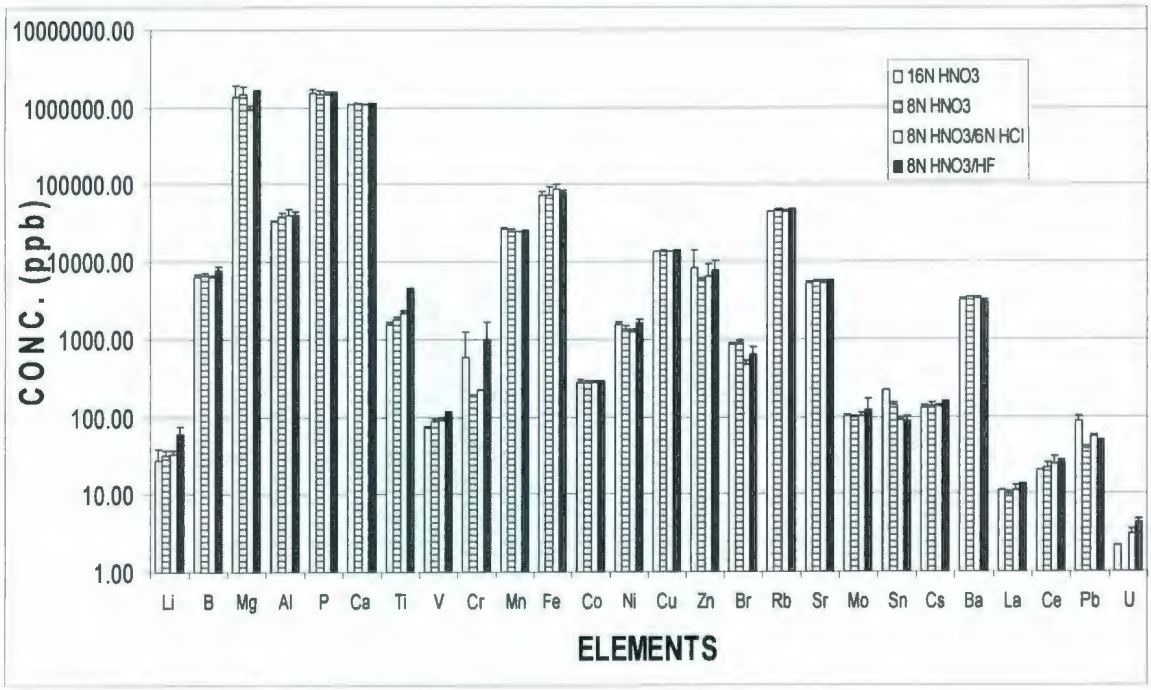

Fig. 4. 1: Concentration of trace elements (ppb) recovered by the leaching agents in the in-house coffee material.

ICP-MS results for elements such as Be, As, Cl, Si, S, Br, Se, Hg, Mo, Ag, Cd, Sn, Sb, I, Tl, and Bi which were below detection limits in the in-house tea and coffee samples (Figs. 4.1 and 4.2). It could however not be ascertained whether the low concentrations were natural or were due to losses by volatilization during dry ashing and dissolution.

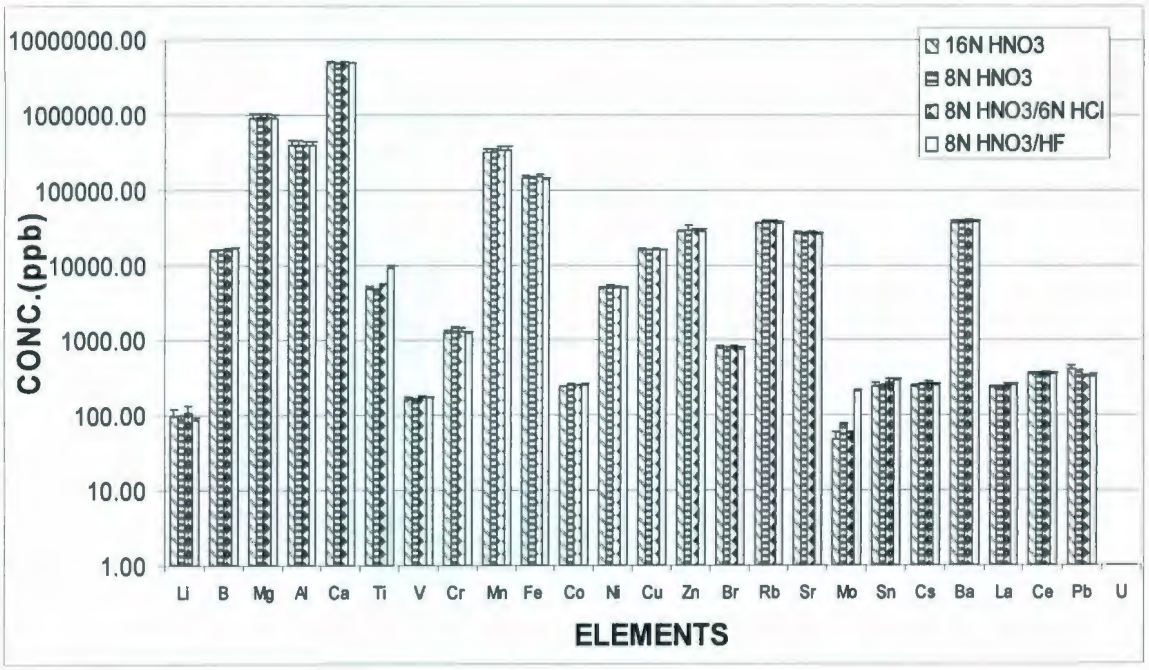

Fig. 4. 2: Concentration of trace elements (ppb) recovered by the leaching agents in the in-house tea sample

Uranium recovery by all four reagent combinations in the tea in-house reference sample was below the detection limit. This may be due to low levels of uranium in the sample or due to losses. There was good agreement between the element recoveries by all four reagent mixtures except for Mo where recovery with 8 M HNO_3/HF far exceeded the recoveries by the other three reagent mixtures. The recovery by 8 M HNO_3/HF is 30 % higher. Again 3.6 ppb and 2.6 ppb of uranium were recovered by 8 M HNO_3/HF and 8M HNO_3/HCl respectively in the in-house spruce reference material (Table 4.1) but recovery by 16 M and 8 M nitric was below the detection limit. Recoveries of all other elements by all four reagents are quite good with no significant differences except for uranium which was not detected. The recovery of Mn, Cr, and Mo however recorded

RSDs higher than 20% while concentrations for elements such as Be, Si, Ti, As, Br, Se, Hg, Tl, and Bi were below detection limit.

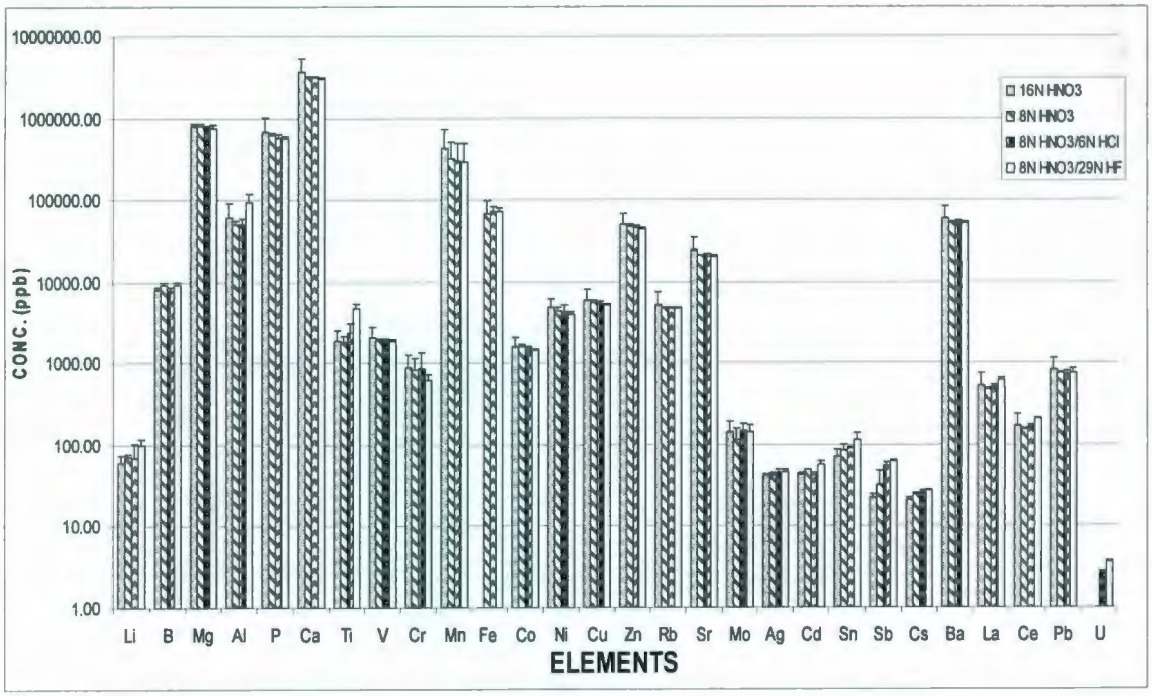

Fig. 4. 3: Concentration of trace elements (ppb) recovered by the leaching agents in the in-house spruce sample.

The efficiency of leaching by all four reagent mixtures was assessed by analysing a certified reference material, NIST 1575 pine needles. The analytical results for the NIST SRM 1575 were satisfactory for all four reagent mixtures with recoveries in the range of 40 % and higher. Recovery of silicate bound elements (lithophile elements), such as Al, Ni, Ti, and U vary for all four reagents. Their recovery was most satisfactory with the 8N HNO_3/HF reagent mixture, with recoveries ranging from 75 – 81 %, while V and Co exceeded 100 % recovery (Table 4.4). The source of contamination of these

elements could however not be ascertained. The recovery of Al, Ni, and U with nitric acid and HNO_3/HCl reagents was 72%, 75%, and 68% respectively. The use of HF has been reported to give complete digestion (Gorsuch 1970; Hoenig 2000; Mader *et al,* 1997; and Xinbang Feng *et al*, 1999).

The recovery of uranium ranges between 58 to 82 % with 8 N HNO_3/HF recovering 82 % while 16 M nitric acid recovered the lowest at 58 %. Similar patterns of results were also observed in the in-house reference material. The recoveries of As, Cd, Sb, and Tl were above 60 % in the SRM 1575 pine needle reference material. This strongly suggests that the below detection limit recorded for these elements in tea and coffee in-house reference material cannot entirely be attributed to losses through any of the routes stated above. The concentration of these elements may naturally be low in the tea and coffee samples hence the low concentrations recorded.

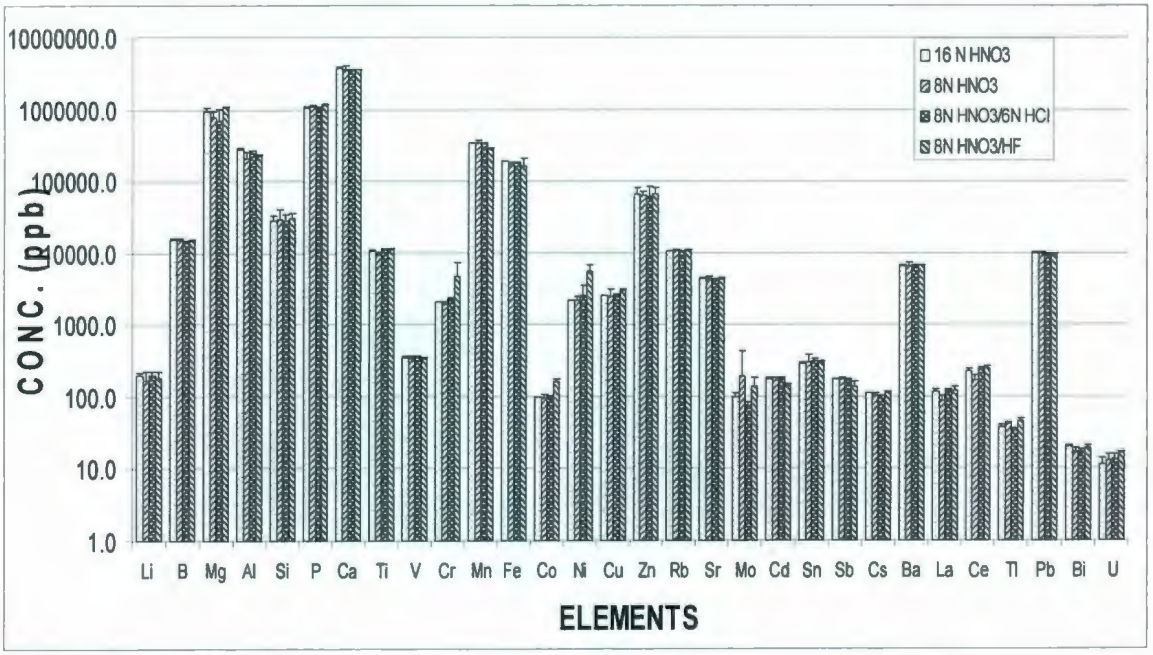

Fig. 4. 4: Concentration of trace elements (ppb) recovered by the leaching agents in the SRM 1575 pine needle.

Mercury and Br were below the detection limit in all four samples (i.e. in-house reference material and SRM 1575 pine needle) when leached with each of the four reagent mixtures after an initial dry ashing at 500 °C for 16 hours.

Mader *et al,* (1997) reported that losses of volatile elements such as Hg, As, and Se could be minimized by the use of ashing aids in the dry ashing procedure. They explained that ashing aids – generally MgO and/ or $Mg(NO_3)_2$ can sometimes lead to the formation of less volatile Se and As compounds during ashing. However, Hoenig (2000) observed that ashing aid efficiency is strongly dependent on the initial analyte form and may not apply to all sample types. For example, Vassileva *et al,* in Hoenig (2000) noticed after dry ashing of terrestrial plants that, As and Se recoveries are very consistent but that

this was not the case for plants of aquatic origin. Therefore utilization of ashing aids is particularly questionable because a few successful examples cannot be generalized for routine use. Their use in a procedure necessitates a serious and time-consuming validation for each type of samples analyzed. In addition, the utilization of ashing aids may significantly increase the total content of dissolved salts in solutions, limiting the application of this approach for ICP-MS analysis and also prevent the often required determination of the sample Mg concentration.

Consensus exists in the literature that moderate heating prior to ashing (charring phase) is of critical importance to avoid losses of analyte from the sample through local overheating and subsequent loss of the analyte due to its removal from the sample in the form of solid particles of smoke (Mader *et al*, 1996; Mader *et al,* 1997). This necessitated the slow rate of temperature ramp (18 °C per hour) adopted for the initial ashing programme. Mader *et al*, (1997) also observed that the removal of organic components from plant materials proceeds through an initial charring temperature below 200 °C. The choice of a base line initial temperature of 50 °C allows adequate time for the sample to dry and expel bound water.

In order to improve elemental recoveries and mitigate losses by volatilization, the ashing temperature was varied from 400 °C to 500 °C and its impact on elemental recovery was assessed. The samples were ashed at 400 °C and 450 °C for 8 hours and at 500 °C for 16 hours after a gradual temperature ramp of 17 °C per hours. All samples (both in-house and certified reference material) ashed at 400 °C for 8 hours were charred into charcoal particles and the walls of test tubes were covered with soot: an indication of

incomplete decomposition. However, complete decomposition of the plant materials was achieved when ashing temperature was increased to 450 °C and 500 °C and held for 8 and 16 hours respectively. All plant materials examined were completely ashed forming a white to grey ash with clear sample tubes. This contradicts the suggestion by Mader *et al,* (1997) that a decrease in ashing temperature from 500 °C to 450 °C leads to incomplete decomposition of even readily decomposable plant materials such as potato tubers. The recovery of the elements in all samples ashed at 450 °C was significantly higher than the quantities recovered in samples ashed at 500 °C. For instance, U recovery in in-house spruce and coffee reference material increased from 3.7 ppb to 13.4 ppb and 4.0 to 7.0 ppb respectively (Tables 4.10 and 4.11). A reduction of the ashing temperature from 500 °C to 450 °C also improved the recovery of Be and Tl in the in-house spruce sample (i.e. 28.2 ppb and 15.5 ppb respectively) (Table 4.10).

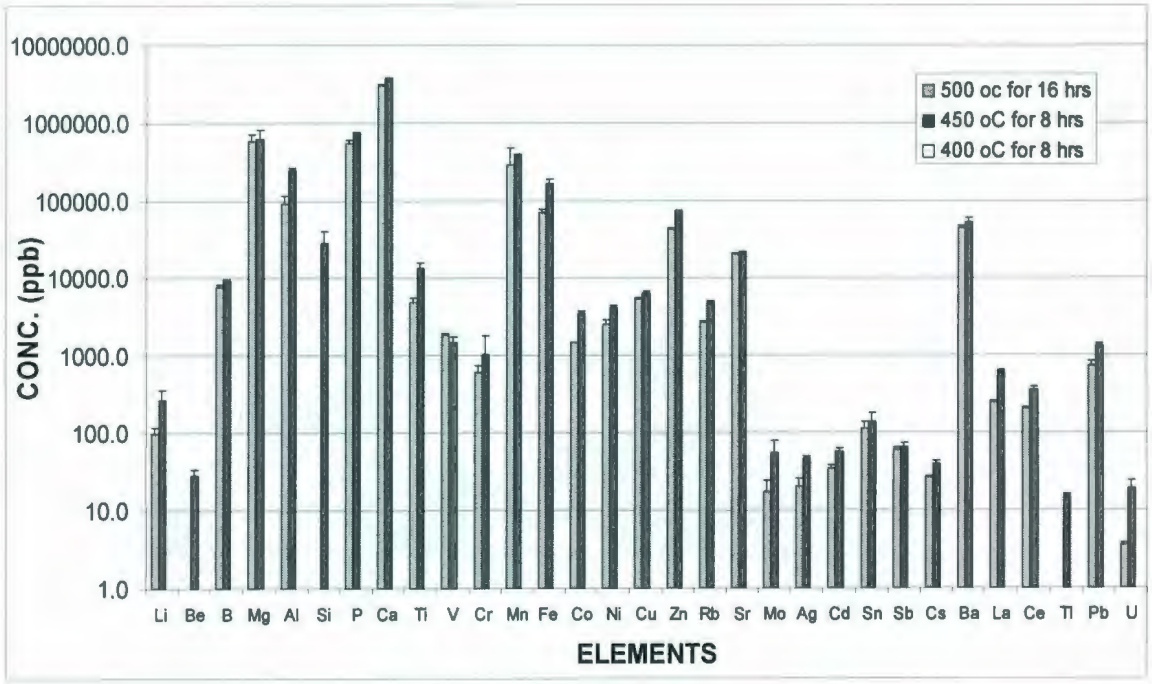

Fig. 4. 5: Concentration of trace elements (ppb) recovered at varying ashing temperatures in the in-house spruce sample.

The in-house tea reference sample recorded 4.9 ppb of uranium when ashed at 450 °C for 8 hours but it recorded concentration below detection limit when ashed at 500 °C for 16 hours. Other elements, Be, Br, and Tl were recovered in relatively high quantities when ashed at 450 °C as compared to the below detection limit recorded when ashed at 500 °C. Appreciable increases were observed for Cr, Mo, and Co with element concentrations increasing by 15 %, 50 %, and 20 % respectively in comparison with concentrations recovered when the sample was ashed at 500 °C (Fig. 4.5, Table 4.12).

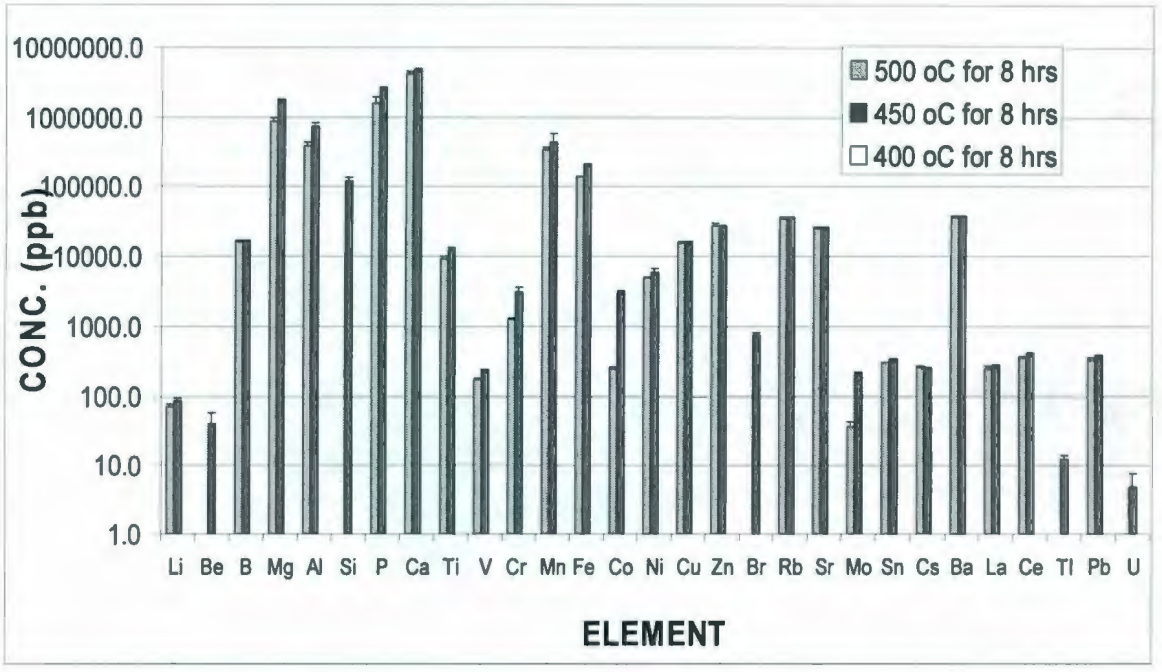

Fig. 4. 6: Concentration of trace elements (ppb) recovered at varying ashing temperatures in the in-house tea sample.

A similar phenomenon was observed with the in-house coffee reference material where uranium recovery increased by 66 % from 4.0 ppb to 6.0 ppb (Fig. 4.6, Table 4.11) when the ashing temperature was reduced from 500 °C to 450 °C. All other elements increased marginally but the differences in the recoveries are not as significant as in the tea and black spruce in-house reference materials. Coffee has high fat content and as stated in the literature, materials rich in fat are much more difficult to decompose (Mader *et al,* 1997; Radojevic and Bashkin 1999). Besides Li and U whose concentrations increased appreciably when ashed at 450 °C (Fig. 4.7), the difference in the mean elemental recovery for all other elements is not statistically significant at 95% confidence limit.

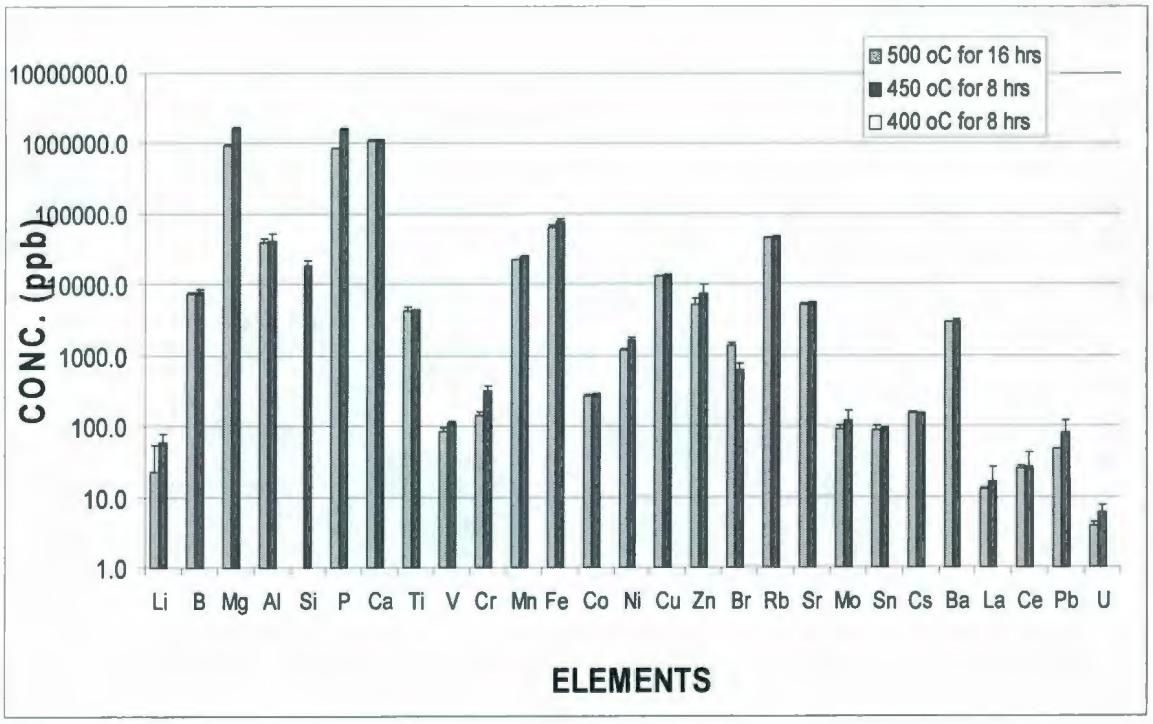

Fig. 4. 7: Concentration of trace elements (ppb) recovered at varying ashing temperatures in the in-house coffee sample.

Figure 4.8 shows results of varied periods (i.e. 4, 8, and 16 hours) for ashing the plant materials. All samples that were ashed for 4 hours at 450 °C after a gradual temperature ramp of 18 °C per hour resulted in a partially decomposed sample containing charcoal particles. Coloured solutions of varying degrees were obtained for ICP-MS analysis unlike the clear solutions obtained for completely decomposed samples. Ashing for 4 hours recovered 161 ppb of Arsenic. But ashing for longer periods recorded As concentrations below detection limit (Table 4.16). Recovery for all other elements was low with high RSD's in comparison with samples ashed for 8 hours. Uranium recovery dropped by 23% in the in-house spruce reference sample (Fig. 4., Table 4.13) while other

lithogenic elements, such as Al, Ti, and Fe, also recorded significant drops in their recovery. This is because the elements bound to the undecomposed matrix were not recovered. The recovery of Mo was highest in spruce and coffee in-house materials (92 ppb and 114 ppb respectively) (Tables 4.13 and 4.14) after 4 hours of ashing. Concentrations of metals recovered after 4 hours of ashing were imprecise, inaccurate, and were characterised by high RSDs.

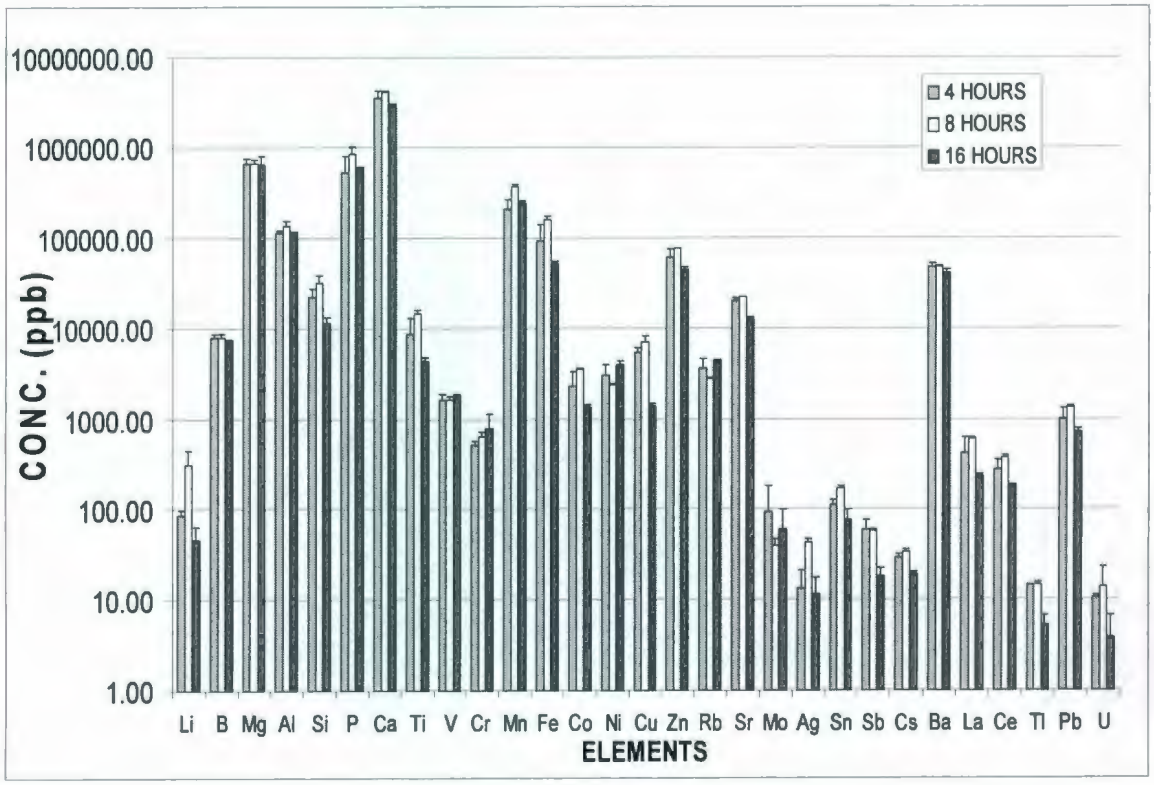

Fig. 4. 8: Concentration of trace elements (ppb) recovered at varying durations of ashing (in-spruce sample)

A similar pattern of element recovery was observed for the in-house coffee sample but with small differences in mean element recoveries between samples ashed for

4, 8, and 16 hours. Uranium recovery was much higher in samples ashed for 4 hours than those ashed for 16 hours (Fig 4.9). The highest elemental recovery was obtained for samples ashed for 8 hours (Table 4.15). The low element recovery observed after 4 hours of ashing was due to the incomplete decomposition of the plant material and the adherence of some of the elements onto the undecomposed matrices hence their unavailability for measurement.

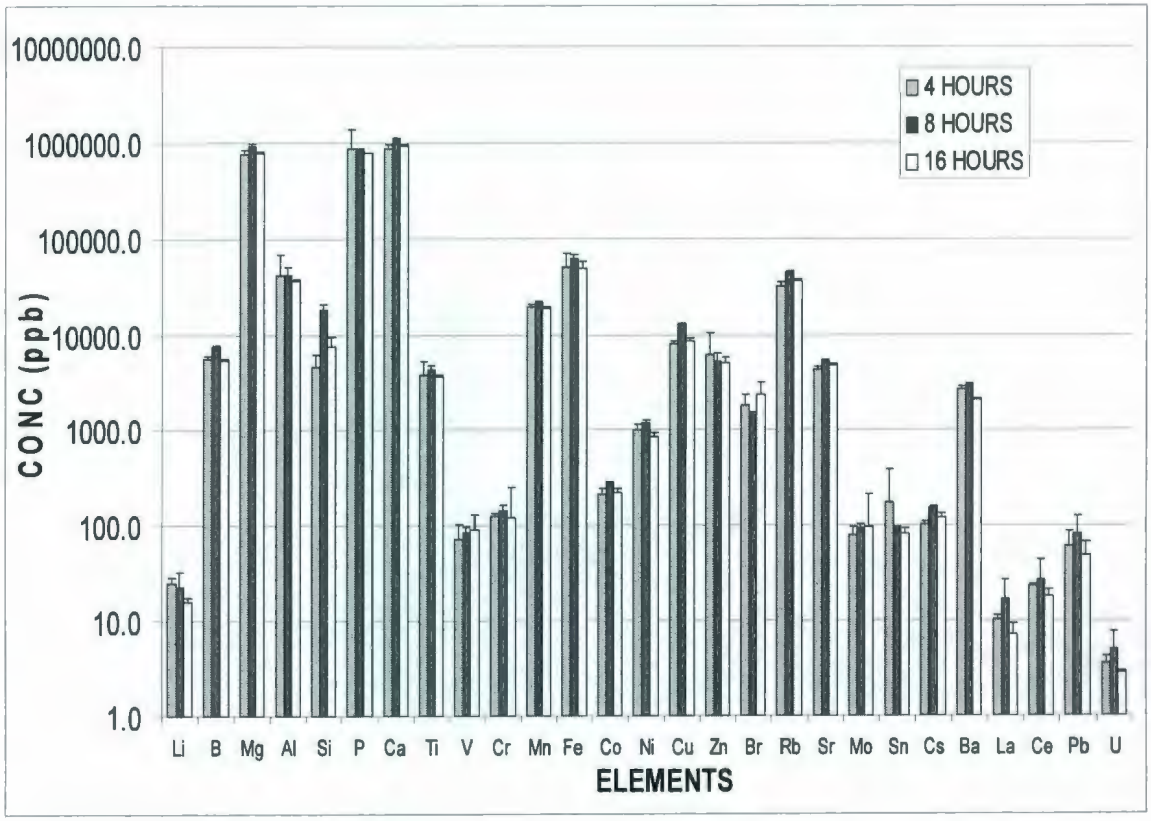

Fig. 4. 9: Concentration of trace elements (ppb) recovered in the in-house coffee sample after varied periods of ashing.

The differences in the mean recoveries of some of the elements were however marginal and statistically insignificant at 95% confidence limit (e.g. B, Mg, Al, Ca, and Mn) between samples ashed for 4, 8, and 16 hours. The RSD's were also relatively higher in samples ashed for 16 hours (Table 4.13 - 4.18).

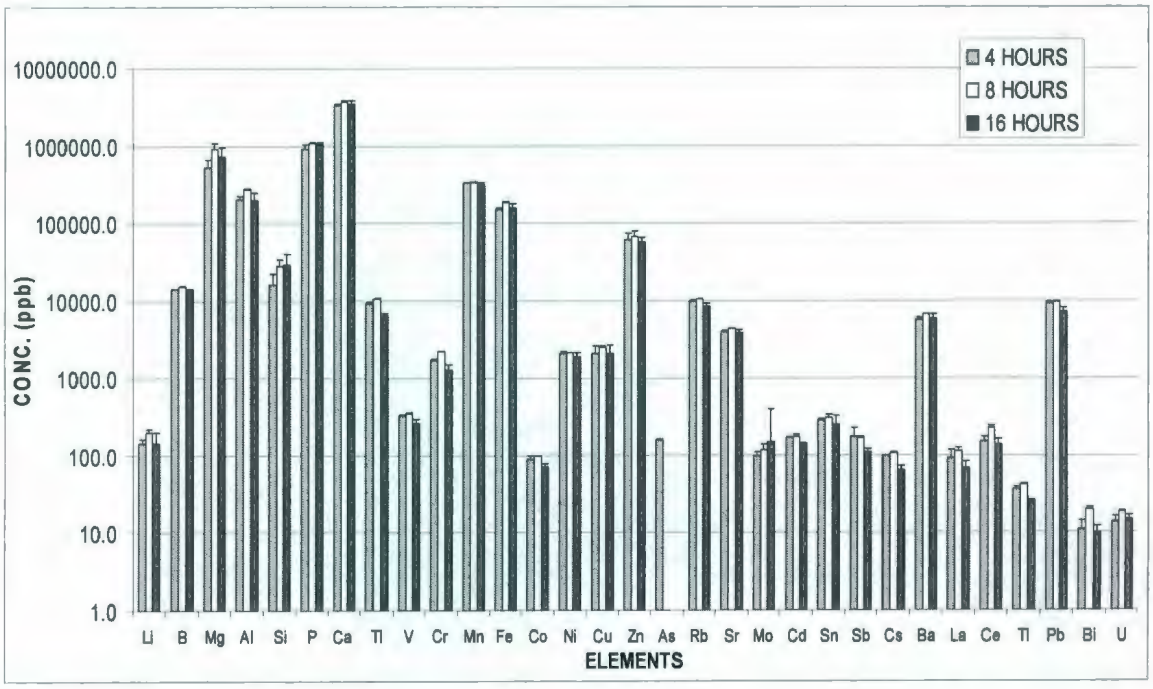

Fig. 4. 10: Concentration of trace elements recovered in the SRM 1575 pine needle after varied periods of ashing.

For the in-house tea material, uranium was recovered in samples ashed for 4 and 8 hours but was totally lost when ashed for 16 hours. The mean concentrations recovered for both U and Tl in samples ashed for 4 hours were very close to the detection limits (i.e. 0.63 ppb and 1.91 ppb respectively) (Fig 4.10). A large variation was observed in the

mean element recovery of Co with increasing duration of ashing. The recovery dropped from 8.7 ppm when ashed for 4 hours to 3.1 ppm after 8 hours of ashing at 450 °C and to 0.2 ppm when ashed at 500 °C for 16 hours (Table 4.15). Molybdenum was completely lost when ashed at 500 °C for 16 hours.

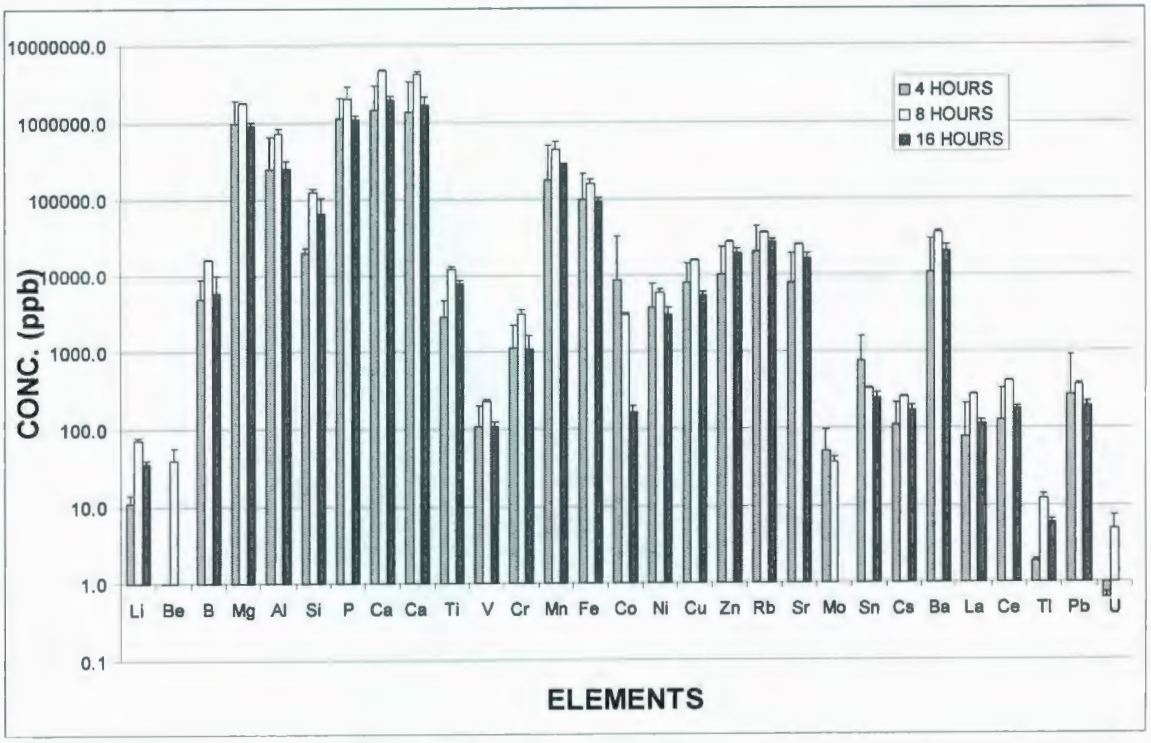

Fig. 4. 11: Concentration of trace elements (ppb) in the in-house tea sample after varied periods of ashing.

The result, thus far suggest that not only does ashing at elevated temperatures lead to losses of analytes but prolonged times of ashing also contribute to element loss. This therefore corroborates the observation by Mader *et al,* (1997) that ashing at lower temperature assures a decreased loss of sample components during the dry ashing procedure.

The effect of the rate of temperature rise on the efficiency of decomposition and retention of volatile sample components was studied. The samples were ashed by using rates of temperature rise of 50 °C/hr, 18 °C/hr, and 10 °C/hr. In general, element recovery increased with a slower rate of temperature rise for all examined samples. All samples ramped at 50 °C/hr were only partially decomposed into charcoal and the test tubes were covered with soot, an indication of incomplete combustion. Ramping at rates of 18 °C/hr and 10 °C/hr resulted in complete decomposition of the plant material indicating that a slower temperature ramp increases the efficiency of the decomposition process. For example, there was a significant increase in the recovery of Be, Mg, Si, Ag, and Cs when the rate of temperature ramp was slowed from 18 °C/hr to 10 °C/hr in the CLV-1 reference material (Fig 4.12). The increase was marginal for all other elements, for example, U recovery increase from 73 ppm to 74 ppm (Tables 4.17). These increases in recovery are not statistically significant.

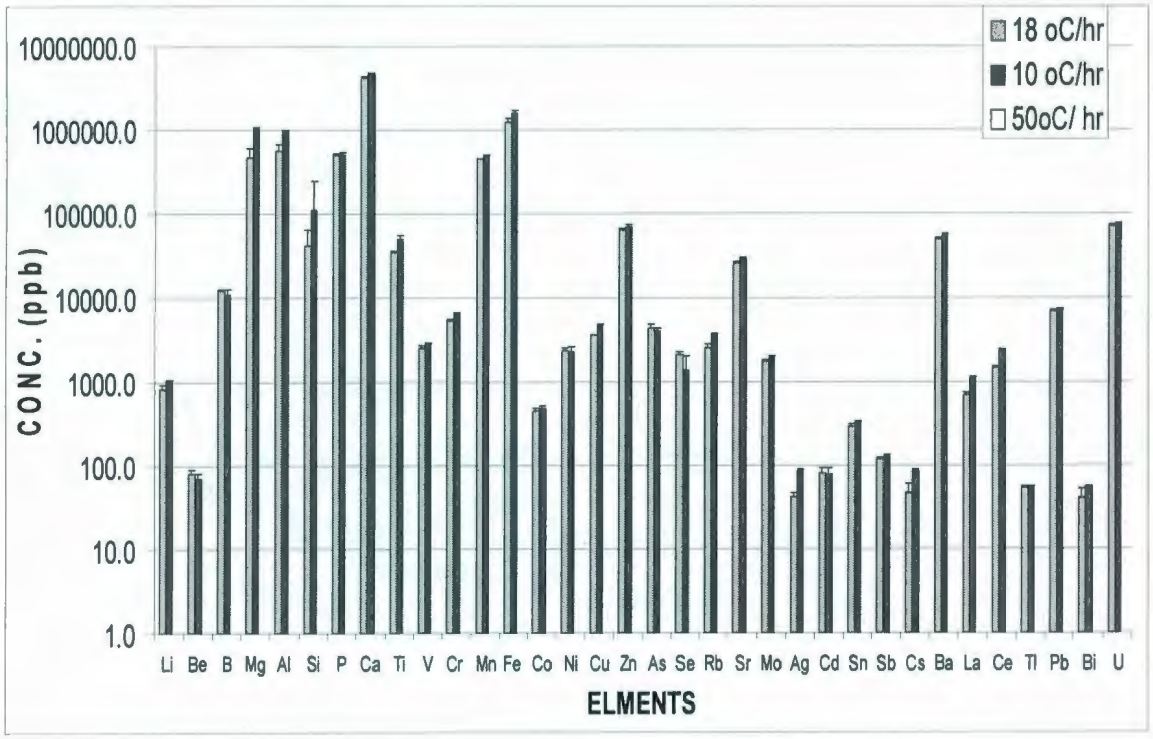

Fig. 4. 12: Concentration of trace elements recovered in CLV-1 at different rates of temperature rise.

The difference in element recovery in the case of CLV-2 reference material was however not as distinct as observed for the CLV-1. The only elements whose recovery increased marginally when the rate of temperature ramp was reduced from 18 ^{o}C/hr to 10 ^{o}C/ hr were Mg, Al, Ti, and Ag (Fig 4.13). The recovery of Ag increased by approximately 50 % i.e. from 14.3 ppb to 23.3 ppb while As and U increased from 276 ppb and 3.0 ppm to 311 ppb and 3.3 ppm respectively (Tables 4.18). Lithium also increased by 14%, from 263 ppb to 299 ppb. Besides Ag whose recovery increased by approximately 50 %, the increase observed for all other elements was less than 15 %.

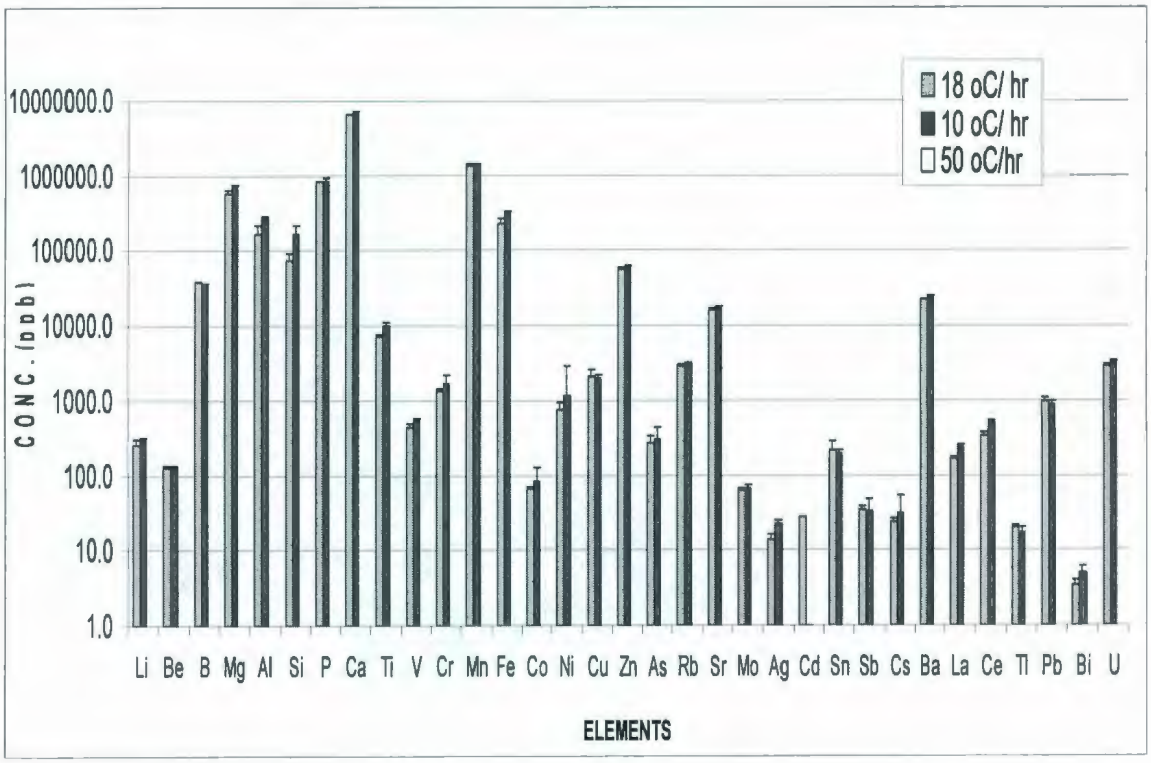

Fig. 4. 13: Concentration of trace elements (ppb) recovered in CLV-2 at different rates of temperature rise.

The element recovery in SRM 1547 peach leaves (Fig 4.14) is similar to the pattern observed in the CLV-1 and CLV-2 samples. There was a slight increase in element recovery when the rate of temperature rise was reduced from 18 °C/hr to 10 °C/hr. This difference in element recovery is, however, not statistically significant. The concentration of U recovered when 1547 peach leaves was ashed by varying the temperature ramp at rates of 18 °C/ hr and 10 °C/hr were 21 ppb and 24 ppb respectively, about 30% higher than the concentration given in the certificate of analysis (i.e. 15 ppb) (Table 4.19).

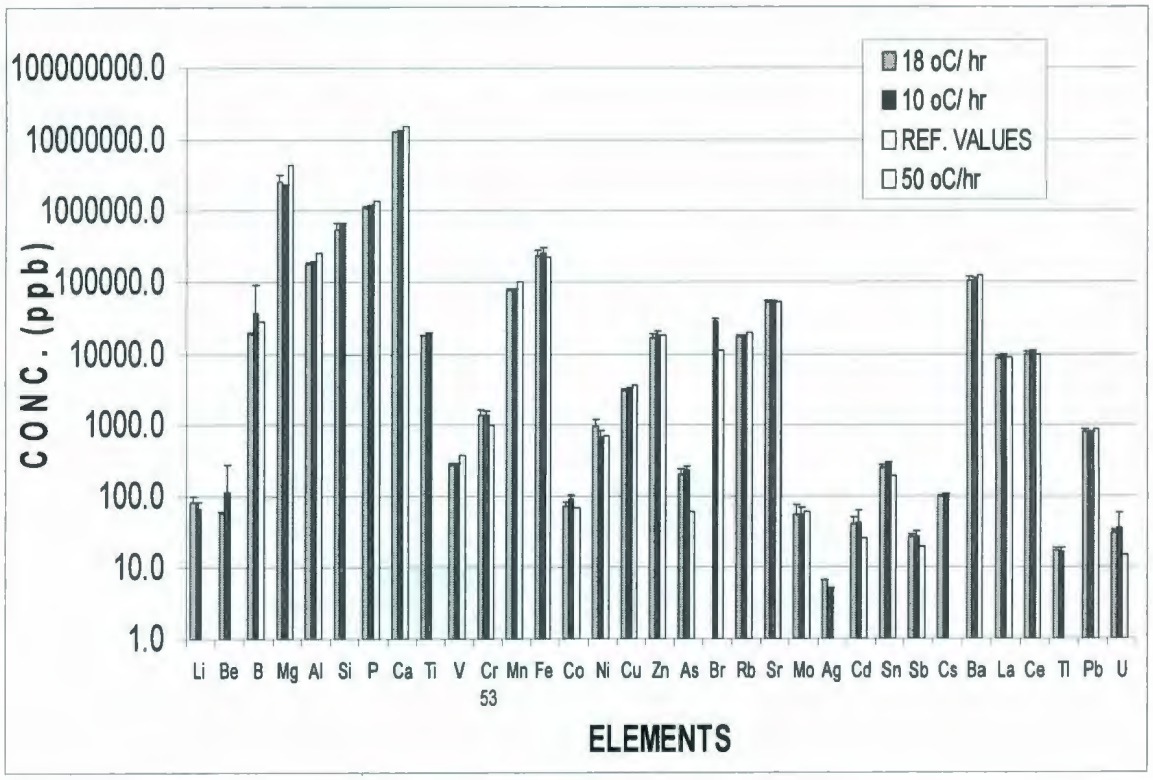

Fig. 4. 14: Concentration of trace elements recoverd from 1547 peach leaves at different rates of temperature rise.

There was no significant difference in the elemental concentrations recovered when black spruce in-house reference material was decomposed at rates of 18 °C/hr and 10 °C/hr except for Bi whose concentration increased from 6.74 ppb to 13.44 ppb. The U concentration also increased from 17.4 ppb to 19.5 ppb. This implies that increasing the temperature at a slower rate increases the efficient of decomposition while minimizing the loss of volatile sample components. This is in agreement with the observation by van Paemel *et al*, (2005) that a moderate charring stage is of critical importance to avoid losses of analytes from samples through local overheating and subsequent loss of solid

particles as smoke. The comparison of the element recovery by decreasing the temperature at a rate of rise from 18 °C/hr to 10 °C/hr for all elements that recorded concentrations above detection limit including U showed no statistically significant differences in the mean element concentrations. Therefore, increasing the temperature at a rate of 18 °C/ hr was preferred because it is a compromise with respect to time and cost.

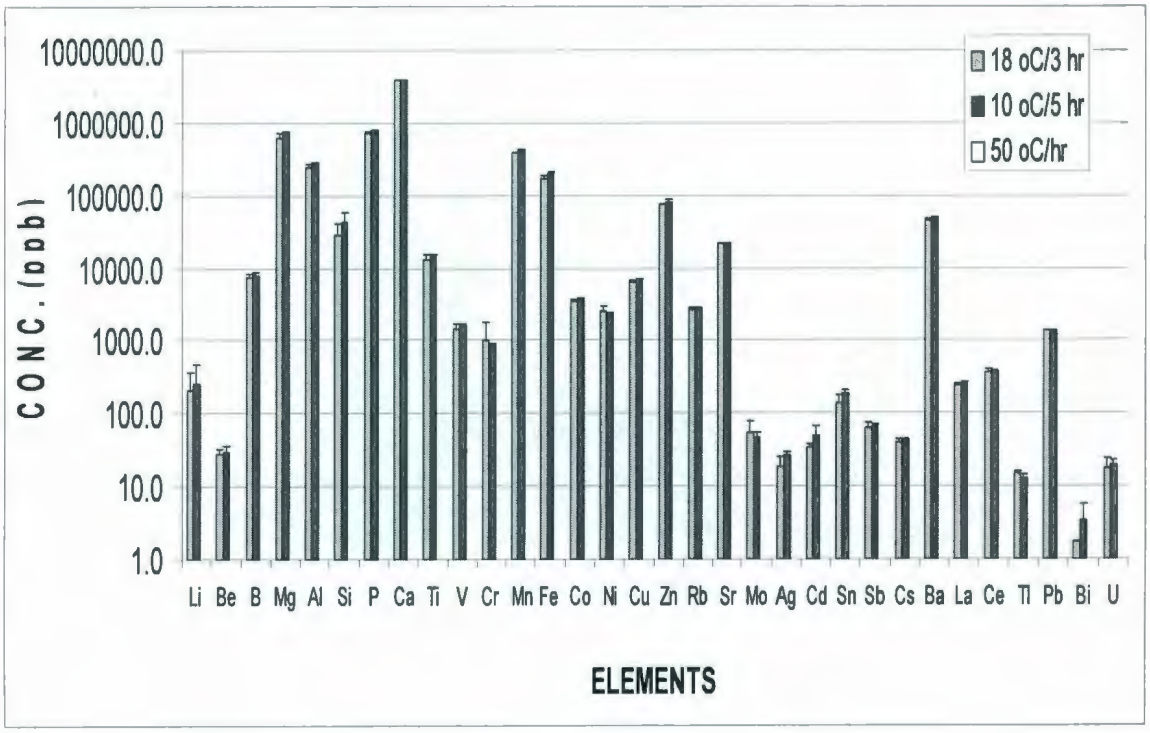

Fig. 4. 15: Concentration of trace elements (ppb) recovered from in-spruce coffee sample at varying rates of temperature rise.

The trial digestion experiments and the optimisation of parameters such as ashing temperature, rate of temperature rise (charring stage) prior to ashing, and varying duration of ashing led to a more optimised dry ashing procedure. For a successful dry ashing the final ashing temperature should be kept as low as possible to minimise the loss

of volatile sample components but it must be high enough to ensure complete combustion (oxidation) of all the organic matter. Thus a procedure where the sample is ashed at 450 °C for 8 hours after an initial temperature ramp of 18 °C/hr resulted in complete decomposition of plant materials and improved recovery.

Factors which were considered in the selection of this procedure included the completeness of digestion and reproducibility in analyte recovery from matrix, its ability to handle a representative sample, and its economic efficiency with regard to the time required for sample preparation, labour, reagent consumption, and equipment cost.

Increasing the temperature (i.e. the charring stage) at a rate of 18 °C/hr reduced the total ashing time to 32 hours compared to 48 hours when the temperature was increase a rate of 10 °C/hr. The comparison of the element recoveries for all samples ashed by increasing the temperature at rates of 18 °C/hr and 10 °C/hr showed no statistically significant differences. This demonstrates that increasing the temperature at a rate of 18 °C/hr is as good as 10 °C/hr. Another important consideration for the selection of a digestion method for the determination of trace metals in biological and environmental materials is the required digestion time. The comparison of the dry ashing procedure with the wet ashing procedure used by the ICP-MS Group at MUN showed significant differences.

To verify the accuracy of the proposed procedure, three certified reference materials: pine needle (1575) by NIST and black spruce radionuclide samples (CLV-1 and CLV-2) from Cluff Lake uranium mining area in northern Saskatchewan were treated by the proposed dry ashing procedure and analysed using ICP-MS with the operating

conditions stated in Table 3.2. The results of the analysis indicated that there is agreement between measured and certified values with relative deviations less than 10 %, except for elements such as Ni, Mo, and Co which consistently recorded RSDs more than 10% (Tables 4.2 to 4.5). A student t-test of the mean recoveries at 95 % confidence limit showed good agreements between the measured concentrations and the certified values for most analytes. Elemental recoveries in all SRM were in the range of 75 to 120 %.

Both dry ashing and wet digestion procedures are found to be suitable for ICP-MS determination of trace elements in plant samples. Consistent analytical results and satisfactory precision were noted for both procedures when the element concentrations were compared with the certified values of the standard reference materials. Considering time, reagent, and need for supervision, the dry ashing procedure seems to be more convenient decomposition method for sample preparation. For many of the elements the wet digestion procedure gave slightly higher recoveries. However it cannot be recommended for the determination of Al, Fe, Cr, Ti, and Mn because of the significant losses and relatively higher RSD's associated with these elements.

Table 4. 2: Concentration of trace metals (ng/g) determined using ICP-MS after dry and acid digestion, n = 4

ELEMENT	MEAN CONCENTRATION (ng/g)						
	ACID DIGESTION	RSD %	CERTIFIED VALUE	% RECOVERY	DRY ASHING	RSD %	% RECOVERY
Li	0.83	6			1.05	2	
Be					0.07	10	
B	12.2	7			11.2	13	
Mg	1030	7			1020	4	
Al	450	3	1430	31.51	1000	3	69.62
Si	41	3			11.0	7	
P	490	6			500	6	
Ca	5000	6	6300	79.06	4700	4	74.69
Ti	10.3	3			51	14	
V	2.67	9	3.6	74.16	2.93	2	81.30
Cr	4.2	3			6.6	2	
Mn	470	7	612	76.38	480	5	78.42
Fe	1320	15			1610	5	
Co	0.76	4			0.49	14	
Ni	<DL				2.27	13	
Cu	4.8	22			4.7	5	
Zn	60	6			69	7	
Se	2.29	7			1.40	9	
Rb	2.98	4			3.6	3	
Sr	25.2	5			29.7	2	
Mo	2.22	29			2.0	3	
Ag	0.06	9			0.09	5	
Cd	<DL				0.08	15	
Sn	3.7	12			0.32	4	
Sb	0.10	11			0.13	5	
Cs	0.06	8			0.09	4	
Ba	50	5	170	29.70	57	2	33.76
La	0.64	3			1.11	3	
Ce	1.38	2			2.34	3	
Tl	0.020	1			0.05	1	
Pb	7.3	3			7.4	2	
Bi	0.06	4			0.05	4	
U	78	6	86.80	89.60	77	4	88.38

Table 4. 3: Concentration of trace metals (ng/g) in CLV-2 determined using ICP-MS after dry and acid digestion, n = 4

ELEMENT	MEAN CONCENTRATIONS (ng/g)						
	ACID DIGESTION	RSD %	CERTIFIED VALUE	% RECOVERY	DRY ASHING	RSD %	% RECOVERY
Li	0.258	3			0.3	4	
Be					0.13	3	
B	38	2			37	2	
Mg	810	3	<1.00		740	3	
Al	219	7	320.00	68.49	297	4	92.94
Si	>DL				170	26	
P	870	2			850	5	
Ca	7500	2	7700.00	97.40	7000	2	90.91
Ti	2.55	1			10.3	5	
V	0.51	7	0.76	66.86	0.56	2	73.36
Cr	3.9	18			4.7	15	
Mn	900	3	1940.00	46.14	1410	3	72.56
Fe	239	2			320	6	
Co	<DL				0.08	23	
Ni	<DL				1.15	17	
Cu	1.55	10			2.03	5	
Zn	61	3			61	3	
Se	<DL				0.31	27	
Rb	3.1	2			3.2	2	
Sr	17.1	2			17.7	4	
Mo	<DL				0.07	9	
Ag	0.02	6			0.02	4	
Cd	<DL				<DL		
Sn	2.16	21			0.2	9	
Sb	0.03	9			0.03	6	
Cs	0.02	10			0.03	4	
Ba	24.1	2	28.00	85.90	24.5	3	87.48
La	0.28	17			0.25	6	
Ce	0.29	6			0.51	8	
Tl	0.02	2			0.02	12	
Pb	0.91	4			0.89	7	
Bi	0.01	1			0.01	5	
U	3.3	4	3.60	92.41	3.3	3	92.56

Table 4. 4: Concentration of trace metals (ng/g) determined in SRM 1575 using ICP-MS after dry and wet digestion

ELEMENT	MEAN CONCENTRATION (ng/g)						
	ACID DIGESTION	RSD %	CERTIFIED VALUE	% RECOVERY	DRY ASHING	RSD %	% RECOVERY
Li	0.180	4			0.200	9	
B	14.8	6			15.4	3	
Mg	1050	3			940	5	
Al	470	5	550	85.40	480	3	87.18
Si	<DL				28.3	22	
P	1060	3			1080	3	
Ca	3600	3			3800	2	
Ti	5.3	4			10.8	3	
V	0.35	2			0.36	2	
Cr	2.24	4	2.60	85.98	2.31	6	89.04
Mn	540	3	680	79.37	540	7	80.36
Fe	185	2	200	92.31	191	2	95.32
Co	0.120	7	0.100	117.50	0.100	1	98.02
Ni	<DL		3.5		2.17	11	62.07
Cu	2.68	2	3.00	89.37	2.56	2	85.29
Zn	56	12			68	12	
Rb	10.5	3	11.7	89.96	10.8	2	92.09
Sr	4.3	4			4.4	1	
Mo	<DL				0.120	13	
Cd	0.160	10			0.170	7	
Sn	3.5	7			0.300	15	
Sb	0.210	46	0.200	105.94	0.180	3	91.29
Cs	0.110	4			0.110	1	
Ba	7.1	2			6.7	2	
La	0.12	4	0.20	57.85	0.120	6	58.89
Ce	0.23	5	0.40	57.41	0.230	5	57.02
Tl	0.04	7	0.05	83.51	0.040	3	85.59
Pb	9.7	5	10.8	89.48	9.7	3	90.03
Bi	0.024	13			0.0200	4	
U	0.018	3	0.020	89.73	0.0190	2	94.19

A summary of uranium recovery by both methods in the certified reference materials is given in table 4.5. The repeatability of the procedure was also examined by analysing in-house reference materials (i.e. coffee, tea, and black spruce samples). There was agreement between the mean concentrations of repeated digestions with relative standard deviations less than 10 % for most of the elements. The proposed dry ashing procedure is efficient for simple, rapid, and reliable determination of trace metals in plant tissues.

Table 4. 5: Uranium recovery (%) in certified reference materials using dry and acid digestion methods, n = 4.

MATERIAL	CERTIFIED CONCENTRATION (mg/kg)	% RECOVERY WET DIGESTION	% RECOVERY DRY ASHING
CLV-1	87	90	88
CLV-2	3.6	92	93
1575 Pine needle	0.02	90	94
1547 peach leaves	0.015	-	206

Table 4. 6: Concentrations of trace elements (ng/g) recovered with four different leaching agents in the in-house black spruce reference material, n = 4.

ELEMENT	16M HNO_3		8M HNO_3		8M HNO_3/6M HCl		8M HNO_3/29M HF	
	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %
Li	0.06	20	0.07	9	0.07	33	0.10	13
B	8.2	5	9.2	5	7.8	8	9.4	3
Mg	800	6	810	4	760	5	770	6
Al	60	34	50	6	50	13	93	32
P	690	30	610	6	570	9	560	7
Ca	3700	28	3200	2	3200	4	3100	2
Ti	1.88	35	1.83	15	2.39	21	4.9	14
V	2.1	21	1.97	2	1.92	5	1.88	3
Cr	0.89	36	0.82	29	0.85	14	0.62	16
Mn	430	54	320	41	298	42	294	33
Fe	49	12	67	32	72	14	73	11
Co	1.6	19	1.59	6	1.51	6	1.46	2
Ni	4.9	23	4.5	9	4.4	15	4.1	6
Cu	6.0	22	5.7	6	5.5	6	5.4	3
Zn	51	22	47	4	45	6	44	3
Rb	5.3	29	4.9	2	4.8	4	4.8	2
Sr	24.2	28	20.9	3	20.6	5	20.6	2
Mo	0.14	46	0.11	9	0.15	28	0.14	30
Ag	0.04	2	0.04	4	0.05	6	0.05	5
Cd	0.04	2	0.05	2	0.04	5	0.06	8
Sn	0.07	16	0.08	15	0.09	4	0.11	14
Sb	0.02	8	0.03	33	0.05	17	0.06	5
Cs	0.02	5	0.02	4	0.03	7	0.03	3
Ba	59	26	52	3	53	5	52	2
La	0.52	29	0.47	2	0.5	8	0.62	4
Ce	0.17	26	0.160	8	0.16	8	0.21	3
Pb	0.83	27	0.75	5	0.74	5	0.76	9
U	<DL		<DL		0.003	16	0.004	14

<DL = below detection limit

Table 4. 7: Concentration of trace elements (ng/g) recovered from the in-house coffee reference material with four different leaching agents, n = 4.

ELEMENT	16M HNO_3		8M HNO_3		8M HNO_3/6M HCl		8M HNO_3/29M HF	
	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %
Li	0.03	26	0.03	12	0.03	11	0.03	19
B	6.3	9	6.7	5	6.51	3	6.5	9
Mg	1360	43	1470	30	950.77	10	950	3
Al	33	5	39	10	39.99	19	40	9
P	1540	10	1480	10	1484.82	9	1480	3
Ca	1100	2	1140	4	1093.90	2	1090	3
Ti	1.58	6	1.84	5	2.16	8	2.16	2
V	0.070	7	0.090	11	0.09	7	0.1	5
Cr	0.32	22	0.190	3	0.22	2	0.22	7
Mn	26.6	5	24.8	5	24.71	1	24.7	3
Fe	72	14	72	24	88.10	11	88	7
Co	0.280	8	0.280	6	0.28	3	0.28	6
Ni	1.56	13	1.35	10	1.26	4	1.26	8
Cu	13.4	2	13.5	3	13.52	2	13.5	3
Zn	8.2	23	5.7	8	6.37	21	6.4	14
Br	0.85	8	0.9	9	0.48	16	0.48	17
Rb	44	2	46	4	45.07	4	45	2
Sr	5.4	2	5.5	3	5.42	3	5.4	2
Mo	0.100	10	0.1	8	0.10	10	0.1	27
Sn	0.220	10	0.14	8	0.09	8	0.09	14
Cs	0.130	9	0.14	6	0.14	4	0.14	2
Ba	3.3	4	3.5	3	3.39	4	3.4	10
La	0.01	9	0.01	10	0.01	12	0.01	5
Ce	0.02	1	0.02	20	0.03	19	0.03	11
Pb	0.09	14	0.04	8	0.06	2	0.06	3
U	0.002	13	<DL		0.003	12	0.003	10

Table 4. 8: Concentration of trace elements (ng/g) recovered from the in-house tea sample with four different leaching agents, n = 4.

ELEMENT	16M HNO_3		8M HNO_3		8M HNO_3/6N HCl		8M HNO_3/29M HF	
	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %
Li	0.10	24	0.09	11	0.11	20	0.09	7
B	15.5	4	15.7	3	15.5	5	16.7	3
Mg	900	14	920	13	910	11	890	12
Al	390	15	390	14	390	13	390	13
Ca	4900	5	4800	2	5100	3	4900	2
Ti	4.9	8	4.8	2	5.5	3	9.3	7
V	0.17	7	0.17	2	0.17	4	0.180	2
Cr	1.25	9	1.45	5	1.34	10	1.27	3
Mn	320	8	320	9	340	8.	340	11
Fe	145	4	143	4	147	7	140	2
Co	0.25	1	0.26	3	0.25	2	0.25	3
Ni	5.0	9	5.1	4	5.1	2	5.0	2
Cu	16.0	1	15.8	2	16.0	2	15.7	2
Zn	27.9	2	29.3	10	29.1	5	27.9	5
Br	0.79	6	0.73	9	0.81	4	0.75	11
Rb	36	3	38	3	38	4	36	2
Sr	25.9	5	26.1	4	26.6	3	25.5	3
Mo	0.05	34	0.08	1	0.06	1	0.08	4
Sn	0.25	8	0.23	6	0.28	7	0.3	3
Cs	0.25	4	0.25	4	0.27	5	0.26	3
Ba	36	4	37	3	38	4	38	2
La	0.24	3	0.24	4	0.240	6	0.25	4
Ce	0.36	4	0.35	5	0.36	6	0.36	3
Pb	0.42	6	0.37	8	0.33	4	0.34	6
U	<DL		<DL		<DL		<DL	

Table 4. 9: Concentration of trace elements (ng/g) recovered from the SRM 1575 pine needle materials with four different leaching agents, n = 4.

ELEMENT	16M HNO_3		8M HNO_3		8M HNO_3/6M HCl		8M HNO_3/29M HF	
	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %	MEAN (ng/g)	RSD %
Li	0.2	10	0.17	27	0.2	10.30	0.19	17
B	15.4	3	15.7	4	14.3	2.19	14.9	2
Mg	940	25	760	25	690	33.07	1090	3
Al	275	3	205	24	266	0.91	420	2
Si	28.3	22	29.5	30	29.0	21.72	11.9	20
P	1080	3	1090	4	1080	1.18	1140	2
Ca	3800	2	3700	8	3600	1.25	3600	1
Ti	10.8	3	9.7	6	11.3	3.50	11.2	2
V	0.36	2	0.34	13	0.36	1.69	0.34	4
Cr	2.10	2	1.93	13	2.26	6.69	4.8	25
Mn	340	1	340	5	340	0.68	280	3
Fe	191	3	168	12	184	2.24	212	9
Co	0.1	2	0.1	7	0.10	2.54	0.16	11
Ni	2.17	1	2.19	11	2.63	22.82	2.65	3
Cu	2.56	4	2.51	34	2.55	2.65	2.99	3
Zn	68	12	65	8	62	23.89	67	13
Rb	10.8	1	10.7	6	10.7	1.18	10.9	2
Sr	4.4	1	4.3	7	4.4	1.53	4.4	1
Mo	0.1	9	0.19	8	0.08	9.15	0.14	22
Cd	0.17	4	0.18	3	0.17	5.22	0.1	3
Sn	0.29	4	0.3	16	0.31	6.32	0.3	5
Sb	0.17	3	0.18	4	0.17	2.60	0.14	13
Cs	0.11	1	0.1	11	0.10	1.52	0.11	1
Ba	6.7	2	6.6	10	6.7	1.84	6.8	2
La	0.12	6	0.09	18	0.12	3.63	0.12	8
Ce	0.23	5	0.17	18	0.24	2.77	0.24	6
Tl	0.04	6	0.04	7	0.04	4.42	0.05	5
Pb	9.7	3	9.7	7	9.5	2.86	9.5	3
Bi	0.02	4	0.02	13	0.02	7.42	0.02	4
U	0.012	19	0.013	26	0.014	11.93	0.016	12

Table 4. 10: Concentration of trace elements recovered after ashing the in-house spruce twigs at 500 °C and 450 °C

ELEMENT	MEAN CONCENTRATION (ng/g)			
	500 °C	RSD %	450 °C	RSD %
Li	0.100	14	0.27	11
Be	<DL		0.03	9
B	7.8	4	9.2	4
Mg	600	18	630	14
Al	93	32	249	15
Si	<DL		28.9	23
P	560	7	730	4
Ca	3100	2	3900	1
Ti	4.9	15	13.3	10
V	1.88	3	1.50	11
Cr	0.62	16	1.04	8
Mn	294	43	390	3
Fe	73	12	169	11
Co	1.46	2	3.5	4
Ni	2.52	12	4.1	5
Cu	5.4	3	6.4	7
Zn	44	3	77	2
Rb	2.70	3	4.9	2
Sr	20.6	2	21.7	3
Mo	0.02	28	0.05	13
Ag	0.02	25	0.05	5
Cd	0.04	8	0.06	7
Sn	0.11	15	0.14	25
Sb	0.06	5	0.06	7
Cs	0.03	3	0.04	6
Ba	45	5	54	6
La	0.25	5	0.63	3
Ce	0.21	3	0.36	8
Tl	<DL		0.02	4
Pb	0.76	9	1.36	2
U	0.004	4	0.013	9

Table 4. 11: Concentration of trace elements (ng/g) recovered after ashing in-house coffee material at 500 °C and 450 °C, n = 4

ELEMENT	MEAN CONCENTRATION (ng/g)			
	500 °C	RSD %	450 °C	RSD %
Li	0.023	10	0.06	19
B	7.8	2	7.8	8
Mg	940	4	1640	3
Al	39	9	42	16
Si	<DL		18.5	17
P	880	1	1570	3
Ca	1100	2	1100	3
Ti	4.4	12	4.5	2
V	0.09	11	0.11	5
Cr	0.14	12	0.32	22
Mn	22.4	3	24.5	3
Fe	64	8	79	7
Co	0.28	2	0.28	6
Ni	1.20	6	1.63	8
Cu	13.4	1	13.5	3
Zn	5.3	15	7.6	13
Br	1.43	6	0.64	17
Rb	46	2	46	2
Sr	5.3	2	5.5	2
Mo	0.09	12	0.12	17
Sn	0.088	24	0.092	5
Cs	0.15	2	0.15	2
Ba	3.00	4	3.1	10
La	0.01	5	0.02	8
Ce	0.03	11	0.03	9
Pb	0.05	3	0.08	4
U	0.004	8	0.007	10

Table 4. 12: Concentration of trace elements (ng/g) recovered after ashing the in-house tea material at 500 °C to 450 °C, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)			
	500 °C	RSD %	450 °C	RSD %
Li	0.07	7	0.088	5
Be	<DL		0.040	28
B	16.7	3	16.3	3
Mg	890	12	1770	3
Al	390	13	730	15
Si	<DL		122	11
P	1620	16	2660	3
Ca	4300	9	4900	2
Ti	9.3	7	12.2	8
V	0.180	2	0.23	2
Cr	1.27	4	3.1	10
Mn	340	11	440	13
Fe	140	1	208	2
Co	0.250	3	3.1	5
Ni	5.0	2	6.0	8
Cu	15.6	5	15.7	2
Zn	27.9	5	27.3	2
Br	<DL		0.75	11
Rb	36	2	36	2
Sr	25.5	3	25.6	2
Mo	0.04	19	0.210	4
Sn	0.3	3	0.33	4
Cs	0.26	3	0.260	2
Ba	37	3	38	3
La	0.25	5	0.270	1
Ce	0.36	3	0.42	1
Tl	<DL		0.012	9
Pb	0.34	6	0.37	6
U	<DL		0.0050	15

Table 4. 13: Concentration of trace elements (ng/g) recovered in the in-house black spruce material after ashing for 4, 8, and 16 hours, n = 4

ELEMENT	MEAN CONCENTRATION (ng/g)					
	4 HOURS	RSD %	8 HOURS	RSD %	16 HOURS	RSD %
Li	0.09	36	0.31	19	0.05	27
B	8.3	11	8.3	6	7.4	5
Mg	680	16	690	17	670	15
Al	117	6	140	7	117	2
Si	22.5	25	34	12	11.8	26
P	540	42	860	14	620	3
Ca	3600	17	4200	2	2970	4
Ti	8.9	34	15.0	10	4.5	6
V	1.66	17	1.67	14	1.85	4
Cr	0.53	10	0.66	11	0.81	29
Mn	210	18	380	4	243	12
Fe	144	4	161	10	53	3
Co	2.40	25	3.7	3	1.39	4
Ni	3.1	34	2.43	4	4.1	6
Cu	5.6	8	7.2	12	1.42	5
Zn	61	25	77	3	46	3
Rb	3.7	32	2.81	4	4.5	3
Sr	21.0	5	22.5	2	13.4	5
Mo	0.11	67	0.04	13	0.06	54
Ag	0.01	44	0.04	13	0.01	24
Sn	0.11	9	0.17	6	0.08	46
Sb	0.06	19	0.06	4	0.02	29
Cs	0.03	9	0.03	7	0.02	5
Ba	48	8	48	5	42	6
La	0.42	52	0.62	2	0.23	11
Ce	0.28	30	0.38	11	0.18	5
Tl	0.01	1	0.01	3	0.01	25
Pb	1.03	34	1.40	3	0.72	9
U	0.01	17	0.013	10	0.004	45

Table 4. 14: Concentration of trace elements (ng/g) recovered in the in-house coffee material after ashing for 4, 8, and 16 hours, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)					
	4 HOURS	RSD %	8 HOURS	RSD %	16 HOURS	RSD %
Li	0.025	15	0.022	13	0.016	13
B	5.7	6	7.8	2	5.5	9
Mg	770	10	940	4	800	6
Al	42	44	42	16	37	5
Si	4.7	28	18.5	12	7.8	21
P	860	40	880	1	790	2
Ca	880	9	1100	2	950	3
Ti	3.9	29	4.4	11	3.7	4
V	0.07	35	0.09	9	0.08	15
Cr	0.13	6	0.14	11	0.08	5
Mn	19.8	6	22.4	2	19.4	4
Fe	52	29	64	8	51	13
Co	0.22	9	0.28	2	0.22	6
Ni	1.01	8	1.20	6	0.85	11
Cu	8.1	7	13.4	1	8.7	6
Zn	6.4	50	5.3	14	5.2	12
Br	1.80	22	1.43	6	2.39	34
Rb	32	8	46	2	37	3
Sr	4.4	7	5.3	2	4.8	3
Mo	0.08	32	0.09	12	0.06	17
Sn	0.18	83	0.09	4	0.08	11
Cs	0.1	6	0.15	2	0.12	8
Ba	2.65	10	3.0	4	2.12	3
La	0.01	9	0.02	14	0.01	21
Ce	0.02	4	0.03	14	0.02	16
Pb	0.06	29	0.10	8	0.05	32
U	0.004	19	0.010	12	0.003	6

Table 4. 15: Concentration of trace elements (ng/g) recovered in the in-house tea material after ashing for 4, 8, and 16 hours, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)					
	4 HOURS	RSD %	8 HOURS	RSD %	16 HOURS	RSD %
Li	0.01	22	0.07	7	0.04	8
Be	<DL		0.040	14	<DL	
B	5.1	65	16.3	3	6.0	74
Mg	980	87	1770	3	920	11
Al	125	18	730	15	256	16
Si	20.0	14	122	11	65	48
P	1150	77	2020	4	1110	9
Ca	1370	37	4300	9	1680	20
Ti	2.84	51	12.2	7	7.8	9
V	0.110	80	0.230	2	0.11	14
Cr	1.13	77	3.1	10	1.08	41
Mn	85	5	440	13	272	7
Fe	98	56	160	11	94	10
Co	0.41	3	3.1	5	0.17	13
Ni	3.8	51	6.0	7	3.1	17
Cu	8.0	34	15.6	5	5.5	12
Zn	10.3	61	27.3	2	19.7	10
Rb	20.5	48	36	2	27.7	9
Sr	8.1	26	25.6	3	16.8	13
Mo	0.09	5	0.040	19	<DL	
Sn	0.05	11	0.33	4	0.25	15
Cs	0.11	37	0.260	2	0.17	13
Ba	4.8	20	37	3	21.1	15
La	0.08	42	0.270	1	0.11	11
Ce	0.13	19	0.42	3	0.18	9
Tl	<DL	8	0.0100	9	0.01	13
Pb	0.27	15	0.37	5	0.190	14
U	<DL		<DL		<DL	

Table 4. 16: Concentration of trace elements (ng/g) recovered in the SRM 1575 pine needle material after ashing for 4, 8, and 16 hours, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)					
	4 HOURS	RSD %	8 HOURS	RSD %	16 HOURS	RSD %
Li	0.150	9	0.20	9	0.14	32
B	14.2	2	15.4	3	13.8	4
Mg	540	16	940	12	750	11
Al	206	16	275	3	204	24
Si	16.2	46	28.3	22	29.3	34
P	930	26	1080	3	1060	4
Ca	3400	2	3800	1	3600	8
Ti	9.4	2	10.8	3	6.6	8
V	0.33	3	0.36	1	0.260	16
Cr	1.71	5	2.21	2	1.31	18
Mn	340	1	340	1	310	5
Fe	154	6	191	6	161	13
Co	0.09	12	0.1	1	0.07	9
Ni	2.08	6	2.17	7	1.88	12
Cu	2.13	24	2.56	6	2.10	41
Zn	62	14	68	12	58	9
As	0.160	28	<DL		<DL	
Rb	10.1	2	10.8	6	8.6	7
Sr	4.1	2	4.4	9	3.9	7
Mo	0.1	9	0.12	13	0.15	41
Cd	0.17	4	0.17	6	0.14	4
Sn	0.29	4	0.3	15	0.26	20
Sb	0.18	22	0.17	2	0.11	7
Cs	0.10	4	0.11	7	0.06	19
Ba	5.8	4	6.7	3	5.9	12
La	0.09	24	0.12	6	0.07	22
Ce	0.16	13	0.23	6	0.14	23
Tl	0.04	6	0.04	3	0.03	8
Pb	9.3	3	9.7	3	7.4	11
Bi	0.011	21	0.02	4	0.01	23
U	0.014	10	0.019	2	0.015	10

Table 4. 17: Concentration of trace elements (ng/g) recovered from in-house spruce twigs at varying rates of temperature ramp, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)			
	18 °C/hr	RSD %	10 °C/hr	RSD %
Li	0.82	10	1.05	7
Be	0.08	8	0.070	11
B	12.6	2	11.2	12
Mg	480	9	1020	4
Al	560	27	970	3
Si	42	14	111	8
P	500	2	500	6
Ca	4200	4	4700	3
Ti	35	4	51	14
V	2.57	5	2.93	2
Cr	5.4	2	6.6	2
Mn	450	1	480	5
Fe	1250	7	1610	4
Co	0.46	4.	0.49	4
Ni	2.33	10	2.27	13
Cu	3.7	1	4.7	6
Zn	66	2	69	7
As	4.3	10	4.1	13
Se	2.16	5	1.40	49
Rb	2.56	11	3.6	3
Sr	26.1	3	29.7	2
Mo	1.78	2	2.0	3
Ag	0.04	9	0.09	5
Cd	0.08	15	0.08	14
Sn	0.29	6	0.32	4
Sb	0.12	3	0.13	5
Cs	0.05	23	0.09	34
Ba	51	2	57	2
La	0.7	5	1.11	3
Ce	1.48	4	2.34	3
Tl	0.05	2	0.05	2
Pb	7.1	2	7.4	4
Bi	0.04	11	0.05	8
U	73	4	74	10

Table 4. 18: Concentration of trace elements (ng/g) recovered from SRM CLV-2 at varying rates of temperature ramp, n = 4

ELEMENT	MEAN CONCENTRATION (ng/g)			
	18 °C/hr	RSD %	10 °C/hr	RSD %
Li	0.26	14	0.3	4
Be	0.13	4	0.13	3
B	38	5	37	2
Mg	560	12	740	4
Al	169	22	267	3
Si	75	17	170	26
P	830	4	850	5
Ca	6300	6	7000	1.6
Ti	7.1	8	10.3	5
V	0.45	9	0.56	2.
Cr	1.35	5	1.71	16
Mn	1330	6	1410	3
Fe	234	11	320	3
Co	0.07	9	0.08	33
Ni	0.74	19	1.15	28
Cu	2.16	20	2.03	5
Zn	57	4	61	3
As	0.28	17	0.31	27
Rb	2.99	3	3.2	4
Sr	16.4	3	17.7	1
Mo	0.06	5	0.07	9
Ag	0.01	21	0.02	10
Cd	0.03	8	<DL	
Sn	0.22	35	0.2	9
Sb	0.04	12	0.03	23
Cs	0.02	7	0.03	41
Ba	22.2	3	24.5	3
La	0.17	9	0.25	6
Ce	0.34	9	0.51	8
Tl	0.02	7	0.02	18
Pb	0.97	14	0.89	7
Bi	<DL	12	0.01	15
U	3.0	3	3.2	3

Table 4. 19: Concentration of trace elements (ng/g) recovered from SRM 1547 peach leaves at varying rates of temperature ramp, n = 4.

ELEMENT	MEAN CONCENTRATION (ng/g)			
	18 °C/hr	RSD %	10 °C/hr	RSD %
Li	0.21	18	0.25	15
Be	0.03	11	0.03	22
B	7.7	4	8.10	7
Mg	620	17	755.40	4
Al	249	18	276.81	3
Si	28.9	26	43.05	37
P	730	4	763.98	6
Ca	3900	1	3947.54	3
Ti	13.3	12	14.75	4
V	1.50	14	1.70	1
Cr	1.04	43	0.88	6
Mn	390	3	413.97	5
Fe	169	12	197.42	7
Co	3.5	5	3.73	3
Ni	2.61	13	2.37	5
Cu	6.4	7	7.01	4
Zn	77	3	81.81	8
As	<DL		0.05	9
Rb	2.73	6	2.82	3
Sr	21.7	3	22.51	6
Mo	0.05	26	0.05	12
Ag	0.02	34	0.03	5
Cd	0.03	6	0.05	24
Sn	0.14	28	0.19	8
Sb	0.06	8	0.07	6
Cs	0.04	7	0.04	3
Ba	46	5	48.97	5
La	0.24	7	0.26	2
Ce	0.36	8	0.39	2
Tl	0.02	4	0.01	11
Pb	1.36	2	1.36	3
Bi	<DL		0.003	27
U	0.02	10	0.02	13

Chapter 5: Results of biomonitoring

5.1 Factors affecting metal availability in soils

The uptake of trace elements from soils depends on the bioavailability and the mobility of the metal in soil. Bioavailability is the proportion of total metals that are available for incorporation into biota (bioaccumulation) (Kabata-Pendias, 2001). Plants cannot usually access the total pool of a metal present in the growth substrate hence the total metal concentrations do not necessarily correspond with metal bioavailability. For example, sulphide minerals may be inclusions in quartz or other low solubility minerals, and despite high total concentrations of metals in sediment and soil containing these minerals, metals are not readily available for incorporation in the biota; hence their associated environmental effects may be low (Davis *et al*, 1994).

Metals present in a soil can be divided into a number of fractions including; the soluble metal in the soil solution, metal-precipitates, metal sorbed to clays and other soil particles, hydrous oxides and organic matter, and metals within the matrix of soil minerals. These different fractions are in dynamic equilibrium with each other (Norvell, 1991). However, while the soluble metal in the soil solution is directly available for plant uptake other soil metal pools are less available (del Castilho *et al*, 1993). Hence, factors which affect the concentration and speciation of metals in the soil solution will affect the bioavailability of metals to plants. Soil factors which affect metal bioavailability include the total metal present in the soil, pH, clay and hydrous oxide content, organic matter, and redox conditions.

5.1.1 Total metal concentration

The total metal concentration in a soil includes all the metal, from the readily available to the unavailable. Other soil factors, such as pH, organic matter, clay and redox conditions, determine the proportion of total metal which is found in solution in the soil. Hence, while total metal provides the maximum pool of metal in the soil, other factors have a great importance in determining how much of this soil pool will be available to plants (Wolt, 1994). It has been observed that while total metal correlates with bioavailable soil pools of metal it is inadequate by itself to reflect bioavailability (Peijnenburg *et al*, 2000).

5.1.2 pH

The equilibrium between metal speciation, solubility, adsorption, and exchange on solid phase sites is closely connected to solution pH (Sauve *et al*, 1997). Numerous studies have found soil pH to have a large effect on metal bioavailability (McBride *et al*, 1997). Most elements especially Mo, Mn, and Zn are highly affected by soil pH (Turner, 1994). As soil pH decreases, Mn and Zn competes with the the high concentrations of H^+ and Al^{3+} for positions on the exchange sites. The solubility of Mn and Zn increases in the soil solution and a greater proportion is present as highly available free metal ions in the soil solution (Sauve *et al*, 1997). This increases the concentrations of Mn and Zn in the directly bioavailable fraction, i.e., the soil solution (Jeffery and Uren, 1983). In accordance with the changes in metal bioavailability associated with a change in pH,

many studies have shown that plant metal uptake varies under different pH conditions. For example, it has been observed that plant uptake of Mn and Zn increases as soil pH decreases (Kabata-Pendias 2001). Uptake of uranium by plants may be influenced by pH. Accumulation of uranium in shoots of peas (*Pisum sativum*) grown in nutrient solution at pH 6.0 and pH 8.0 was less than 20% and 5%, respectively compared to the uptake at pH 5.0 (Ebbs *et al*, 1998 in CCME). At pH 5.0 the uranium was present primarily as the free uranyl cation which may be more readily taken up and translocated than other uranium species (Ebbs *et al*, 1998 in CCME).

Conversely, the solubility of Mo increase with the increasing pH of soil. Abnormally high concentrations of Mo were found in native plants grown in neutral or alkaline soils (Kabata-Pendias 2001). Mo concentration in plants therefore has been observed to reflect the soluble Mo pool and the uptake by plants is a function of soil pH. Adsorption of U onto goethite in the pH range of 4.5 to 6.5 increases with increasing $(UO_2)_3(OH)_5^+$ and then decreases because of the formation of carbonates. A similar tendency occurs with hematite, ferric amorphous oxy-hydroxide and other Fe oxides, and also with smectite by edge co-ordination reaction with increasing pH. The mobility of Al is pH dependent and increases sharply in acid soils with pH below 5.5. A sudden increase of the Al solubility occurs in the narrow pH range from 4.5 to 4.0 and its solubility in soils is also increased by soil acidification due to the atmospheric deposition of S (mainly as SO_2) resulting from both anthropogenic and natural events.

5.1.3 Organic matter

Metal ions can be complexed by organic matter altering their availability to plants. The COO^- groups in both solid and dissolved organic matter form stable complexes with metals (Baker and Senft, 1995; Kabata-Pendias, 2001). Hence, as the amount of organic matter present in soil increases the opportunity for forming stable metal-organic matter complexes increases. In general, plants are unable to absorb the large metal-complexes and so the bioavailability of metals decreases.

Copper ions form strong coordination complexes with organic matter (Stevenson, 1991). Hence, Cu is often predominantly found bound to the organic matter fraction in the soil and soil organic matter can be the most important soil factor in determining Cu bioavailability (del Castilho *et al*, 1993). The amount of organic matter found in soils also affects the bioavailability of Zn (del Castilho *et al*, 1993). However, while Zn readily forms complexes with organic matter it does not compete for these sites as well as Cu (Cavallaro and McBride, 1984) and other more prevalent cations such as Ca^{2+} (Fotovat *et al*, 1997). In soil solution, the activity of the highly bioavailable Zn^{2+} in the soil solution decreases as organic matter increases across a range of contaminated soils (McBride *et al*, 1997). Across a range of soils greater than 50% of the soil solution Zn was present as the free ion (Lorenz *et al*, 1997).

5.1.4 Clays and hydrous oxides

Clays and hydrous oxides, i.e. oxides of Al, Fe, and Mn, play an important role in the availability of metals. Clays and hydrous oxides determine metal availability mainly by specific adsorption to surface hydroxyl groups (Pampura *et al*, 1993), nonspecific adsorption (exchange), coprecipitation (Martinez and McBride, 1998), and precipitation as the discrete metal oxide or hydroxide (Martinez and McBride, 1998). Hence, increasing clay and hydrous oxide contents in soils provides more sites for adsorption of metals thus reducing the directly bioavailable metal (Qiao and Ho, 1996).

5.1.5 Oxidation and reduction

The oxidation/reduction (redox) conditions of a soil can play a role in the availability of metals. The redox status of the soil can be affected by many factors including water logging and compaction. Redox conditions can affect the availability of metals by affecting the proportion of particular metal species (e.g. Mn(II) vs. Mn(IV) in the soil solution and by affecting the solubility of metals in the soil solution (Patrick and Jugsujinda, 1992; Evangelou, 1998).

Redox state has a large affect on Mn speciation and solubility in the soil solution (Sajwan and Lindsay, 1986). Manganese can exist in soil as Mn(II), Mn(III) and Mn(IV), however only the reduced Mn(II) form is stable in solution (Whitehead, 2000). Manganese (II) is the most soluble form of Mn and so under reducing conditions higher concentrations of Mn^{2+} will be present in the soil solution (Patrick and Jugsujinda, 1992).

Conversely, under more oxidising conditions, soil solution concentrations of Mn decrease because the equilibrium shifts in favour of Mn(III) and Mn(IV) which primarily exist as insoluble hydroxides and oxides. For example, increasingly reducing conditions corresponded with an increase in the highly bioavailable Mn^{2+} in the soil solution and a corresponding increase in Mn uptake by rice plants (Schwab and Lindsay, 1983). Similarly, uranium IV and VI are the oxidation states typically observed in the environment. It occurs in the tetravalent oxidation state under reducing conditions. Under oxidizing conditions, uranyl is reduced to the U(IV) form, which tends to precipitate on clay layers, calcite, or phosphate. The tetravalent oxides of uranium are less soluble than the hexavalent.

5.2 Plant factors

While soil factors have a large impact on the bioavailablity of metals to plants, different species or varieties grown on the same soil can have different metal uptake capabilities (Miles and Parker, 1979). There are species specific factors affecting plant uptake. Jarvis and Whitehead (1981) suggested that a true measure of plant availability of metals will not be attained unless the extent of soil exploitation by the roots is accounted for. However, it should be noted that while two plant species may take up a different amounts of metal within a given time frame it does not necessarily mean they are extracting from different soil pools of metal.

5.2.1 Supply of bioavailable metals for plant uptake

The bioavailable fraction of metals in a soil is generally thought to be the free metal ion in the soil solution. However, this appears to be an over simplification in some circumstances. In deficiency and sufficiency situations the free metal ion activity in the soil solution is low and plants have developed strategies to maximise the potential uptake of metals (Welch, 1995). Plants are able to influence the solubility and speciation of metals in the rhizosphere by exuding chelators (Fan *et al*, 1997) and manipulating rhizosphere pH.

Most of the present understanding of plant metal uptake has come from the study of Fe (Kochian, 1993). For Fe uptake, two different strategies have been identified. In Strategy I plants, i.e. dicots and nongraminaceous monocots, Fe(III)-chelates or -complexes present in the rhizosphere are reduced by plant produced reductants in the rhizosphere for uptake with other sources of free Fe^{2+} across the plasma membrane (Welch, 1995). In addition, plant produced organic acids are excreted which can complex with Fe (Grusak *et al*, 1999). It does not appear as though Strategy I plants are able to directly absorb Fe-chelates or Fe-complexes (Chaney *et al*, 1972). In addition to the above mechanisms, Strategy II plants, i.e. graminaceous monocots, excrete chelates such as mugeneic and avenic acids (Kochian, 1993; Fan *et al*, 1997) which are known as phytosiderophores or phytometallophores depending on their association with Fe alone or all metals respectively, into the rhizosphere (Fan *et al*, 1997). Iron, and other metals, chelate with the phytometallophores, providing a ready supply of metals for reduction

and transport across the plasma membrane. Research has also shown that Strategy II plants can directly absorb the Fe-phytometallophore complex (Grusak *et al*, 1999).

5.2.2.1 Manipulation of rhizosphere pH

The pH of the rhizosphere may vary by up to 2.5 pH units from that of the bulk soil solution depending on plant species, plant age, nutrient supply, and the buffer capacity of the soil (Romheld *et al*, 1984). This is primarily as a result of an imbalance in cation/anion uptake, and hence, excretion of H^+/OH^-(or HCO_3^-), excretion of organic acids, production of CO_2, and microbial activity in the rhizosphere (Marshner, 1993). Hence, the solubility, speciation, and corresponding availability of metals in the rhizosphere may be different from that in the bulk soil solution.

5.2.2.1 Role of mycorrhizae

Mycorrhizae are mutualistic associations between certain soil fungi and the roots of plant species (Brundrett *et al*, 1996). The mycorrhizal fungi benefit from the association by obtaining photosynthates, and in exchange, mycorrhizal fungi increase the plant uptake of P (Burgess *et al*, 1993) and trace metals (Pahlsson, 1989). Mycorrhizal fungi achieve this increase in plant nutrition by increasing the surface area of the soil explored compared with non-mycorrhizal roots (Clarkson, 1985) and increase the solubility of metals e.g. by producing metal-chelators (Szaniszlo *et al*, 1981).

It has been suggested as well that as assisting nutrient uptake at low metal concentrations, mycorrhizal fungi are able to reduce metal uptake, or at least increase plant metal tolerance by affecting metal translocation, under conditions of metal contamination. Some studies have found this to be the case, for example, mycorrhizal *Trifolium pratense* (red clover) plants grown in acid soils had less Mn in the roots and the shoots than non-mycorrhizal plants (Arines *et al*, 1989).

It appears that the ability of mycorrhizal fungi to increase plant metal tolerance is affected by other growth conditions, the fungal species, and the metal type (Weissenhorn *et al*, 1995). Of special importance is the tolerance of the mycorrhizal fungi to excess metal as the plant could be more tolerant than the fungi.

5.3 DISCUSSION

5.3.1 Introduction

Having demonstrated acceptable accuracy and precision, the digestion method was applied to determine trace elements in spruce twigs collected from the Holyrood area. From the results of the mean metal concentrations for samples collected in both winter and spring, it is evident that Ca, Mn, P, Mg, Al, Fe, Ba, Zn, Sr, and Si were the dominant elements in the samples analysed. The high concentrations and distribution of these elements are indicative of their large flux within the study area which also correlates with their bioavailable concentrations in the soil and bedrock. The result did not show any clear seasonal effect in the uptake of metals by spruce trees. The element

concentrations varied randomly with the seasons and with the sample locations. Some of the elements recorded higher concentrations in winter while for others the concentrations were higher in spring. The only elements which exhited a clear seasonal effect were uranium and phosphorus. Phosphorus recorded a mean concentration of 695 mg/kg in winter while all the spring samples recorded concentrations below the detection limit. The source of the excessively high concentration of phosphorus in the winter samples was not ascertained. The input of these elements in soil is related to weathering of parent rocks, pedogensis, and from anthropogenic sources. The standard deviation values related to the distribution of these metals in the plant samples show a high dispersion around the mean metal concentrations.

The average concentrations of B, Cu, Ti, Ni, Rb, Cr, and V in all samples ranged from 1.0 μg/g to 8.2 μg/g. Most of these elements are lithogenic hence may reflect the crustal and background soil concentrations in the study area. It also implies that uptake of these elements/metals by the spruce trees may occur mainly by the roots rather than aerial absorption. Anthropogenic additions to soil concentrations and absorption through shoots and needles cannot be entirely discounted. Soil additions through leaching from scrap metals, municipal and electronic waste, *etc* dumped at the study site cannot be eliminated from consideration. Also Ni and V have been associated principally with emissions from the Holyrood thermal electric plant.

It should be noted that the elements with the highest crustal abundance also recorded higher concentrations in the spruce samples. In particular, Ca, Mn, P, Mg, Al, Fe, Ba, Zn, Sr, and Si made up a significant weight of the analysed elements, Ca, Mn,

Mg, Al, and Fe being the most abundant. Geogenic and anthropogenic sources may contribute to the load of metals in the twigs. The geogenic sources include the bedrock, soil dust, sea spray, and direct uptake from the soil by roots. The anthropogenic sources within the study area are probably limited to emission from vehicular traffic, emission and discharge from the power plant, house-heating, dust and aerosols from the pyrophyllite mine, and small manufacturing industries.

5.3.2 Uranium in black spruce trees (*Picea mariana*)

Examination of uranium concentration in spruce twigs obtained from the study area revealed extremely low concentrations (close to the detection limit in most samples). Of all the samples, only three samples P13, P14, and P15 collected in winter registered a slight uranium anomaly, with concentrations of 83 ng/g, 111 ng/g, and 20 ng/g respectively. The U concentration in the spring samples were also very low and below detection limit in most samples. The samples SP 2 and SP 5 recorded 49.36 and 71.52 ng/g respectively. The low concentration of U in the spruce twigs reflects its low concentration in soils within the study area. This is evident in the study conducted by Sherwin (1979) in the Hoyrood claims, a site about 500 m south of the current study area. Of the 58 soil samples analysed, only 22.4% had U concentrations above 0.1 μg/g. One sample out of the 22.4% recorded 10.5 μg/g uranium while all others had concentrations below 2.5 μg/g.

In a similar study where vegetation was sampled in the Midwest uranium deposit area of the Athabasca basin (Northern Saskatchewan) uranium concentrations were below analytical detection (<0.2 mg/kg) in black spruce trunks (300 sites). Spruce twigs (69 sites) contained the highest uranium concentrations, ranging between 20 to 130 μg/g (Gordon 1992 in CCME) and uranium concentrations ranged from 8 to 46 μg/g) in Labrador tea stems and from 4 to 39 μg/g in Labrador tea leaves (CCME). The above study shows that spruce twigs are excellent indicators of metal accumulation.

5.4 Statistical analysis

Prior to statistical analysis, analytes which consistently had concentrations below their respective detection limits in all samples were eliminated from further consideration. The elements S, Cl, As, Br, Se, I, Hg, and Bi had concentrations below detection limit in the winter samples and Be, P, S, Cl, As, Br, Se, Cd, Sb, I, Hg, Tl, and Bi were below detection limit in the spring samples. The lower detection limits encountered may be attributed to the low natural abundance of these analytes, or due to high detection limits for the individual elements. The parameter set used for the waters package for analysing environmental samples includes measurements of multiple isotopes for some selected elements. This made it possible to select an isotope which is optimal for a given sample taking into account the backgrounds and interferences. For this study, ^{7}Li, ^{43}Ca, ^{53}Cr, and ^{57}Fe were chosen for the multi-isotopic elements measured.

The distribution of the analytes were treated using stem and leaf plot (Minitab 14.0 for windows) to determined whether they approximated normal error distributions (significance level of 0.05). The skewness and kurtosis for the trace metals and major elements were also examined. It should be noted that parametric statistical tests require the errors to be normally distributed. In order to meet this requirement, the data was logarithmically transformed to make them more closely approximate a normal distribution (Taylor *et al*, 2003). The log transformation also reduced the relative distribution of the data and brought both elements of higher and lower abundances into the same range. This step is also important because elements of widely varying concentrations are included in the same analysis (Yun *et al*, 2002). For all values which were greater than zero, the measured value was used in subsequent statistical analysis. However, since negatives and zeroes cannot be log transformed, these values were set to half of the detection limit (Huelin *et al*, 2006). It was observed that more of the log normalised variables approximated a normal distribution compared to the original data.

Pearson's correlation coefficients were used to measure association between the analytes (i.e. the extent to which values of two analytes are proportional to each other). Pearson's R range from +1 to -1 and because of the linear relationship there is an assumption that residuals are normally distributed (Gardiner, 1997). A normal correlation assumes that outliers are highly unlikely and therefore meaningful, giving them a large influence on correlation coefficients. The robustness of the Pearson R values were evaluated by examining a probability value associated with each correlation. The probability value (p) is the probability that an observed relationship occurred by pure

chance, so for p = 0.05, there is 95 % probability that the relationship can be reproduced (Gardiner, 1997). The correlation matrix is then examined for high values expressing similarity between variables. If two variables are highly correlated, it implies they will give similar information, and one of them can therefore be eliminated, as a ratio of 4 or 5 observations relative to variables is recommended to produce a stable model. A high correlation was obtained for La and Ce, with a Pearson correlation coefficient of 0.823 hence a high probability of exhibiting similar characteristics.

5.4.1 Multivariate analysis

Multivariate analysis comprises a set of techniques dedicated to the analysis of data sets with more than one variable. These methods comprise descriptive techniques which can simultaneously analyse a large number of variables (characters or attributes). In many cases, they represent the generalization of classical univariate statistical methods. Their main task is to reveal the underlying structure of the data to help identify points of similarity and dissimilarity as well as inter-relationships between the experimental units and their response measurements (Taylor *et al*, 2003). Multivariate statistics have been used for chemical fingerprinting in environmental studies which rely on a large amount of chemical data (Berg *et al*, 1994; Yun *et al*, 2002; Taylor *et al*, 2003; and Huelin *et al*, 2006). Certain difficulties have been encountered in applying statistical methods in chemical data. Multivariate analysis do not account for analytical uncertainties. Hence errors may arise when analytes with concentrations close to

detection limits are included in the statistical analysis as the RSD associated with analytes with such low concentrations is high. The multivariate method used assumed the residuals are normally distributed (a normal distribution would assume that for each element, 68% of the determined concentrations are within 1 standard deviation of the mean, 95% of the concentrations are within 2 SD, and 99% are within 2.5% SD).

5.4.2 Principal component analysis

Principal component analysis (PCA) was used to obtain an overview of the data, to show the differentiation between the metals at the study site, and to identify their possible sources. PCA, a data reduction technique, identifies patterns in a data set and expresses the data in such a way as to highlight their similarities and differences. The technique reduces a large number of variables into a smaller set of dimensions by analyzing the inter-relationships between variables (Gardiner, 1997). The components give the best linear combination of variables, and identify variables which account for more of the contribution of that component. For each component, a loading is assigned to each variable and an absolute value of the loading indicates the magnitude of contribution of each variable to the component, with values ranging between 0 and ±1. The sign, positive or negative, of the loading describes whether the variable is positively or negatively correlated with other variables in that component. Variables with loadings less than 0.5 were not considered to contribute significantly to a factor or component.

Each component is a linear combination of a number of variables. The first component accounts for the largest possible amount of variance. The second component, formed from the variance remaining after that associated with the first component has been extracted, accounts for the second largest amount of variance, *etc*. The principal components are extracted with the restriction that they are orthogonal. Geometrically, they may be viewed as dimensions in a given dimensional space where each dimension is perpendicular to each other dimension (Gardiner, 1997).

Rotation of the component matrix redistributes the variance to achieve a different component pattern, ideally where loadings are high for a few variables and low for other variables in the analysis, to give a clear grouping of variables. The reduction of ambiguities that accompanied initial unrotated factor loadings improved the interpretation of factors in the rotated component matrix. The total variance explained by each component is determined by an eigenvalue, which is reported as a percentage. Each eigenvalue represents the amount of variance that has been captured by one component or factor. The number of significant principal components to retain was determined by examining the scree plots and eigenvalues for each set of data (Ratha and Sahu, 1993). The Kaiser criterion states that only factors or principal components having eigenvalues greater than 1 should be retained. Hence components that account for less variance than does a single variable were discarded.

The characteristics and similarities of elements grouped by PCA were evaluated by reviewing their chemical properties according to the periodic law and ionic potential. According to the periodic law, elements in the same group, or columns, of the periodic

table, have the same valences and structure, and therefore tend to have similar physical and chemical properties (Bradl, 2004). The grouping of elements with similar properties implies that element mobility influences the uptake of elements by plants. Elements in the same periodic group display geochemical coherence in their distribution in nature (Taylor *et al*, 2003). Element mobility is influenced by the ionic potential of an element, which is quantified as the ratio of valence, or positive ionic charge, to the ionic radius, in picometres (pm) (Rollinson, 1993).

Of the several approaches to performing factor rotation, varimax rotation was used to maximise the variance of the normalized component loadings. Varimax rotation maximises the variance of a column of the factor pattern matrix as opposed to a row of the matrix (Berg *et al*, 1994). PCA analysis was performed on log transformed element concentrations using the SPSS statistical software (version 11.0 for windows). Based on the point of inflection or minima point of cumulative eigenvalues (Ratha and Sahu, 1993) seven prinicipal components were extracted for subsequent varimax rotation and interpretation. The component loadings for samples collected in winter and spring, and their respective percent variance are given in tables 5.1 to 5.4. Elements which did not contribute significantly to any of the components, or had high analytical uncertainty were excluded from the analysis.

5.5 Discussion on the sources of Elements

For winter samples, the PCA analysis produced seven principal components whose eigen values are greater 1. The unrotated component matrix (Table 5.1) set for these samples indicate that Be, Bi, Fe, Co, Mo, Cr, Ag, P, Cs, Al, Ni, and Ce are associated, and they display high values in the first component. The second component is explained by V, La Li, Ce, Pb, Ti, Si, Sb, and Ni, while Rb, Mn, B, and U loaded in the third component. Component four loaded Al, Ca, Cu, and Ba; and Sb, Mg, and Ba were explained by the fifth component. The element, Zn, is isolated in the sixth component. The seventh component had an eigen value greater than 1 but displayed low loading for all variables.

The rotation of the matrix aided in the clarification of the ambiguities associated within the unrotated component matrix (Facchinelli *et al*, 2001). Again seven components were retained, accounting for 83.1% of the total variance. The first rotated component contributing 25.1% of the total variance loaded V, Li, Pb, Sb, La, P, Ce, and Ni with a negative correlation for P. This factor suggests crustal erosion (weathering) as the main source with a minor anthropogenic contribution from atmospheric fallout. The elements Ni, V, and Pb could account for emissions from the Holyrood power plant and vehicular exhaust. Burning of heavy fuels emits V, Ni, and Pb and as estimated by Laveskog *et al* in Yun *et al* (2002), approximately 20 kg of V, 6 kg of Ni, and 0.3 kg of Pb are released when 1,000 tons of heavy fuels are combusted. The geographical variables such as Sb, La, P, and Ce are explained by weathering of the parent rock. The second rotated component explains approximately 18.8% of the total variance containing

Be, Bi, Cr, Cs, Fe, Sn, Co, and Ce which can be interpreted as contribution from the source rock lithology (magnetite and ilmenite), and abrasion and leaching from scrap metals dumped in the study area.

The third rotated component, contributing 11.0% of the total variance, has a significant contribution from Fe, Al, and Ti suggesting a soil source. The variability of these elements appears to be controlled by parent rocks. A small quantity of Ti and Fe may be coming from anthropogenic sources such as pigment and paint, and corrosion from cast iron, alloys, and machinery dumped at the site. The fourth component, which contributed 9.7% of the total variance, shows significant loading from Sr, Ba, Ca, Si, Mg, and Mo and suggests a carbonate rock origin. All elements except Si and Mo in this component belong to the alkaline earth group with similar ionic potential, solubility, and bioavailability hence similar geochemical properties. The elements Mg, Ca, and Sr are well known seaspray constituents (Yun *et al*, 2002). Depositions from these elements are probably through atmospheric fallouts since the study site is located a few kilometres from Conception Bay. The negative relation of Mo in relation to Sr, Ba, Ca, Si, and Mg suggest its chalcophilic nature and possible replacement by other cations. The association of Si with the alkaline earths may arise from minerals such as beryl and asbestos. The fifth component loaded Rb, B, and U and contributed to 7.7% of the total variance. This component represents a lithogenic source; U may also have anthropogenic contributions from the Holyrood thermal power plant.

The sixth rotated factor, explaining 5.9% of the total variance, contains Mn, Zn, Ag, and Cd, all of which except Mn are chalcophilic and therefore have affinity for

sulphide phases. The contribution of these elements to this component is likely a consequence of atmospheric pollution from vehicular exhaust fumes. There may be geogenic addition to since Ag, Zn, and Cd are abundant in the volcanic rocks of Newfoundland (Yun, *et al*, 2002). The seventh and last component reflects the mineralization of the bedrock. It displayed a high loading for U, Co, Tl and Cu with a total variance of 4.8%.

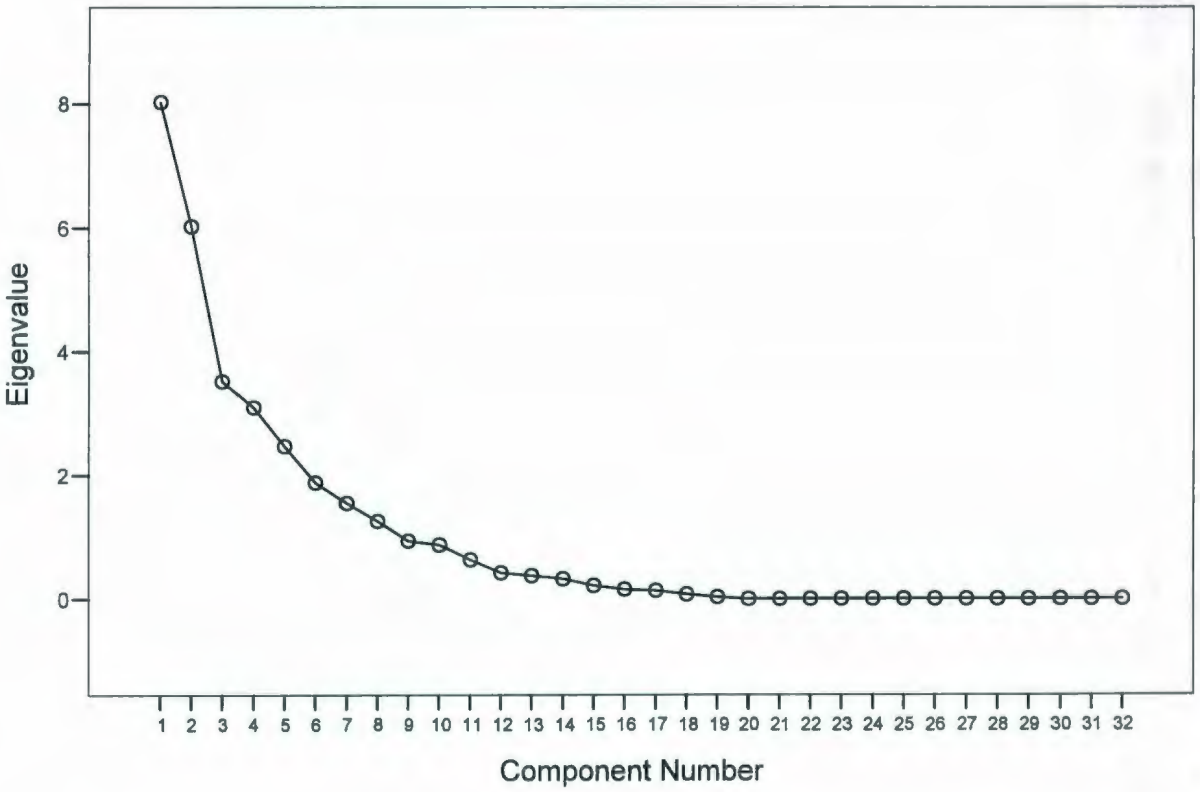

Fig. 5. 1: A scree plot of eigenvalues versus component number for the winter samples.

Table 5. 1: Unrotated component matrix showing loadings of analytes in the winter samples

Variables	Components						
	1	2	3	4	5	6	7
Be	0.880	0.064	0.054	-0.185	-0.017	0.248	0.188
Bi	-0.823	0.402	0.095	0.266	-0.011	-0.213	0.002
Fe	0.815	0.480	-0.118	-0.072	0.233	-0.150	0.028
Co	-0.765	0.063	0.449	0.082	-0.099	0.036	0.295
Mo	0.729	0.385	-0.098	0.131	-0.349	-0.036	0.287
Cr	0.727	0.200	-0.175	-0.293	0.386	0.085	-0.068
Ag	-0.725	-0.061	-0.097	0.197	0.275	0.289	0.243
P	0.618	-0.332	0.329	0.425	0.201	-0.038	-0.151
Cs	0.574	-0.008	0.319	-0.351	0.103	0.468	-0.089
Al	0.572	0.193	-0.038	0.527	0.223	-0.354	0.282
Cd	-0.465	0.063	-0.428	0.317	0.362	0.122	-0.039
V	-0.041	0.951	-0.120	0.085	-0.136	0.153	0.053
La	0.327	0.886	-0.068	-0.250	0.047	0.075	-0.048
Li	-0.201	0.846	-0.223	0.022	-0.132	-0.013	0.078
Pb	-0.485	0.711	-0.238	0.125	-0.167	0.214	0.125
Ti	0.485	0.609	0.082	0.319	0.244	-0.338	0.072
Si	-0.476	0.602	0.502	-0.038	0.196	-0.055	0.002
Sb	-0.283	0.582	-0.228	-0.285	-0.500	0.247	-0.232
Ni	-0.518	0.523	0.164	0.221	0.049	0.031	0.102
Rb	0.317	0.157	0.736	0.213	-0.123	0.247	-0.256
Mn	-0.104	-0.130	-0.670	0.349	0.394	0.224	0.161
B	0.184	0.233	0.655	0.491	-0.107	0.233	-0.059
U	0.258	-0.226	0.588	0.094	-0.180	0.368	0.469
Sr	-0.208	0.367	0.457	-0.224	0.439	-0.026	-0.337
Ca	-0.477	0.076	0.376	-0.661	0.224	-0.007	0.093
Cu	0.097	0.346	-0.101	0.585	0.021	0.335	-0.385
Mg	-0.129	0.042	0.369	0.169	0.722	-0.172	0.130
Ba	-0.446	0.098	-0.080	-0.534	0.553	0.228	-0.025
Zn	-0.118	-0.098	-0.073	0.339	0.217	0.616	0.157
Sn	0.380	-0.077	-0.165	-0.272	0.111	0.282	0.493
Tl	-0.378	0.066	0.299	-0.159	-0.216	-0.289	0.432

Table 5. 2: Rotated component matrix showing loadings of analytes in the winter samples

Variables	Components						
	1 (25.1%)	2 (18.8%)	3 (11.0%)	4 (9.7%)	5 (7.7%)	6 (5.9%)	7 (4.8%)
V	0.958	0.039	0.189	0.028	0.111	0.038	-0.064
Li	0.885	-0.105	0.154	0.035	-0.104	0.001	0.004
Pb	0.858	-0.282	-0.074	0.011	-0.055	0.274	0.057
Sb	0.795	-0.051	-0.474	-0.086	-0.058	-0.182	-0.116
La	0.778	0.447	0.245	0.198	0.031	-0.209	-0.139
P	-0.563	0.200	0.425	-0.112	0.464	-0.029	-0.281
Ni	0.522	-0.439	0.087	0.272	0.151	0.200	0.149
Be	-0.038	0.867	0.206	-0.220	0.238	-0.112	0.000
Bi	0.443	-0.809	0.023	0.277	-0.032	0.117	0.146
Cr	0.021	0.795	0.302	0.143	-0.122	-0.087	-0.284
Cs	-0.100	0.746	-0.158	0.168	0.398	-0.082	-0.081
Fe	0.242	0.665	0.620	-0.008	-0.017	-0.235	-0.224
Sn	-0.042	0.637	0.026	-0.126	-0.122	0.235	0.304
Co	0.145	-0.600	-0.222	0.274	0.237	0.178	0.551
Al	-0.045	0.139	0.897	-0.243	0.092	0.043	-0.045
Ti	0.324	0.159	0.833	0.059	0.152	-0.153	-0.140
Sr	0.162	-0.065	0.024	0.797	0.192	-0.156	-0.125
Ba	0.111	0.113	-0.322	0.720	-0.369	0.264	0.066
Ca	0.064	-0.020	-0.366	0.708	-0.109	-0.148	0.444
Si	0.479	-0.337	0.088	0.639	0.274	-0.048	0.216
Mg	-0.224	-0.131	0.462	0.627	0.095	0.238	0.110
Mo	0.335	0.488	0.435	-0.537	0.191	-0.209	0.057
Rb	-0.008	0.104	0.051	0.122	0.876	-0.203	-0.127
B	0.089	-0.084	0.219	0.013	0.873	0.028	-0.038
U	-0.238	0.272	-0.039	-0.149	0.657	0.144	0.503
Mn	-0.042	-0.007	0.152	-0.162	-0.429	0.733	-0.242
Zn	-0.016	0.053	-0.111	-0.030	0.220	0.725	-0.069
Ag	0.071	-0.428	-0.200	0.238	-0.119	0.676	0.199
Cd	0.132	-0.353	0.040	0.124	-0.321	0.577	-0.239
Tl	0.105	-0.303	-0.021	0.078	-0.009	-0.179	0.661
Cu	0.317	-0.112	0.153	-0.129	0.364	0.294	-0.609

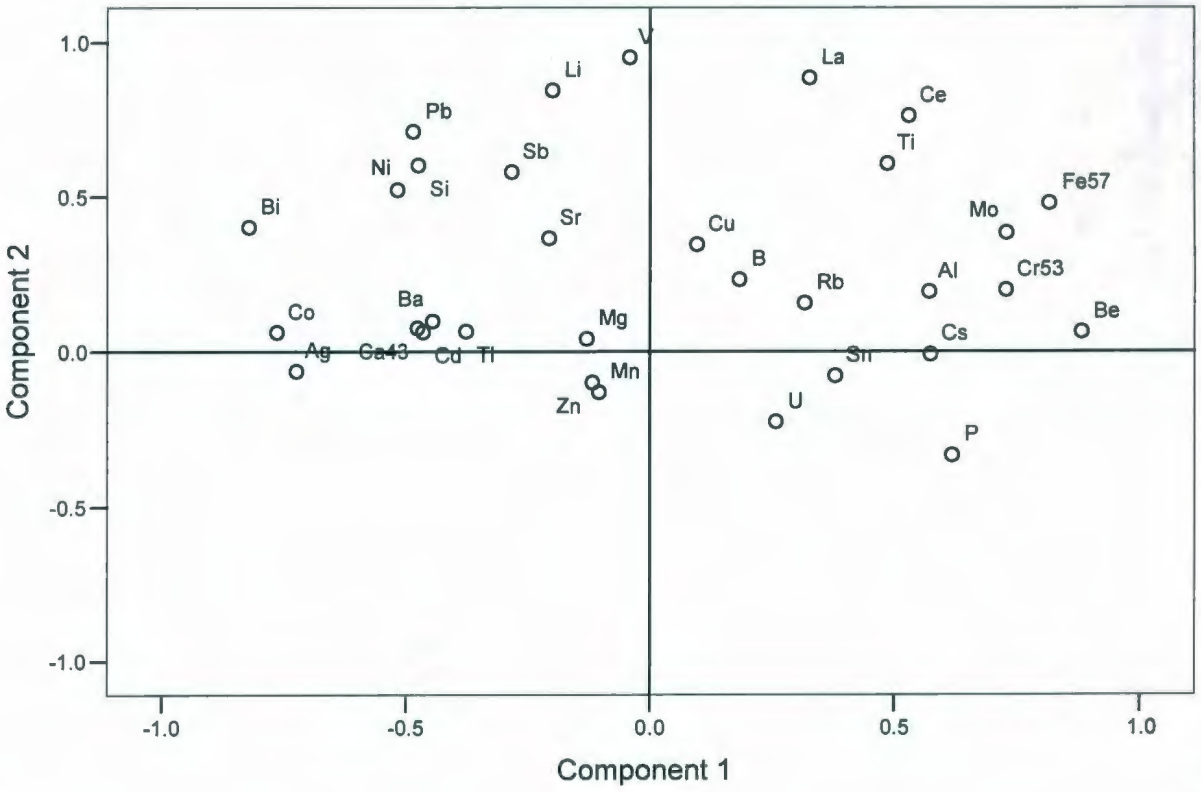

Fig. 5. 2: Unrotated plot of components 1 and 2 of the winter samples.

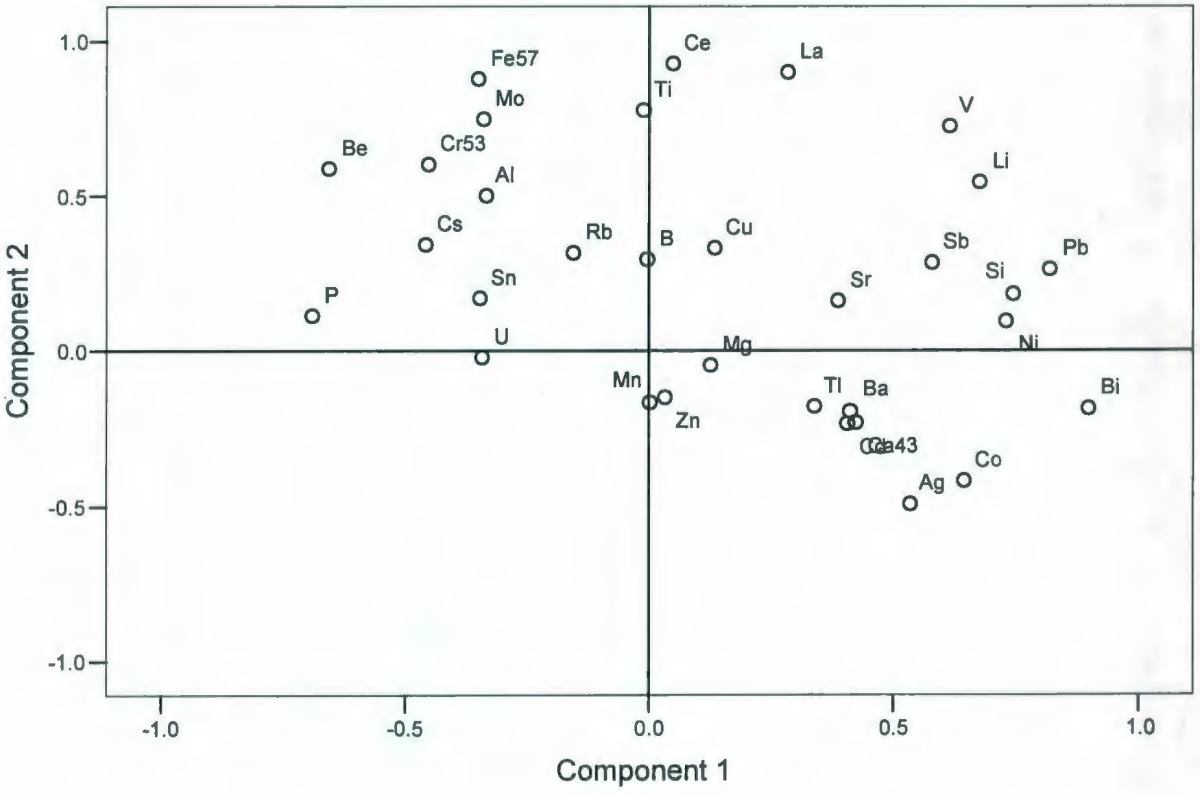

Fig. 5. 3: Rotated plot of components 1 and 2 of the winter samples.

For samples collected in the spring, the principal component analysis using SPSS produced seven unrotated components with eigen values greater than 1 (Table 5.3). The first component loaded high positive loadings for Fe, Ti, La, Al, U, Pb, V, Li, Mo, and Zn. The second component is explained by Cr, Ba, Ca, Rb, Ag, Mn, and Co. The third component loaded Cs, Mg, Cu and Rb; while the fourth component contained Cu and Zn.

The fifth component explains Li, B, and Si. Only Ag was in the sixth component. The seventh component had a total variance of 5.2%, and did not record any high loading for any element. Nickel and strontium recorded very low loadings in all seven components.

The application of varimax rotation to the data set maximised the loadings of individual elements on single factors and also eased the interpretation of the results. This created a new set of a smaller number of composite variables to replace the original set of unrotated variables. The component loadings and percent variance after varimax rotation of each component are presented in Table 5.4. Seven components explaining 83.8% of the total variance in the spring samples resulted. The first rotated component, contributing 29.7% of the total variance is a lithogenic component; it depicts the high influence of La, Fe, Ti, Li, U, V, Pb, and Al. This explains the general chemical weathering of the source rocks. According to the definition of the enrichment factor, the less enriched elements Ce, La, U, V, and Li mainly represent soil contribution. The relatively high U concentration observed in sample SP13 and SP14 could be associated with the granitic bedrock of the area. The rather low to less than detection limit for uranium in most samples attest to the fact that anthropogenic addition may be negligible and therefore concentrations recorded may reflect a U anomaly in such locations.

Lithium is widely distributed in the Earth's crust. It is very mobile in geochemical processes, and readily absorbed by clay minerals (Kabata-Pendias, 2001). The soluble Li in soils is readily available to plants; therefore, the plant content of this element is thought to be a good guide to the Li status of the soil. High Ti content is normally associated with highly weathered soils and soils derived from Ti-rich parent rocks.

Anthropogenic activities which produce effluents or emissions from certain industries (Ti alloys, Ti paint production) may contribute significantly to the bioavailability of Ti in soils and to plants. Levels in plants vary rather considerably within the range of 0.15 to 1500 ppm (DW) (Kabata-Pendias, 2001), and the average concentration in the spruce trees in the study area falls within this range i.e 5.05±0.43 μg/g.

The second rotated component, contributing to15.6 % of the total variance, shows high loadings for Rb, Ca, Mo, and Ag, which primarily represent soil contribution. The third component loaded Ba, Ca, Sr, and Co, contributed to a total variance of 11%. This component relates to contributions from carbonate rocks and soils because of the high mobility and bioavailability of the cations of groups IIA (alkaline earth metals). Chromium, Zn, and Mn showed a strong association with component four (8.8% of the total variance) and is likely a consequence of anthropogenic pollution, including emissions from activities such as use of fungicides, anti-knock agents, batteries, and process from alloy industries. The occurrence of Cr in components three and fouth indicates a mixed source, i.e. lithogenic and anthropogenic inputs. The fifth rotated component loaded Sn, Cu, and Pb and explains 7.2% of the total variance. This component could be anthropogenic with the Pb and Cu may originating from motor vehicle exhaust and leachate from scrap metals (automobiles, fridges, etc) dumped at the study site. Soil additions of Pb, Cu, and Sn can also originate from atmospheric deposition of particulates from the nearby pyrophillite mine. Contributions of Pb therefore have lithogenic and anthropogenic sources as it loaded in components one and five. Component six contributed to 6.7% and has high loadings for Mg, B, and Si; and

could be related to the natural occurrence of these elements in plant composition. Silicon and Ni are also in the seventh component accounting for a total variance of 4.8%.

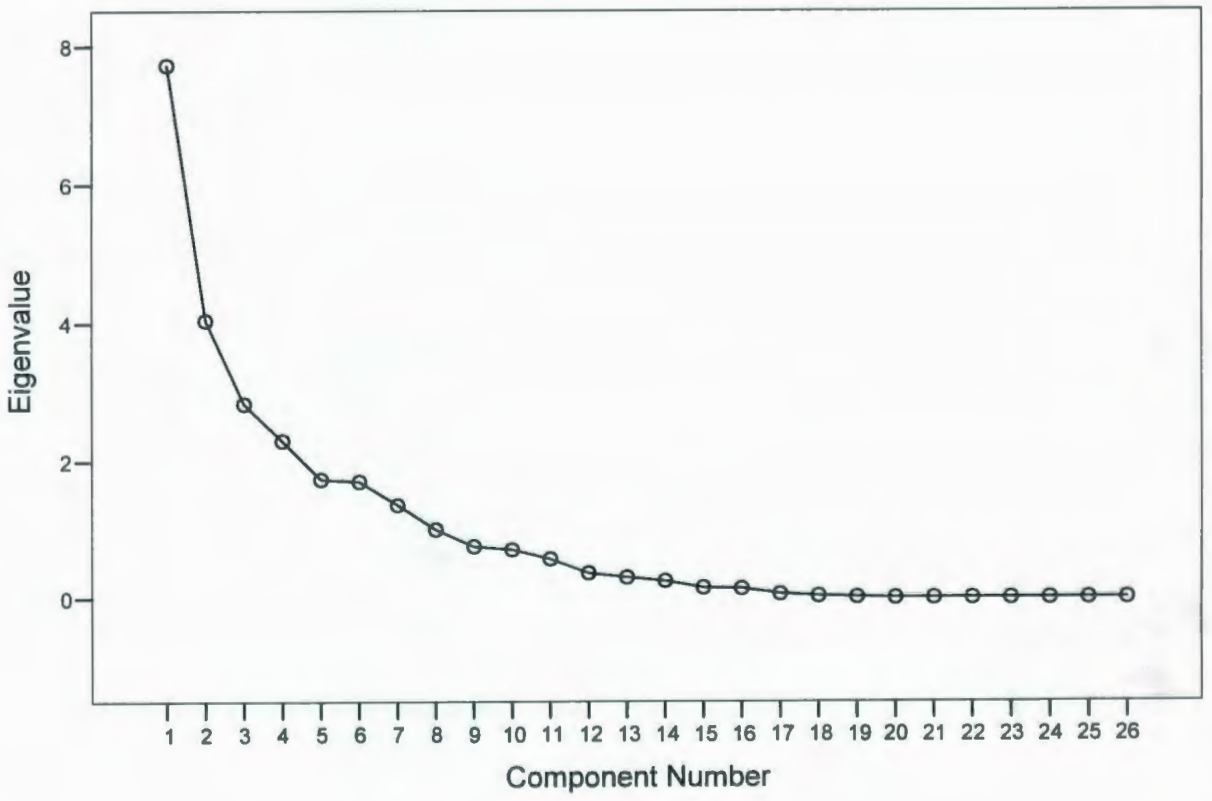

Fig. 5. 4: A scree plot of eigenvalues versus component numbers of the spring samples.

Table 5. 3: Unrotated component matrix showing loading of analytes in the spring samples

Variables	Components						
	1	2	3	4	5	6	7
Fe	0.931	0.002	0.147	-0.141	-0.081	-0.037	-0.067
Ti	0.878	-0.277	0.084	-0.141	-0.152	-0.128	-0.120
La	0.836	0.123	0.062	0.175	-0.025	-0.244	0.302
Al	0.785	-0.313	-0.150	0.075	-0.079	0.198	-0.185
U	0.783	-0.278	0.157	-0.131	-0.119	-0.118	-0.055
Pb	0.727	0.055	-0.424	0.491	0.037	-0.015	0.005
V	0.708	0.311	-0.268	0.221	0.094	-0.303	0.231
Li	0.627	-0.112	0.044	-0.114	-0.535	-0.247	0.192
Mo	0.610	0.606	-0.220	-0.181	0.209	0.080	-0.065
Zn	0.606	-0.160	-0.066	-0.558	0.010	0.236	-0.314
Ni	0.493	-0.065	-0.222	0.265	0.097	0.478	0.473
Cr	-0.091	0.778	0.318	0.158	-0.034	-0.276	-0.080
Ba	0.177	0.723	0.240	0.179	-0.092	0.253	-0.271
Ca	-0.154	0.620	-0.402	-0.148	0.301	-0.184	0.094
Rb	-0.035	-0.618	0.526	0.373	-0.113	0.269	-0.065
Ag	-0.014	0.599	-0.169	-0.323	-0.091	0.529	0.111
Mn	-0.480	0.508	0.303	0.471	-0.091	-0.222	0.100
Co	0.480	0.504	0.330	-0.283	0.167	0.279	0.109
Cs	0.225	0.284	0.655	0.165	-0.039	0.453	0.144
Mg	0.049	-0.382	0.552	0.082	0.429	-0.113	0.461
Sr	0.200	0.305	0.437	0.408	-0.352	0.130	-0.225
Cu	0.110	-0.327	-0.512	0.636	0.191	0.336	-0.065
Sn	0.389	0.144	-0.238	0.483	0.293	-0.054	-0.442
B	0.258	-0.116	0.252	-0.156	0.649	0.134	0.082
Si	0.225	-0.132	0.495	0.003	0.570	-0.240	-0.407

Table 5. 4: Rotated component matrix showing loadings of analytes in the spring samples.

Variables	Components						
	1 (29.7%)	2 (15.6%)	3 (11.0%)	4 (8.8%)	5 (7.2%)	6 (6.7%)	7 (4.8%)
La	0.892	0.101	0.116	-0.082	0.138	0.156	0.177
Fe	0.820	0.006	0.236	0.412	0.075	0.135	-0.015
Ti	0.816	-0.174	-0.003	0.462	0.090	0.068	-0.084
Li	0.804	-0.133	-0.026	0.116	-0.210	-0.284	0.039
U	0.743	-0.204	0.004	0.387	0.025	0.125	-0.063
V	0.735	0.412	0.020	-0.148	0.334	0.048	0.164
Al	0.563	-0.208	0.020	0.533	0.389	-0.014	0.162
Rb	-0.061	-0.915	0.082	0.005	0.056	0.183	0.063
Ca	-0.155	0.812	-0.013	-0.198	0.051	-0.006	-0.011
Mo	0.391	0.675	0.373	0.265	0.204	0.087	0.082
Ag	-0.212	0.504	0.468	0.240	-0.207	-0.200	0.366
Ba	0.028	0.236	0.830	-0.064	0.155	-0.100	-0.080
Cs	0.105	-0.248	0.763	-0.062	-0.154	0.270	0.245
Sr	0.199	-0.290	0.679	-0.185	0.134	-0.179	-0.114
Co	0.291	0.331	0.611	0.228	-0.199	0.331	0.146
Cr	0.045	0.358	0.565	-0.507	-0.065	-0.026	-0.334
Zn	0.328	0.086	0.063	0.856	-0.037	0.074	-0.069
Mn	-0.242	0.033	0.356	-0.807	-0.030	-0.094	-0.154
Sn	0.183	0.127	0.142	0.025	0.805	0.072	-0.153
Cu	-0.116	-0.207	-0.218	0.039	0.797	-0.065	0.441
Pb	0.600	0.123	0.009	0.030	0.697	-0.092	0.287
Mg	0.138	-0.367	-0.151	-0.253	-0.228	0.747	0.138
B	0.045	0.053	0.026	0.220	0.036	0.743	0.079
Si	0.106	-0.138	0.112	0.104	0.198	0.692	-0.538
Ni	0.300	-0.017	0.070	0.112	0.255	0.124	0.801

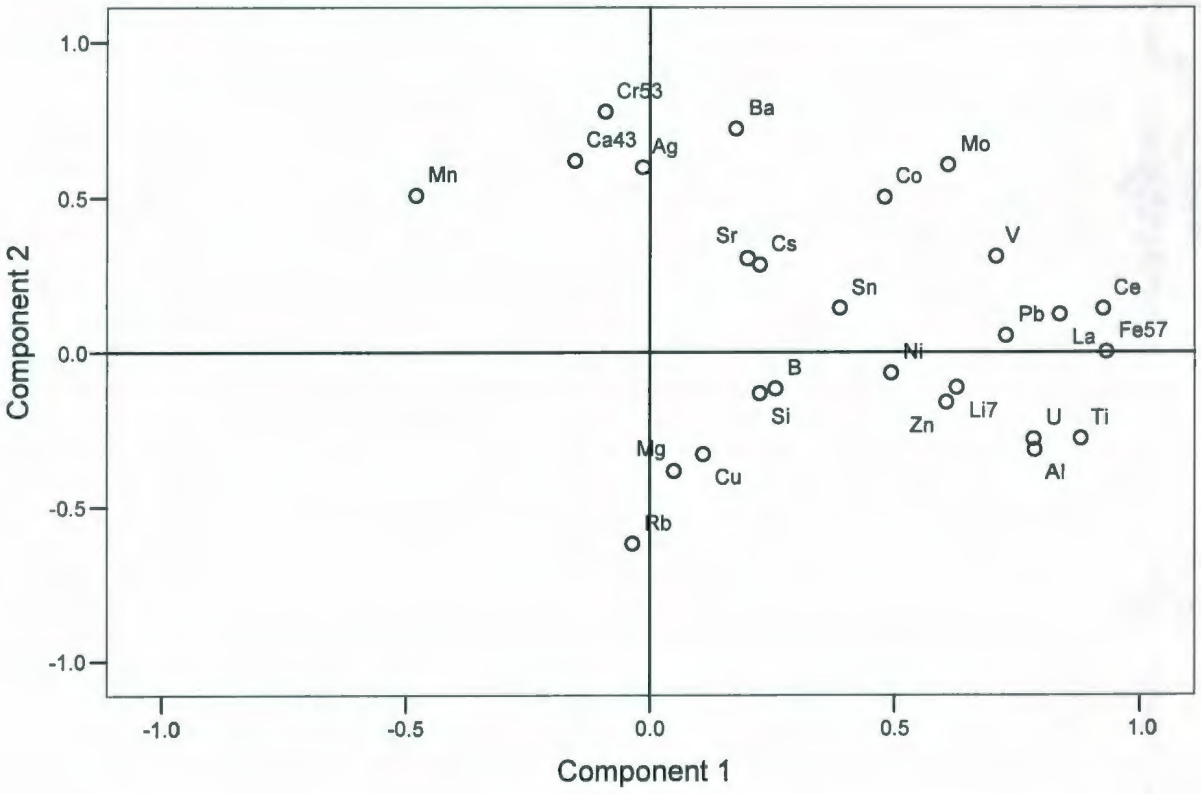

Fig. 5. 5: Unrotated plot of components 1 and 2 of the spring samples.

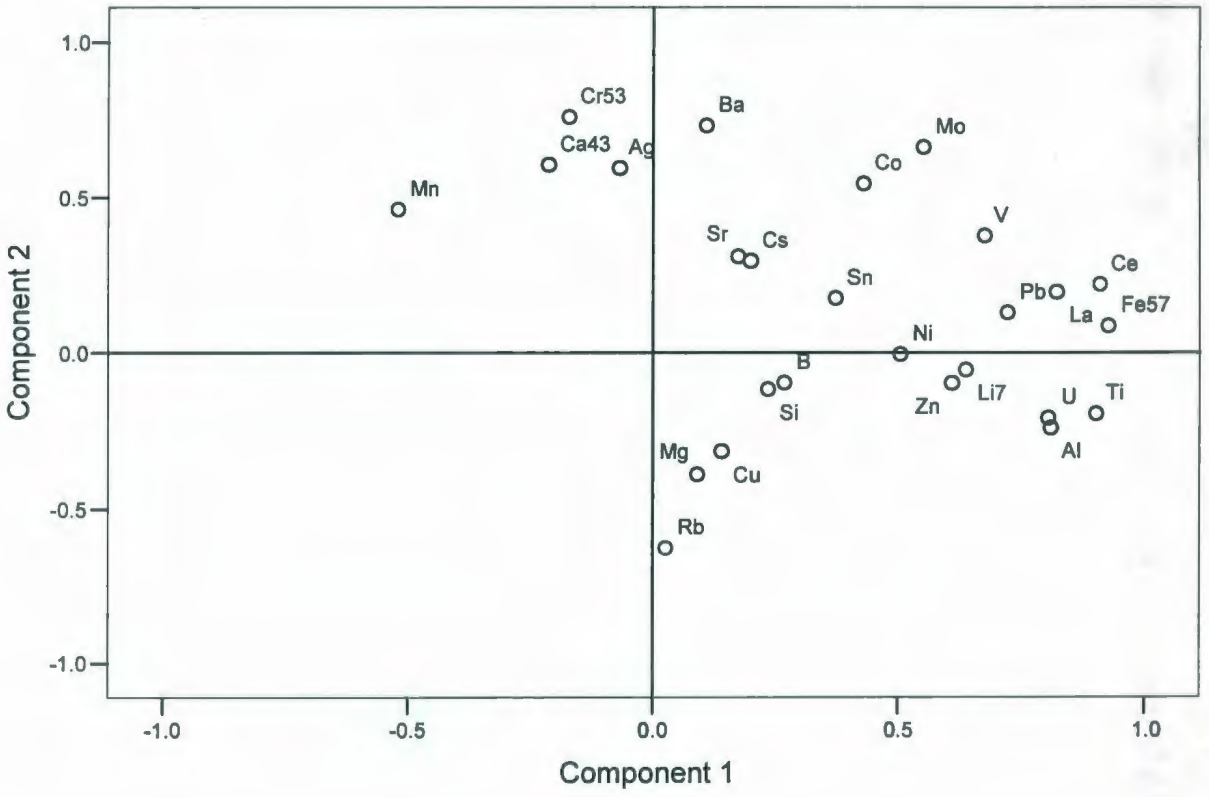

Fig. 5. 6: Rotated plot of components 1 and 2 of PCA analysis of spring samples

Chapter 6: conclusion

6.0 Introduction

The effect of the sample-preparation step on the quality of the analytical results is universally recognized (Novozamsky *et al*, 1995; Mader *et al*, 1996; Mader *et al*, 1997; Mader *et al*, 1998a; Poykio *et al*, 2000; and Hoenig, 2001). The application of appropriate digestion procedure and its effective combination with the separation and detection methods are of major importance in the analysis of trace and heavy metals in biological matrices. The high tendency of losing volatile sample components, the low concentrations to be determined in the varied matrices, and numerous interferences seriously limit the direct application of even highly sensitive and selective spectrometric techniques, *e.g.* ICP-MS, to the examination of the samples. Complete digestion of the biological samples and a quantitative transformation of the analytes into solution are therefore very essential in quantitative determination of trace metals.

The following factors were considered in developing this sample preparation procedure for environmental analysis: the amount of sample, quantities of the elements in the sample, the need for total or partial digestion, and the instrumental methods available for element determination. The efficiency of the procedure as regards low reagent consumption and contamination, low residue generation, the integrity of the sample, and the uncertainty in the measurements were also given a careful consideration.

The application of a wet digestion treatment, widely used for the decomposition of many materials is limited; it is deficient in completely decomposing silicate minerals. This is partly because the maximum digestion temperature cannot exceed the ambient-

pressure boiling point of the acid/acid mixture and also due to the fact that most acids do not efficiently oxidize the biological matrices. Other limitations associated with acid digestions include contaminations arising from use of large volumes of acids, longer periods for digestion, and lower sample throughput.

Dry ashing, though prone to losses via volatilization and reaction with container material; leads to more complete decomposition of the organic matrices and high sample throughput. This study involved the development of a classical dry ashing procedure for digesting matrices of plant origin. The effect of different acid mixtures, type of digestion vessel, temperature of ashing, duration of ashing, and rate of temperature ramp were evaluated for optimum digestion conditions. The study revealed the importance of a digestion vessel in enhancing the rate and completeness of a digestion process. Open (uncapped) test tubes made of quartz proved to be the best vessels for complete decomposition of plant materials in the dry ashing process even at high temperatures. Crucibles, though useful could not supply adequate air to enhance combustion as the open (uncapped) test tubes did, hence less efficient a digestion medium. The suitability of vessels for a digestion procedure was estimated according to the following criteria: heat resistance and conductance, mechanical strength, resistance to acids and alkalis, surface properties, and reactivity and contamination. In addition to the afore-mentioned requirements, vessels for dry ashing must be able to supply adequate air for combustion while minimizing contamination and loss of sample components.

The ashing temperature was evaluated to determine the optimum temperature for complete decomposition of plant material and minimum loss of volatile sample

components. Ashing at 450 °C was most efficient as all samples were completely ashed and at the same time recovered significant quantities of the analytes as compared to ashing at 500 °C. Ashing at 400 °C caused a partial decomposition of the plant samples. Ashing for 8 hours was optimum while ashing for 4 hours resulted in incomplete decomposition and longer duration; 16 hours of ashing caused higher loss of analytes. It can therefore be inferred from this study that high temperature and prolong ashing promote losses of analytes. Hence the ashing temperature and duration of ashing play significant roles in recovery of metals in biological tissues.

In evaluating the effect of temperature ramp on efficiency of digestion and possible retention of volatile sample component, ramping the temperature at a rate 18 °C/hr was observed to be the best with regard to time and cost effectiveness. A temperature ramp at 18 °C/hr also improved element recovery while ramping at 50 °C/hr produced incomplete digestion. The study also revealed that a slower rate enhances efficiency and also reduce loss of volatile sample components during a digestion process. This corroborated the observation by Mader (1996) that losses may be minimized when a moderate charring regime is adopted in a dry ashing procedure. The mechanism by which this occurs is however uncertain. Ramping at a rate of 50 °C/5 hrs produced relatively higher element concentrations, but the difference in concentrations are statistically insignificant in relation to ramping at 50 °C/3 hrs.

On the choice of a leaching acid mixture, HNO_3/HF (2:1) and two drops of H_2O_2 is the most suitable reagent combination for quantitative determination of trace elements in vegetation. The other three reagents (16 N HNO_3, 8 N HNO_3, and 8 N HNO_3/6 N HCl)

exhibited varying degrees of inadequacies in dealing with the lithophile elements. Decomposition of silicates under the hydrofluoric acid treatment is generally required for releasing the silica-bound minerals.

There is no universal sample preparation method, but from the results of the study, washing the plant material thoroughly with nanopure water and subsequent ashing at 450 °C for 8 hours after an initial temperature ramp of 50 °C/3 hrs followed by leaching with a mixture of HNO_3/HF (2:1) plus 2 drops of H_2O_2 proved to be a powerful and a fast method of sample preparation. The advantage for this dry ashing procedure is its ability to digest large samples and to perform total elemental analysis including major, minor and trace element in the same run. Moreover, this method is less time consuming, requires less amounts of reagents and it is easy to control. It is useful in environmental studies where conclusions are drawn on the basis of a large number of samples.

Although there was generally high recovery for most heat stable elements, volatile elements such as Se, Hg, As, and I were completely lost or partially as a result of the high ashing temperature. The precision was also poor for Cd resulting in relatively high RSD. The proposed procedure produced very accurate and precise results when applied to determine metal concentrations in tea, coffee, and spruce twigs. The relative standard deviation of heavier elements were <10% while Ni, Mo, Co and most light elements were <15%. The procedure was validated by the use of standard reference materials SRM 1575, SRM 1547, CLV-1, and CLV-2. Element recoveries were in the range of 75 – 120%. A comparison of the proposed procedure with the wet digestion method used by the ICP-MS group at MUN also yielded good agreement in the mean recoveries.

Biomonitoring

The proposed procedure was applied in a biomonitoring study using black spruce as indicator of metal pollution in the Holyrood area. Knowledge of the source of the trace metals and their relative concentrations is necessary for taking effective control measures to prevent pollution. The metal concentrations determined using ICP-MS and PCA analysis of the chemical data revealed varied sources of pollution within the study area. The advantage of the PCA is that it reduces a large number of variables into a smaller set of dimensions by analyzing the inter-relationships between variables (elements) and explains them in terms of common underlying factors.

A multivariate statistical analysis produced seven principal components of the element data from samples collected both in winter and spring. Each set of samples had contributions from anthropogenic and geogenic sources. The rotated components interpreted metal sources including crustal weathering, the source rock lithology (bedrock), sea spray, atmospheric deposition of soil particles, emission from the thermal electric generation plant and vehicular exhaust, leaching from scrap, electronic, and municipal waste dumped at the study site. The study however did not show any clear seasonal variation in trace metal concentrations as the concentrations varied randomly with season and from one sample to the other. The reason for this pattern may be attributed to the long winters and diffused climate pattern in Newfoundland. The high U concentrations recorded in a few of the samples were indicative of a U anomaly in such as areas and a confirmation of earlier observation that black spruce is an excellent indicator of U anomaly.

6.1 Future work

Future work should include analysis of both soil and plant samples in order to establish a correlation or otherwise between plant and soil metal concentration. Also a comparative study involving samples obtained from highly polluted sites and others from pristine sites (areas of low industrial activity) to assess the usefulness of black spruce of Newfoundland as indicators of pollution. Future work on the seasonal variation in metal uptake by spruce tree should include samples taken in all four seasons.

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Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P1		P2		P3		P4	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	%
Li	0.082	2	0.047	9	0.046	5	0.058	14
Be	0.005	4	<DL		<DL		0.004	1
B	11.0	8	10.1	1	7.9	3	9.4	4
Mg	700	8	780	7	620	17	710	6
Al	174	6	59	12	64	8	164	8
Si	31	31	28.2	8	24.0	7	17.3	9
P	600	5	610	3	670	4	460	6
Ca	2410	8	3900	2	3100	3	2820	4
Ti	8.5	2	4.7	11	4.0	2	6.5	18
V	6.0	1	0.97	3	1.03	1	2.28	1
Cr	1.32	11	0.82	17	0.50	5	0.36	7
Mn	1070	9	590	13	520	9	610	17
Fe	106	10	53	12	55	7	82	9
Co	1.90	23	1.29	4	0.89	3	2.07	4
Ni	6.8	7	4.3	1	2.68	1	4.5	8
Cu	7.0	3	5.0	2	5.9	4	4.7	2
Zn	45	4	43	2	45	3	40	5
Rb	4.6	26	6.3	2	5.9	1	3.2	1
Sr	20.0	4	21.0	2	23.2	1	20.2	1
Mo	0.128	4	0.002		<DL		<DL	
Ag	0.061	9	0.044	1	0.044	2	0.033	6
Cd	0.119	2	0.042	14	0.045	3	0.040	5
Sn	0.176	6	0.121	1	0.130	16	0.113	10
Sb	0.175	5	0.039	5	0.058	3	0.053	10
Cs	<DL		0.035	8	0.044	2	<DL	
Ba	51	3	50	5	68	1	45	1
La	1.51	9	0.37	1	0.46	1	0.51	4
Ce	0.40	7	0.140	4	0.146	2	0.243	5
Tl	0.009	3	0.009	2	DL		0.02	2
Pb	1.04	11	0.59	2	0.56	4	0.99	3
Bi	0.003	2	0.001	34.5	0.002	10	0.003	3
U	0.008	7	0.009	13	0.004	23	0.005	17

Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P5		P6		P7	
	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%
Li	0.052	7	0.046	8	0.032	11
Be	<DL		<DL		<DL	
B	8.6	5	7.7	1	7.5	1
Mg	930	7	440	25	860	13
Al	70	5	162	1	102	3
Si	21.5	18	12.7	11	13.8	21
P	740	6	740	9	660	9
Ca	2340	4	1570	4	2230	2
Ti	6.2	1	5.8	5	3.8	13
V	1.47	3	0.93	9	0.53	11
Cr	3.0	14	0.37	21	0.58	23
Mn	1310	4	940	9	1350	10
Fe	83	9	57	7	51	17
Co	0.98	15	0.55	10	0.56	9
Ni	6.9	12	2.86	39	2.62	16
Cu	5.1	4	5.2	7	5.5	7
Zn	52	5	48	11	43	11
Rb	3.5	1	2.86	4	2.21	3
Sr	22.5	2	11.3	2	15.7	1
Mo	<DL		<DL		<DL	
Ag	0.032	1	0.025	10	0.070	4
Cd	0.066	3	0.033	5	0.102	3
Sn	0.132	2	0.146	5	0.116	7
Sb	0.053	4	0.034	8	0.024	10
Cs	<DL		<DL		<DL	
Ba	66	3	18.3	3	50	4
La	0.39	5	0.203	9	0.187	2
Ce	0.165	5	0.101	13	0.078	14
Tl	0.012	1	0.02	4	0.01	4
Pb	0.66	2	0.63	9	0.48	6
Bi	0.002	5	0.001	8	0.001	19
U	0.005	22	0.009	6	0.003	28

Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P8		P9		P10		P11	
Element	MEAN ppm	RSD %	MEAN ppm	RSD %	MEAN ppm	RSD %	MEAN ppm	RSD %
Li	0.036	15	0.051	9	0.40	11	0.046	15.3
Be	<DL		<DL		<DL		<DL	
B	9.2	1	7.0	2	75	6	8.6	6
Mg	750	27	590	5	4800	9	790	11
Al	96	2	76	2	560	3	96	1
Si	12.3	13	11.0	9	144	29	20.6	3
P	800	7	590	5	6800	3	540	3
Ca	2350	3	2570	5	29900	2	3300	2
Ti	2.30	5	2.39	6	24.5	7	4.0	3
V	0.40	7	0.55	5	5.8	1	1.32	2
Cr	1.03	14	0.43	9	4.8	29	0.80	17
Mn	1160	13	1300	18	7700	16	1340	23
Fe	43	11	43	13	370	19	55	15
Co	0.45	9	0.82	3	38	17	1.45	2
Ni	3.2	32	3.0	9	27.4	13	16.2	8
Cu	4.9	6	4.7	4	54	5	4.9	2
Zn	47	11	41	9	450	5	53	4
Rb	4.8	2	2.27	6	14.8	6	2.70	2
Sr	17.7	1	13.5	3	246	2	17.7	2
Mo	<DL		<DL		<DL		<DL	
Ag	0.036	2	0.055	10	0.31	2	0.07	2
Cd	0.084	16	0.091	6	0.62	5	0.08	3
Sn	0.132	8	0.19	3	1.92	7	0.2	9
Sb	0.018	2	0.026	8	0.22	4	0.034	2
Cs	<DL		<DL		<DL		<DL	2
Ba	44	2	42	3	670	2	57	3
La	0.143	7	0.169	10	1.99	5	0.35	9
Ce	0.057	15	0.068	17	0.77	2	0.17	10
Tl	0.023	1	<DL		0.08	7	0.021	3
Pb	0.35	6	0.44	8	5.0	2	1.02	1
Bi	0.001	18	0.001	22	0.01	6	0.002	12
U	0.002	22	0.006	13	0.005	10	0.004	5

Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P12		P13		P14		P15	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	%
Li	0.049	9	0.032	9	0.039	12	0.034	5
Be	<DL		0.028	7	0.03	1	0.025	11
B	4.1	7	10.1	3	9.7	2	9.4	5
Mg	750	4	760	10	560	15	1010	12
Al	67	2	65	6	83	3	560	1
Si	17.8	6	12.9	16	13.7	21	13.6	13
P	440	2	680	9	910	3	1330	4
Ca	4000	1	2930	4	2480		2240	6
Ti	3.1	12	2.49	12	3.0	3	12.6	3
V	0.79	1	0.57	7	0.67	8	0.45	4
Cr	1.00	11	1.01	15	1.16	13	3.0	2
Mn	1420	4	670	6	800	10	1030	5
Fe	49	13	52	6	60	6	159	4
Co	1.46	1	1.57	5	0.97	3	0.271	3
Ni	2.24	17	1.99	12	2.05	30	1.71	10
Cu	4.2	2	4.4	4	5.2	7	5.2	4
Zn	45	6	50	4	48	6	47	3
Rb	1.18	2	5.8	5	6.2	2	4.2	3
Sr	16.7	1	15.9	5	16.5	3	19.5	2
Mo	<DL		0.03	10	0.03	20	0.052	17
Ag	0.104	3	0.04	7	0.03	6	0.027	10
Cd	0.254	1	<DL		0.13	4	0.053	14
Sn	0.166	7	0.18	17	0.17	10	0.192	6
Sb	0.025	1	0.022	19	0.026	23	<DL	
Cs	<DL		0.051	10	0.08	7	0.023	6
Ba	101	2	49	7	41	7	36	3
La	0.272	3	0.227	8	0.24	5	0.28	4
Ce	0.126	6	0.109	10	0.11	8	0.23	4
Tl	0.038	1	0.034	6	0.006	6	<DL	
Pb	0.64	2	0.35	6	0.49	8	0.254	2
Bi	0.001	42.	<DL		<DL		<DL	
U	0.004	28	0.083	1	0.111	3	0.02	6

Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P16		P17		P18		P19	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	%
Li	0.04	2	0.04	7	0.05	7	0.05	8
Be	0.02	2	0.02	13	0.02	2	0.02	3
B	9.2	5	9.2	6	7.6	2	7.3	5
Mg	570	4	570	7	610	2	620	12
Al	127	5	127	4	103	3	116	2
Si	9.1	12	9.1	14	15.3	5	14.4	29
P	750	4	750	7	610	4	900	5
Ca	1970	2	1970	4	2440	2	2670	1
Ti	4.7	6	4.7	9	4.2	2	7.0	4
V	1.50	4	1.50	4	0.84	3	1.03	2
Cr	2.97	5	2.97	5	5.8	4	5.6	3
Mn	1760	5	1760	15	1070	13	570	11
Fe	104	5	104	4	148	2	158	2
Co	0.10	4	0.10	5	0.11	5	0.12	5
Ni	2.11	12	2.11	5	1.53	6	3.3	4
Cu	6.8	4	6.8	2	4.4	2	5.0	2
Zn	47	5	47	3	38	1	37	4
Rb	3.0	2	3.0	2	2.78	2	4.1	2
Sr	15.9	1	15.9	3	17.1	2	17.7	2
Mo	0.05	3	0.05	3	0.06	1	0.07	5
Ag	0.03	5	0.03	3	0.02	6	<DL	
Cd	0.05	9	0.05	7	0.004		0.004	27
Sn	0.16	5	0.16	4	0.2	7	0.18	5
Sb	0.04	13	0.04	9	0.05	11	0.05	1
Cs	0.02	2	0.02	10	0.04	6	0.03	4
Ba	38	2	38	4	81	2	31	4
La	0.46	1	0.46	3	0.76	17	0.62	21
Ce	0.19	3	0.19	4	0.46	12	0.36	8
Tl	<DL		<DL		0.01	18	0.02	1
Pb	0.62	6	0.62	2	0.42	9	0.4	5
Bi	<DL		<DL		<DL		<DL	
U	0.004	18	0.004	19	0.005	20	0.004	28

Appendix 1: Element concentrations in spruce twigs collected in winter.

Sample	P20	
	MEAN	RSD
Element	ppm	%
Li	0.042	2
Be	0.0160	11
B	6.3	8
Mg	490	3
Al	124	3
Si	10.5	7
P	530	2
Ca	2840	1
Ti	5.9	3
V	1.43	1
Cr	3.6	3
Mn	610	6
Fe	119	5
Co	0.141	4
Ni	2.12	2
Cu	4.2	2
Zn	50	2
Rb	2.63	1
Sr	18.4	1
Mo	0.051	6
Ag	0.022	5
Cd	0.050	10
Sn	0.164	14
Sb	0.072	12
Cs	0.026	7
Ba	39	1
La	0.79	2
Ce	0.259	1
Tl	<DL	
Pb	0.49	4
Bi	<DL	
U	0.003	13

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P1		P2		P3		P4	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	%
Li	0.060	11	0.050	16	<DL		0.06	13
B	7.6	6	7.6	2	6.3	6	5.6	6
Mg	650	11	550	4	720	1	560	1
Al	330	7	145	5	81	7	174	2
Si	17.0	11	26.0	4	14.5	11	21.5	4
Ca	3200	4	2400	2	2400	1	3000	3
Ti	5.9	1	4.9	2	1.93	11	5.8	4
V	2.03	5	0.89	1	0.250	3	1.74	3
Cr	13.7	10	6.0	3	7.7	5	13.3	7
Mn	1700	3	740	3	1700	2	2380	2
Fe	201	3	133	1	72	3	155	4
Co	0.35	4	0.14	5	0.28	6	0.37	6
Ni	1.89	3	2.48	19	1.57	18	2.67	14
Cu	3.7	2	5.6	3	4.5	9	4.3	8
Zn	47	1	57	2	40	4	58	2
Rb	2.07	2	3.9	1	4.9	7	1.88	2
Sr	13.0	13	12.0	9	13.0	3	28.0	14
Mo	0.1	6	0.06	13	<DL		0.08	16
Ag	0.06	9	0.07	22	0.04	17	0.07	11
Sn	0.16	16	0.19	7	0.14	5	0.15	19
Cs	0.05	7	0.07	11	0.05	15	0.06	12
Ba	66	1	90	1	41	7	149	3
La	0.59	3	0.64	3	0.2	4	0.81	5
Ce	0.35	9	0.24	3	0.09	16	0.31	7
Pb	0.60	2	0.57	3	0.27	3	0.76	9
U	0.01	12	0.05	9	<DL		<DL	

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P5		P6		P7	
Element	MEAN	RSD	MEAN	RSD	MEAN	RSD
	ppm	%	ppm	%	ppm	%
Li	0.06	13	0.04	7	0.04	5
B	5.7	9	5.5	9	6.8	8
Mg	490	6	550	1	730	2
Al	140	5	85	2	117	3
Si	18.5	8	0.07	3	10.0	11
Ca	2950	2	2070	1	1980	1
Ti	5.4	8	2.69	7	4.5	3
V	1.22	4	0.54	1	0.39	4
Cr	12.8	2	5.3	4	6.0	1
Mn	1810	1	1030	1	1330	1
Fe	161	12	71	1	84	2
Co	0.15	5	0.14	8	0.12	8
Ni	1.93	7	2.36	14	2.00	15
Cu	3.6	3	3.4	1	4.0	7
Zn	44	3	46	2	56	2
Rb	2.03	2	6.0	1	2.90	1
Sr	17.5	4	17.0	4	10.5	7
Mo	0.06	13	<DL		<DL	
Ag	0.05	16	0.03	28	0.03	28
Sn	0.11	13	<DL		<DL	
Cs	<DL		0.03	3	<DL	
Ba	50	3	46	7	20.7	2
La	0.55	3	0.29	5	0.28	5
Ce	0.220	10	0.1	7	0.11	7
Pb	0.59	1	0.29	4	0.32	4
U	0.070	8	<DL		<DL	

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P8		P9		P10		P11	
Element	MEAN ppm	RSD %	MEAN ppm	RSD %	MEAN ppm	RSD %	MEAN ppm	RSD %
Li	0.05	1	0.11	7	0.11	14	0.04	20
B	7.6	7	7.1	4	7.1		8.0	10
Mg	600	1	690	1	690	1	680	1
Al	310	11	610	6	610	1	112	1
Si	11.0	10	18.1	7	18.1	5	14.0	5
Ca	1550	8	550	2	550	1	1970	1
Ti	7.0	3	9.9	4	9.9	4	4.1	6
V	0.65	5	0.76	4	0.76	6	0.85	1
Cr	6.2	3.	6.8	2	6.8	3	6.5	2
Mn	930	9	1270	2	1270	3	1430	1
Fe	134	12	226	4	226	6	83	1
Co	0.13	9	0.26	3	0.26	9	0.07	5
Ni	1.71	8	3.9	11	3.9	11	2.06	1
Cu	4.0	2	8.4	2	8.4	1	8.1	5
Zn	44	4	48	5	48	2	43	3
Rb	3.2	3	7.2	4	7.2	1	3.6	1
Sr	11.7	5	14.0	1	14.0	5	11.0	4
Mo	<DL		<DL		<DL		<DL	
Ag	0.03	3	0.06	4	0.06	20	<DL	
Sn	0.1	6	0.1	7	0.10	12	0.120	5
Cs	<DL		0.05	8	0.05		<DL	
Ba	28.1	3	42	3	42	1	36	1
La	0.45	5	0.69	4	0.69	3	0.45	2
Ce	0.2	13	0.32	2	0.32	6	0.140	10
Pb	0.39	2	0.86	5	0.86	2	0.78	3
U	<DL		<DL		<DL		<DL	

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P12		P13		P14		P15	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	%
Li	0.03	13	0.06	12	0.05	15	0.1	14
B	6.9	14	8.6	6	8.2	3	7.2	1
Mg	880	1	640	9	680	8	530	5
Al	88	2	360	4	297	2	2250	2
Si	15.0	9	30.0	4	25.0	6	18.0	8
Ca	3100	1	2150	2	1690	2	2040	11
Ti	3.3	14	6.1	1	5.5	4	20.5	2
V	0.84	2	1.55	8	1.36	1	1.16	1
Cr	4.6	4	5.5	2	4.6	2	3.3	1
Mn	920	2	1110	3	790	3	173	3
Fe	75	2	118	5	103	8	350	2
Co	0.1	7	0.11	7	0.11	3	0.24	3
Ni	3.3	5	2.00	10	2.37	2	2.79	1
Cu	7.3	1	7.0	9	5.3	7	6.0	2
Zn	43	2	48	2	39	3	140	2
Rb	4.7	1	3.8	2	4.7	1	2.46	4
Sr	16.0	3	14.0	2	13.0	2	12.0	1
Mo	<DL		<DL	5	<DL		0.11	4
Ag	0.05	16	<DL		<DL		0.05	16
Sn	0.15	11	0.26	26	0.16	14	0.18	8
Cs	0.02	14	<DL		<DL		<DL	
Ba	37	4	37	7	27.7	3	34	3
La	0.41	7	1.07	4	0.34	4	0.54	5
Ce	0.13	5	0.27	10	0.16	5	0.36	2
Pb	0.50	5	0.89	6	0.49	2	0.83	3
U	<DL		<DL		<DL	7	0.01	15

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P16		P17		P18		P19	
	MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
Element	ppm	%	ppm	%	ppm	%	ppm	
Li	0.08	9	<DL		0.03		0.03	20
B	6.4	8	6.9	7	6.93	1	6.9	6
Mg	730	2	540	1	500.4	2	500	15
Al	1440	3	490	1	523	5	520	6
Si	197	17	13.0	7	16.50	13	16.5	9
Ca	660	7	2830	1	2689.5	3	2690	1
Ti	13.3	1	3.9	2	3.54	1	3.5	4
V	0.45	2	1.23	4	1.11	3	1.11	3
Cr	6.3	1	7.2	2	4.93	2	4.9	2
Mn	1130	1	1400	2	1029.7	5	1030	2
Fe	234	2	102	4	76.5	11	77	8
Co	0.12	6	0.14	10	0.09	8	0.09	6
Ni	2.14	8	3.7	9	1.47	1	1.47	8
Cu	5.7	1	12.2	2.	6.63	3	6.6	7
Zn	60	1	44	2	46.23	2	46	1
Rb	20.3	3	4.5	1	5.0	1	5.0	2
Sr	25.5	3	14.0	3	20.0	3	20.0	6
Mo	<DL		0.06	6	<DL		<DL	
Ag	<DL		0.03	8	0.04	5	0.04	13
Sn	0.08	11	0.16	14	0.20	14	0.20	3
Cs	0.07	10	<DL		0.04	20	0.04	
Ba	47	2	34	1	49.44	2	49	1
La	0.53	1	0.29	2	0.27	3	0.27	17
Ce	0.23	6	0.13	4	0.14	9	0.14	16
Pb	0.56	10	0.99	2	0.70	1	0.70	4
U	0.01	13	<DL	15	<DL	7	<DL	

Appendix 1: Element concentrations in spruce twigs collected in spring.

Sample	P20	
	MEAN	RSD
Element	Ppm	%
Li	0.19	14
B	8.8	9
Mg	650	3
Al	2880	2
Si	0.07	8
Ca	2540	5
Ti	9.9	5
V	4.2	3
Cr	4.8	2
Mn	770	3
Fe	269	24
Co	0.21	3
Ni	4.0	3
Cu	7.4	6
Zn	48	4
Rb	3.4	2
Sr	15.7	4
Mo	0.06	16
Ag	0.03	10
Sn	0.2	12
Cs	0.03	6
Ba	37	2
La	1.96	2
Ce	0.57	14
Pb	1.58	10
U	0.01	16

Appendix 3: Pearson's R correlation coefficients between element concentrations in spruce twigs

	Li 7	Be	B	Mg	Al	Si	P	Ca	Ti
Be	-0.341								
B	0.187	0.191							
Mg	-0.184	-0.078	0.270						
Al	-0.153	0.301	0.237	0.411					
Si	0.634	-0.432	0.383	0.316	-0.095				
P	-0.428	0.493	0.319	0.295	0.707	-0.242			
Ca	0.091	-0.260	-0.188	0.163	-0.317	0.470	-0.432		
Ti	0.196	0.212	0.281	0.381	0.849	0.220	0.534	-0.258	
V	0.821	-0.146	0.391	-0.015	0.064	0.599	-0.268	-0.075	0.392
Cr	-0.041	0.607	-0.183	-0.021	0.186	-0.242	0.278	-0.249	0.344
Mn	-0.026	-0.089	-0.255	0.177	-0.001	-0.288	-0.056	-0.353	-0.129
Fe	0.094	0.610	0.032	0.092	0.567	-0.109	0.430	-0.314	0.719
Co	0.224	-0.397	0.162	-0.069	-0.231	0.383	-0.327	0.444	-0.212
Ni	0.367	-0.393	0.220	0.276	-0.109	0.467	-0.265	0.222	0.042
Cu	0.357	-0.050	0.379	-0.066	0.110	0.232	0.184	-0.437	0.224
Zn	-0.250	-0.036	0.082	0.149	0.027	-0.076	0.069	-0.139	-0.039
Rb	0.003	0.302	0.706	0.181	0.040	0.382	0.352	0.084	0.105
Sr	0.161	-0.229	0.220	0.238	-0.000	0.554	0.009	0.426	0.182
Mo	0.505	0.222	0.245	-0.261	0.334	0.029	-0.023	-0.426	0.514
Ag	0.144	-0.507	-0.197	0.304	-0.152	0.282	-0.412	0.425	-0.276
Cd	0.181	-0.312	-0.374	0.141	-0.126	0.183	-0.261	0.352	-0.205
Sn	-0.025	0.450	-0.158	-0.199	0.187	-0.216	0.199	-0.021	0.063
Sb	0.814	-0.098	0.255	-0.180	-0.107	0.580	-0.330	-0.039	0.273
Cs	-0.257	0.650	0.266	-0.109	-0.102	0.007	0.201	0.201	-0.134
Ba	0.095	-0.234	-0.430	0.190	-0.352	0.300	-0.450	0.554	-0.341
La	0.709	0.118	0.224	-0.116	0.079	0.492	-0.211	-0.041	0.436
Ce	0.497	0.390	0.106	-0.033	0.254	0.263	0.006	-0.080	0.529
Tl	0.059	-0.133	-0.140	0.146	-0.249	0.109	-0.290	0.416	-0.256
Pb	0.738	-0.437	0.158	-0.017	-0.180	0.532	-0.573	0.130	0.091
Bi	0.693	-0.722	0.244	0.232	-0.120	0.692	-0.456	0.233	0.103
U	-0.327	0.596	0.391	-0.031	-0.028	-0.137	0.296	-0.009	-0.177

	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Rb
Cr	-0.026								
Mn	0.003	0.092							
Fe	0.211	0.871	-0.027						
Co	0.233	-0.571	-0.286	-0.504					
Ni	0.322	-0.200	0.130	-0.168	0.252				
Cu	0.528	-0.058	0.374	0.085	0.001	0.061			
Zn	-0.006	-0.215	0.356	-0.257	0.002	0.356	0.163		
Rb	0.090	-0.082	-0.496	-0.001	-0.106	-0.058	0.143	0.025	
Sr	0.205	-0.029	-0.397	0.022	0.529	0.168	0.110	-0.028	0.206
Mo	0.583	0.196	-0.155	0.478	-0.054	-0.087	0.214	-0.237	-0.008
Ag	0.121	-0.584	0.380	-0.551	0.268	0.312	-0.009	0.147	-0.286
Cd	0.132	-0.319	0.372	-0.340	0.186	0.069	-0.033	0.101	-0.308
Sn	0.004	0.380	0.153	0.352	0.031	0.114	-0.058	0.011	-0.247
Sb	0.936	0.122	-0.074	0.257	0.091	0.193	0.482	-0.092	0.136
Cs	-0.223	0.216	-0.339	0.138	-0.201	-0.003	-0.142	0.096	0.609
Ba	-0.026	0.022	0.222	-0.179	0.302	0.058	-0.179	-0.014	-0.286
La	0.870	0.380	-0.120	0.536	-0.016	0.150	0.361	-0.183	0.092
Ce	0.538	0.716	-0.154	0.828	-0.219	0.033	0.091	-0.415	0.019
Tl	-0.058	-0.226	-0.124	-0.306	0.311	0.173	-0.465	-0.044	-0.078
Pb	0.706	-0.288	0.169	-0.144	0.365	0.675	0.334	0.182	-0.159
Bi	0.590	-0.508	-0.066	-0.336	0.501	0.628	0.144	0.029	-0.027
U	-0.155	-0.151	-0.268	-0.153	0.090	-0.189	-0.137	0.202	0.545

	Sr	Mo	Ag	Cd	Sn	Sb	Cs	Ba	La
Mo	-0.202								
Ag	-0.139	-0.356							
Cd	-0.057	-0.272	0.775						
Sn	-0.200	0.007	-0.032	0.028					
Sb	0.210	0.490	0.021	0.095	0.009				
Cs	-0.014	-0.149	-0.287	-0.212	0.242	-0.110			
Ba	0.369	-0.387	0.447	0.543	0.090	0.035	-0.056		
La	0.224	0.564	-0.125	-0.021	0.171	0.934	-0.010	0.044	
Ce	0.139	0.583	-0.350	-0.198	0.335	0.608	0.125	0.068	0.830
Tl	-0.164	0.028	0.381	0.319	0.003	-0.156	-0.088	0.270	-0.179
Pb	0.142	0.405	0.318	0.218	-0.142	0.570	-0.212	0.142	0.460
Bi	0.365	0.199	0.399	0.239	-0.362	0.448	-0.437	0.171	0.280
U	-0.171	-0.044	-0.098	0.022	0.169	-0.183	0.687	-0.172	-0.202

	Ce	Tl	Pb	Bi
Tl	-0.110			
Pb	0.198	0.141		
Bi	0.015	0.218	0.788	
U	-0.199	0.166	-0.261	-0.327

Appendix 4: Glossary of terms

1. Bioindicator - a bioindicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or part of an environment) (Market *et al.* 1999).

2. Biomonitor - a biomonitor is an organism (or part of an organism or a community) that contains information on the quantitative aspect of the quality of the environment. (Market *et al*, 1999).

3. Dry ashing is an oxidative process in which organic matter is oxidized by reaction with gaseous oxygen, generally with the application of energy in some form (Gorsuch T.T., 1970).

4. Wet ashing/Acid digestion refers to an oxidation process where the organic part of the sample is oxidized in a liquid phase by the application of energy. The oxidants are usually strong acids (such as sulpheric acid, nitric acid, perchloric acid) in different combinations and proportions (Novozamsky *et al.*, 1995). Aside from the mineral acids, other reagents such as hydrogen peroxide, potassium peroxide sulphate, boric acid and many more are employed.

5. Microwave digestion is an acid digestion procedure where samples are heated directly by absorption of microwave radiation from digestion equipment heated by microwaves.

6. Trace metals are metals in a sample that are present in small concentrations usually in average concentrations of less than 100 parts per million atoms or less than 100 micrograms per gram.

7. Volatile elements are chemical elements that condense (or volatilize) at relatively low temperatures. Volatile elements can be divided into moderately volatile (T_c = 640-1230 K) and highly volatile (T_c < 640 K). Moderately volatile lithophile elements are Mn, P, Na, B, Rb, K, F, and Zn. Moderately volatile siderophile and chalcophile elements are Au, Cu, Ag, Ga, Sb, Ge, Sn, Se, Te, and S. Highly volatile lithophile and atmophile elements are Cl, Br, I, Cs, Tl, H, C, N, O, He, Ne, Ar, Kr, and Xe. Finally, highly volatile siderophile and chalcophile elements are In, Bi, Pb, and Hg (James Wittke, 2007; http://www4.nau.edu/meteorite/Meteorite/Book-GlossaryV.html).