HANDBOOK OF EXPLORATION AND ENVIRONMENTAL GEOCHEMISTRY 9
M. HALE (SERIES EDITOR)

BIOGEOCHEMISTRY IN MINERAL EXPLORATION

COLIN E. DUNN

Handbook of Exploration and Environmental Geochemistry

VOLUME 9
Biogeochemistry in Mineral Exploration

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M. Hale (Series Editor)

1. ANALYTICAL METHODS IN GEOCHEMICAL PROSPECTING
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8. LIFE CYCLE OF THE PHOSPHORIA FORMATION
9. BIOGEOCHEMISTRY IN MINERAL EXPLORATION

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VOLUME 9

Biogeochemistry in Mineral Exploration

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Amsterdam ● Boston ● Heidelberg ● London ● New York ● Oxford
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Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
Linacre House, Jordan Hill, Oxford OX2 8DP, UK

First edition 2007

Library of Congress Cataloguing-in-Publication Data
A catalogue record for this book is available from the Library of Congress

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-444-53074-5
ISBN: 978-0-444-53154-4 (Inserted CD-Rom)
ISSN: 1874-2734

Printed and bound in The Netherlands

07 08 09 10 11 10 9 8 7 6 5 4 3 2 1

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EDITOR'S FOREWORD

The publication of this volume marks a milestone for the series. As part of the preamble to the first volume, published just over 25 years ago, my predecessor and founding editor of the series, Gerry Govett, listed the titles of the volumes he envisaged in the series. These would build a comprehensive set of handbooks covering the various operational techniques used in exploration geochemistry (drainage, regolith, etc.), the methods used in geochemical analysis and the statistics used for extracting exploration information. The realization of this latest volume, dealing with the use of biogeochemistry in mineral exploration, completes his vision.

This volume reveals that the trace element content of plant tissues offers a valuable and probably underused tool in the armoury of the exploration manager. A successful survey, however, rests on bringing together a wide spectrum of multidisciplinary knowledge and a careful adherence to operational protocols. Different species concentrate different trace elements to different degrees in different tissues. Species selection in climatic and geographic zones ranging from boreal forests to humid tropics to deserts is considered. Sampling and sample preparation call for meticulous contamination-avoidance precautions due to the very low concentrations of trace elements found in plant tissues. On the other hand, recent advances in analytical instrumentation now make the determination of these low concentrations relatively straightforward and routine. The same advances are opening possibilities to determine an even wider range of elements in plant tissues. A fascinating source of biogeochemical data is the plants of the climate-controlled bio-domes of the Eden project in southwest England. The biogeochemical behaviour of most elements of the periodic table is catalogued in this volume and a unique bibliography of biogeochemistry in mineral exploration is included. Case histories describing biogeochemical surveys in exploration for gold, platinum group elements, nickel, uranium and kimberlites illustrate the practical application of biogeochemistry. Going beyond this thorough review of the current state-of-the-art, a well-founded glimpse into future developments includes the potential of both biogeochemistry and geomicrobiology in mineral exploration.

The content fully reflects the fact that the author, Colin Dunn, is supremely qualified to contribute an expert volume on biogeochemistry in mineral exploration. One of the features of this volume that I find – and I hope other readers find – most striking and exciting is the way in which it is imbued with his personal experience. Furthermore, Colin Dunn has not only devoted much of his career to the application of biogeochemistry to mineral exploration, but also, in the course of his career, met

and worked with almost all of the other scientists who made notable contributions to biogeochemistry during the second half of the 20th century. Not surprising then, that the incisive knowledge and distilled wisdom to be found in this volume are unsurpassable.

Martin Hale, The Netherlands
January 2007

PREFACE

To a large extent, this book is a personal odyssey through almost 30 years of biogeochemical research, involving many tens of thousands of samples and millions of analytical determinations. It aims at emphasizing the practical aspects of field operations and the precautions that need to be taken, with details of sample preparation, choice of analytical method and data interpretation. The pitfalls that may be encountered along the way are indicated, with the intention of making this a reference book and a true handbook of the how, why, when, what and where to proceed with a mineral exploration survey using plant tissues. 'Case History' accounts from around the world provide follow-up results of a number of surveys directed towards precious metals, base metals, uranium and diamondiferous kimberlites.

This book has a different bias from the comprehensive texts dealing with similar subject matter by Brooks (1983), Kovalevsky (1987), Kabata-Pendias and Pendias (1992), Brooks et al. (1995) and Kabata-Pendias (2001). Its coverage is significantly different from the 'classic' biogeochemical studies involving mostly the fundamental building blocks of biogeochemistry (C, H, N, O) that are comprehensively dealt with in 'Biogeochemistry' edited by William Schlesinger (2005) as Volume 8 of the parallel series of books by Elsevier entitled *Treatise on Geochemistry*. These valuable texts should be used for further details on the composition of a wide range of plants, foodstuffs and soils. There is no discussion of foodstuffs in the present text, because the intention is to focus upon those common, naturally occurring plant species that are widespread throughout a survey area, and can therefore be systematically collected and analysed to determine the spatial relationships of elements to a mineralized target.

This book is aimed at the geologist. When working in the field with some botanists I have received looks of incredulity when I have asked the name of a plant that I continually see and which appears to be, therefore, of potential value for mineral exploration. To many botanists it is only the unusual plants that are of particular interest, and so to them my question seems frivolous and redundant, yet these common plants represent the key species of value in the biogeochemical exploration for mineral deposits.

As geologists we have our own vocabulary of complex terms and in general we seek to simplify the other sciences that impinge upon our geological knowledge. With this in mind a number of terms are expressed here in a language that is more compatible with the geologist than the chemist; in the tables, elements are listed in alphabetical order of their chemical symbols, rather than grouped together in some other chemically coherent format; and in Chapter 9, the descriptions of the element characteristics of plants are arranged in alphabetical order of their element name.

Concentrations are expressed as parts per million (ppm) and parts per billion (ppb), rather than what are to most geologists the more complex chemical units of $\mu g\,g^{-1}$ or $\mu g\,kg^{-1}$ or $ng\,g^{-1}$.

More than 20 years ago, after some significant relationships between plant chemistry and mineralization had been revealed, I asked an exploration manager if he was considering using the biogeochemical method to assist in his exploration programme. His response was 'No – quite frankly it scares the heck out of me, because I don't understand it'. By which he meant that the numbers tend to be different from soils, and there are other considerations to be taken into account when interpreting the data. Subsequently, many studies and presentations have been designed to try and simplify the complex world of plant chemistry as it relates to minerals concealed in the ground. To this end, the first biogeochemical exploration short course that was sponsored by the former Association of Exploration Geochemists (now the Association of Applied Geochemists) was entitled 'Biogeochemical Exploration – Simplified'. In this the expertise of a 'botanist–biogeochemist' (Jim Erdman), a 'chemist-geochemist' (Gwendy Hall) and two 'geologist–biogeochemists' (Shea Clark Smith and myself) was brought together at a conference in Phoenix to present our combined knowledge, with the emphasis on exploration in arid terrains. Subsequently, I have had the good fortune to work with several of the world's 'old school' of biogeochemists (Harry Warren, Robert Brooks, Alexander Kovalevsky) of whom none are with us today, and to meet and discuss details with many other fundamental contributors to biogeochemistry in mineral exploration including Nils Brundin, Hans Shacklette, Helen Cannon, Jim Erdman, Don Hornbrook, John Fortescue and more recently Shea Clark Smith, Mark Fedikow, David Cohen and Ken Lovstrom. Peter Rogers (Chavin Consulting), a long-time colleague with whom I have collaborated on many projects, was instrumental in helping to launch a number of large biogeochemical surveys in Nova Scotia and elsewhere, and has provided valuable input to the content and structure of several chapters.

Two friends and colleagues deserve special mention for their immense contributions to my understanding of the non-geological aspects of biogeochemistry. For much of the detailed chemistry outlined in Chapters 6 and 7, I have lent heavily on the comprehensive knowledge of Gwendy Hall at the Geological Survey of Canada (GSC) with whom I have had the good fortune to work on many projects over the past 20 years. With her kind permission, I have quoted from many of her publications and my text has benefited from her critical reviews of those two chapters. Similarly, for botanical aspects of Chapter 2, I have based much of my text on publications and discussions with Rob Scagel (Pacific Phytometric Consultants, Surrey, BC). Thanks to many field sessions with Rob, I have learnt almost everything I now know about botany and forestry. The three of us have collaborated on producing a number of workshops and publications (e.g., Dunn et al., 1993a).

Over the years, the generous support and encouragement of managers at the Saskatchewan Geological Survey and the Geological Survey of Canada have greatly assisted in facilitating field programmes and providing the resources to acquire large

amounts of analytical data. Subsequently, while consulting for a number of companies and organizations I have accumulated vastly more data, and these companies have recently granted permission for me to release a considerable amount of the information contained within this book. I thank, in particular the exploration managers, past and present, with Anglo American, AngloGold-Ashanti, BHP Billiton, Falconbridge, Noranda, Merit Mining, Placer Dome, Uravan Minerals and the companies that have granted this permission.

It was late in 1992 that Dr. Gerry Govett, as editor of the series 'Geochemistry in Mineral Exploration', sent me a letter enquiring as to whether I might be interested in compiling a book on biogeochemistry in mineral exploration. Although my interest was high, other commitments precluded for many years any concrete action to produce this book, but the seeds of thought were sown and it encouraged me to steadily accumulate the various pieces of information required for ultimately achieving this goal. After an early retirement from the GSC in 1998, I was able to further develop my ideas, but it was not until the end of 2003 after a decade of gentle persuasion from Gerry that a contract with Elsevier was finally signed. In the interim, Dr. Martin Hale took over the reins as Series Editor and my sincere gratitude goes to Martin for his enormous help in keeping me on track throughout the writing process.

To all of the above, and to the many more individuals with whom I have worked and discussed problems over the years, I extend my sincere gratitude.

For those seeking more information than that provided in these pages, there is a CD in the back pocket that contains data listings, a complete study on halogens in plants and soils (courtesy of Geoscience BC), and abstracts of all papers (about 130) published in the *Journal of Geochemical Exploration* (JGE – 1972–2007) on biogeochemical and geobotanical studies directed towards mineral exploration. The JGE is a primary source of such information, and the digital abstracts each have a hyperlink to Elsevier's website and access to the full papers.

The compilations of data selected to illustrate various topics are drawn primarily from large personal databases, and although there are many pages of references, discussion revolves mainly around projects with which I have had personal involvement. I have not been able to give full credit to the many individuals involved in developing this science, but references are provided to all those that I have been able to track down. This assemblage of words, figures, tables, digital listings and references is designed for 'one-stop shopping' with respect to information on biogeochemistry in mineral exploration. Inevitably, it is a 'work in progress'. The intention is to provide a field manual and a source of reference. It is hoped that the information contained within these pages will help to spark new ideas, and provide a stepping-stone towards further development of the intriguing and ever-surprising interactions among the sciences of geology, botany and chemistry.

Colin E. Dunn, Sidney, BC, Canada
3 January 2007

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Chapter 1

INTRODUCTION

SETTING THE SCENE

Vascular land plants have been evolving for over 425 million years. During that long period they have adapted to survive a remarkably wide range of both physical and chemical conditions. Mechanisms have evolved to absorb and scavenge chemical elements and translocate them through roots into stems, twigs, bark, foliage, flowers, cones and seeds. Some species have developed defences that screen and exclude at the soil/root interface, or at barriers within a plant, those elements that are either not required for metabolic function or are harmful to development. Other species accumulate selected elements and use them to ward off insects and/or diseases. For example, the Douglas-fir has a propensity to accumulate arsenic. Since there is no known metabolic function for this usually toxic element it can be assumed that, until shown otherwise, it is using the arsenic as a defence against either predatory creatures or diseases. Thus, the enrichment of arsenic in Douglas-fir is a facet of knowledge that can be used to advantage by the mineral explorationist, since arsenic is so commonly associated with many metal deposits, especially gold.

The plant can be viewed as a sophisticated geochemical sampling device, as yet not fully understood. An extraordinary wealth of information on plant chemistry is scattered throughout the literature of botanists, plant chemists and geochemists. In spite of this, although past its infancy, the application of biogeochemical methods to mineral exploration must be considered to be only in the early stages of maturity. There is much that we have yet to establish. As research progresses on conditions that control the accumulation of elements by plants, and as field studies continue to augment the databases on plant chemistry, the role of the plant in mineral exploration programmes becomes increasingly important.

BIOGEOCHEMISTRY AND GEOBOTANY

General considerations and distinctions

As the name implies, 'biogeochemistry' involves the integration of the three sciences – biology, geology and chemistry. With minor exceptions, the 'biology' component involves the plant kingdom, i.e., botany, and not the animal kingdom, i.e., zoology. Consequently, the term 'phytogeochemistry' (from the Greek word for

'plant' – 'phyton') would be a more precise term to apply to the activities of the 'biogeochemist'. In some countries, notably France and China, it seems that there is a predisposition to use this as a more accurate term. For the purposes of this book the terms 'biogeochemistry' and 'phytogeochemistry' are considered as synonymous.

Biogeochemical methods of exploration involve the chemical analysis of plant tissues to assess the presence and nature of underlying mineralization, bedrock composition, bedrock structure (faults, joints and folds), and the chemistry of the soil, surficial sediments, and associated groundwater. By contrast, 'geobotanical exploration' relies on the recognition of the occurrence of a plant species (or plant communities) with underlying mineralization and/or bedrock and groundwater. The distinction can be summarized simply as follows:

- Biogeochemistry is the chemical approach, whereas
- Geobotany is the visual approach.

Consequently, they should be considered as closely associated but separate disciplines. With a modicum of training a geologist with some knowledge of geochemistry can soon learn the fundamentals required to effectively conduct a biogeochemical survey. It is a much larger step that is required for a geologist to become a geobotanist, because of the significant training in plant identification that geobotany demands. This step to geobotany is made more easily by a trained botanist. Ideally, a geologist/geochemist needs to team up with a botanist/forester to optimize the information that can be obtained from the distribution and analysis of vegetation.

Geobotanical clues are more subtle in temperate and boreal (i.e., northern) forests than in tropical forests, because there is a lesser diversity of trees and shrubs in the cooler regions, and plants are able to accumulate wide ranges of many elements without exhibiting any visible clues. It is sometimes apparent that when a traverse crosses major differences in composition of the substrate, such as granite to carbonate, some geobotanical signs might be evident, e.g., a change in dominant species, a change in plant population densities, more vigorous growth, or health of a particular species. In Siberia, vegetation on kimberlites, as compared with that on surrounding rocks, develops more profusely because of elevated levels of P in the kimberlitic bedrock (Buks, 1963). Roses grow better in carbonate-rich soils, so the presence of wild roses commonly indicates enhanced carbonate content to the soil and/or underlying bedrock. Similarly, extensive studies have been made of the 'Serpentine floras' of the world (Brooks, 1987), although more strictly these should be referred to as the 'ultramafic' floras, because they do not rely on serpentinization for their development, just the over all bedrock composition with its rich sources of iron, magnesium and calcium.

Most of the geobotanical 'indicator' plants of specific metals occur in the warm parts of the world, notably in central Africa (Brooks et al., 1995; Brooks, 1998). Plant development in cool and cold regions is controlled more by physical than chemical conditions. As a result, there are far fewer species in cold areas than in warm and

moist climates, where plant growth and diversity are not impeded by the harsh physical conditions. In the tropics, ground chemistry plays a more dominant role in the distribution of plants, and some species adapt to a chemical niche. In the 1950s, some of the best examples that guided many exploration activities are the copper/cobalt flora of the Democratic Republic of Congo (DRC) and the copper flora of the Zambian copper belt (Brooks and Malaisse, 1985; Brooks et al., 1995; Brooks, 1998; Brummer and Woodward, 1999). Plants comprising these floral assemblages provide a *geobotanical* indication of copper and cobalt, but they may or may not all be enriched in these metals: certain species may develop barriers to copper/cobalt uptake, and would therefore be poor choices for a *biogeochemical* survey.

Important characteristic floras summarized in Brooks et al. (1995) are as follows:

1. *Calciphilous floras* – characteristic of limestone.
2. *Halophyte floras* – characteristic of saline environments.
3. *Serpentine floras* – characteristic of ultramafic environments.
4. *Selenium floras* – especially of the mid-western United States (Cannon, 1960).
5. *European zinc (Galmei) floras* – notably over base metals in western Germany and Belgium.
6. *Copper/cobalt floras of DRC* – notably the Shaban Copper Arc.

Extensive bibliographies on geobotanical indicators and plant communities as ore indicators are listed in Rose et al. (1974), Brooks (1987), Brooks et al. (1995) and Brooks (1998). Among the names frequently cited there are, in chronological order, Helen Cannon (1953, 1956, 1957), S.V. Viktorov (1955), A. Chikishev (1965), H. Wild (1965), N.G. Nesvetailova (1970), Monica Cole (1973, 1977), F. Malaisse (Malaisse et al., 1978, 1979) and Brooks and Malaisse (1985). This list is by no means exhaustive, but presents some of the authors within whose studies more details can be found. For those familiar with the Russian language, there are many papers and a number of books published only in Russian, that contain a considerable amount of information on geobotanical indicators (e.g., Nesvetailova, 1970).

Biogeochemical surveys commonly rely on the analysis of tissues from trees or shrubs (i.e., vascular plants), although they may involve the collection of any species of plant life, depending on the distribution of that species and its propensity to accumulate metals (e.g., lower-order plants such as lichens, mosses, fungi and seaweed). From a geochemical standpoint, plants can be considered as a *filtered* above-surface extension of the underlying soil and rock, since they contain elements drawn from soil, sediment, rock, groundwater and gaseous compounds contained within the substrate. Typically, there is not a one-to-one relationship between the chemistry of a plant and that of the soil, because of barrier mechanisms established by plants to the uptake of certain elements; hence the 'filtering' of the elements from the ground into the plant tissues. A simple biogeochemical response to changes in the substrate is shown in Fig. 1-1, where sampling commenced on gneiss, traversed an ultramafic body, and finished in metasediments. As might be expected, the elevated levels of

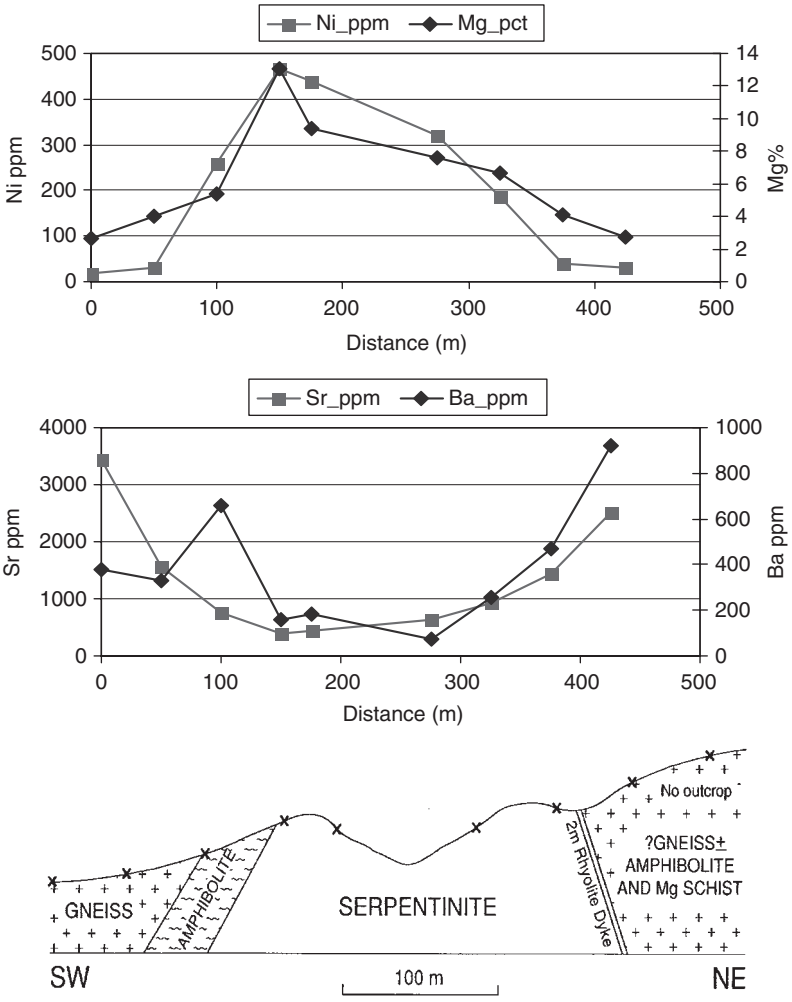


Fig. 1-1. Response of vegetation chemistry to underlying bedrock – *Baccharis trimera* from Cerro Mantiqueira, southern Brazil. *Baccharis* has many common names, notably 'Thola' and 'carqueja', and is widespread throughout much of South America.

Ni and Mg (typical of ultramafic rocks) are evident, whereas conversely there is concurrent depletion of Sr and Ba.

History of geobotany

The application of geobotany to assist in delineating mineralization has been used for many centuries. By contrast, the history of biogeochemical exploration dates back little more than a century, primarily because analytical technology and

instrumentation were inadequate for accurately determining, at reasonable effort and cost, the miniscule traces in plant tissues of many elements that are relevant to mineral exploration.

The earliest of the geobotanical observations appears to be that of Vitruvius who, in 10 BC, noted that certain species are restricted to marshy ground (Brooks, 1983). In India, the 6th century AD Sanskrit writings of Varāhamihira record many relationships among plants, minerals and (notably) groundwater (Prasad, 1980), even listing the depths to aquifers that can be predicted by particular species. Many subsequent studies dealing with geobotany focused on the relationship of plants and plant communities to groundwater (abundance and quality).

In 1841, the Russian geologist Karpinsky advanced the concept of a relationship between plants and geological substrates by noting that a whole plant community should be recognized in helping to characterize the relationship between plants and underlying geology.

History of biogeochemistry

Whereas published accounts on the relationship of plant chemistry to mineralized rock date back to the end of the 19th century, they are scarce until the late 1930s. In an 1898 study at the Omai gold mine in Guyana, ash samples of Baromalli (genus *Catostemma*, from the family *Bombacaceae*) and a species identified as 'ironwood' were found to contain 0.3 ppm Au and 3 ppm Au, respectively (Lungwitz, 1900). Kovalevsky (1987) reports that work on biogeochemical exploration in the USSR began in the 1920s when S.P. Aleksandrov discovered that, when compared to concentrations in plants outside of an ore zone, there were elevated levels of vanadium, radium and uranium in ash of plants growing in the vicinity of a vanadium–uranium deposit. Subsequently, in the 1930s V.I. Vernadsky supported the establishment of a 'Biogeochemical Laboratory of the USSR Academy of Sciences' to which A.P. Vinogradov made significant contributions.

The first published report of biogeochemical methodology was by the Russian worker Tkalich (1938), who cited the discovery that a deposit of arsenopyrite in Siberia could be traced by the iron content of overlying vegetation. At about the same time Nils Brundin in Sweden related biogeochemical data to a vanadium deposit in Sweden and to tungsten in Cornwall, England. He was sufficiently enthused by his findings that he filed a patent on the use of vegetation in exploration, noting that the method would be especially suited for locating ores of gold, silver, lead and zinc (Brundin, 1939).

In the early 1940s, Professor Harry Warren commenced more than half a century of biogeochemical studies from his base at the University of British Columbia in Canada. During the first 30 years of his work, Warren and his associates did much to document the relationships between plant chemistry and concealed mineralization, and to raise the credibility of biogeochemistry from, as he declared, general disbelief,

through 'benevolent scepticism' to general acceptance that, when used properly, it can be a viable exploration tool (e.g., Warren and Delavault, 1950; Warren et al., 1968). Harry Warren rightly earned the distinction of 'father of biogeochemistry' in North America, and it is an enlightening pastime to browse through his more than 200 publications to extract many little gems of information. Not only does he provide many useful pieces of information, but also there are a good many historic data from the analysis of trees over sites that have subsequently been developed as mines (e.g., the Endako Mo mine and the Sullivan Pb/Zn mine, both in British Columbia). What can be more instructive than to look at analytical data from trees of virgin forest, and then review the production data from a mine subsequently developed at that site?

While Harry Warren was advancing the science in Canada, work was also under way in the United States. In the mid-western states Harbaugh (1950) reported the results of a base-metal study of soils and vegetation. Shortly afterwards, when there was a significant push to find deposits of uranium, a number of scientists at the U.S. Geological Survey examined the geobotanical and biogeochemical expressions of uranium roll-front deposits in Colorado and the surrounding states (Cannon, 1957, 1960, 1964). Concurrently, Hans Shacklette and his co-workers were conducting laboratory and field experiments on the metal uptake of plants, and they produced a number of USGS publications on the biogeochemical behaviour of different elements, and several compilations of baseline data on background concentrations in vegetation (Shacklette, 1965; Shacklette et al., 1970).

Following on from his discoveries in the 1930s, Nils Brundin spearheaded the Scandinavian investigations into biogeochemical methods. A monograph on trace elements in plants from Finland provided valuable semi-quantitative information on the composition of a wide range of plants growing on different geological substrates (Lounamaa, 1956). Baseline data were presented for lichens, mosses, ferns, conifers, deciduous trees and shrubs, dwarf shrubs, grasses and herbs.

During this period in the former Soviet Union, numerous papers were published (mostly in Russian, with only a few significant translations into English) that described in general terms the methods that were being employed and results of biogeochemical surveys over various types of mineral deposit. Of importance to the international community was the milestone publication of the first book in English that was devoted exclusively to biogeochemical prospecting for minerals (Malyuga, 1963).

For almost 50 years, until the time of his death in 2001, Alexander Kovalevsky, from his base in Ulan-Ude, Siberia, published the results of a wide range of biogeochemical studies. He produced many papers, some of which have been translated into English. For those with a good working knowledge of Russian, there appears to be an enormous amount of valuable information that can be learnt from the prodigious volumes of tables, figures and text that he produced. Other leading Russian workers on biogeochemical methods during this period included Vinogradov (1954), Tkalich (1959, 1961, 1970), Polikarpochkin and Polikarpochkina (1964), Grabovskaya

(1965), Vernadskii (1965) and Talipov (1966). The 'landscape geochemistry' studies of Perel'man (1961, 1966) contributed, too, to the literature on biogeochemical methods. It is fortunate that Robert Brooks understood Russian and was able to interact with a number of the Russian workers to summarize the complex Russian literature into easily comprehensible English.

In 1960, Professor Robert Brooks (1926–2001) commenced 40 years of fundamental work in biogeochemical, geobotanical and phytomining studies from his base at Massey University in Palmerston North, New Zealand. In 1972, he published the first book from outside the former Soviet Union dealing with geobotany and biogeochemistry in mineral exploration (Brooks, 1972). This was a significant milestone in the science and provided the English-speaking world with an easy to understand review and handbook of biogeochemical methods. It was superseded by a second and expanded edition (Brooks, 1983). Amongst his biogeochemical interests were geobotanical and botanical studies of the flora endemic to serpentine substrates. He initiated, organized and led five expeditions sponsored largely by the National Geographic Society, to remote areas of ultramafic rock outcrop where the 'serpentine flora' had been poorly documented. Thanks to these major efforts several species new to science were discovered, and there are now greatly improved databases and collections housed at several major herbaria around the world. In 1987, he published another significant collation of information and data in his book 'Serpentine and Its Vegetation – A Multidisciplinary Approach' (Brooks, 1987).

In the mid-1960s, the Geological Survey of Canada commenced investigations into the use of biogeochemistry in mineral exploration. A trailer was fitted out to be a mobile biogeochemical laboratory containing basic analytical equipment, a muffle furnace, balances and an emission spectrograph. This was taken into the field and the analytical work carried out close to the survey areas (Fortescue and Hornbrook, 1967, 1969; Hornbrook, 1969, 1970a,b). These studies developed protocols for when and how to sample, and suggested a systematic approach to biogeochemical research. They recommended a 'Visit' lasting one or two days to assess plant distribution and the environment in general (i.e., a brief orientation survey). If results looked promising, then this would be followed by either a 'Pilot Project' of up to 30 days to conduct a comprehensive survey of an area; or, if mining was imminent, a 'Quick Project' to obtain sample material prior to disruption and potential contamination of an area by mining operations. These procedures remain sound advice for conducting biogeochemical investigations today, and high credit should be given to these researchers for their thoughtful and thorough planning that helped to advance biogeochemical methodology in its application to mineral exploration.

The 1974 publication of Kovalevsky's book 'Biogeochemical Exploration for Mineral Deposits' was finally translated into English in 1979 (Kovalevsky, 1979). This work provided new insight into the Russian experience and approach to the science, along with a host of rather complex acronyms that were elegantly deciphered by the translators, and by the biogeochemical/geobotanical group at the USGS – Hans Shacklette, Jim Erdman and Gary Curtin – all of whom in their own right have added

valuable studies to the biogeochemical literature. Subsequently, a revised and extended second edition was published with further elucidation of the Russian terms provided by Robert Brooks (Kovalevsky, 1987). As noted in the Foreword by A.B. Kazhdan, 'this second edition is a greatly expanded version of the first … [with] … discussion of principles governing the formation of biogeochemical haloes for Cu, Hg, Mo, Pb and Zn, and … the biogeochemistry of 45 other indicator elements'. Although many of the species listed are restricted to Siberia, there are very useful analogies to similar species typical of the boreal forests and the cool dry areas of the world. The 1987 edition is well worth browsing through, because it gives insight as to the plant genera and species that might be worth considering for biogeochemical exploration elsewhere. It should not be treated as a 'cook-book', but more of a guide, because some broad generalizations are made, and it seems that results from the Russian environment are not always paralleled by those from other parts of the world. For example, a table indicates that the vast majority of plants have a very low propensity to absorb U. This is partly true, but in certain U-rich environments, such as around the uraniferous Athabasca Sandstone of northern Saskatchewan, there are many plant species that can accumulate U (Dunn, 1981, 1983). Furthermore, Kovalevsky's 1987 text is 20-years old and significant advances have been made in the interim – not least of which is the introduction of inductively coupled plasma mass spectrometry (ICP-MS) analysis of dry tissues.

Kovalevsky wrote a third book that is as yet only available in Russian, therefore its impact on the science is hard to evaluate for the non-Russian-speaking populace (БИОГЕОХИМИЯ РАСТЕНИЙ – meaning 'The Biogeochemistry of Plants', Kovalevsky, 1991). Some information contained in this book appeared in English in book chapters (Brooks et al., 1995) and journal publications (Kovalevsky and Kovalevskaya, 1989; Kovalevsky, 2001, shortly before his death).

During the 1980s, a resurgence of interest in biogeochemical methods of exploration took place in North America, and especially in the south-western United States. A conference held in Los Angeles in 1984 provided a useful 'state-of-the-art' summary of the science, bringing together expertise from a diversity of disciplines (Carlisle et al., 1986). In the United States, big sagebrush (*Artemisia tridentata*) became a common sample medium to assist in the exploration for uranium and gold in the arid terrain of the southwest (Erdman and Harrach, 1981; Gough and Erdman, 1980; Erdman et al., 1985; Erdman and Olson, 1985). The work by S. Clark Smith and J.A. Erdman contributed significantly to the biogeochemical database. In Canada, the preferred sample media were recognized as black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), alder (*Alnus spp.*), Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and lodgepole pine (*Pinus contorta*), among others (Dunn, 1981; Cohen et al., 1987; Dunn, 1989, 1995a).

There has been a tendency for significant advances in analytical instrumentation to coincide with advances in biogeochemical exploration. In the mid-1970s, ICP-ES and instrumental neutron activation analysis (INAA) were developed to provide

accurate and precise multi-element data at low-element concentrations and remarkably low cost. The INAA, in particular, was a great break-through since many elements could be determined at sub-ppm levels from a single irradiation of dry vegetation. During heightened activity for discovering uranium deposits in the late 1970s, delayed neutron counting was used for determining uranium concentrations in vegetation (Dunn, 1983a,b; Dunn et al., 1984), and as renewed interest for gold took over from uranium in the 1980s INAA methods became routine for determination of gold to 0.1 ppb in dry vegetation (Hoffman and Booker, 1986). Within the last 10 years, the most significant advance has been the application of ICP-MS to the analysis of small samples (typically 1 g) of dry plant tissues. This has provided new insight into element concentrations and distributions in and among trees and shrubs, and permitted the systematic examination of the distributions of elements rarely determined previously, because of the time-intensive procedures that were required. As a result, there has been an exponential increase in biogeochemical knowledge, and information, over the past decade.

In 1992, Robert Brooks compiled the book 'Noble Metals and Biological Systems' that covered a broad spectrum of current knowledge of these metals (Brooks, 1992). Three years later, Brooks was lead editor of the book 'Biological Systems in Mineral Exploration and Processing' (Brooks et al., 1995) that contains a wealth of valuable information. The last book that he compiled focussed on those plants that have the ability to concentrate metals to extraordinarily high levels, entitled 'Plants that Hyperaccumulate Heavy Metals' (Brooks, 1998).

In China systematic biogeochemical studies began in the 1970s, and in recent years it appears that the focus has been mainly on the search for deeply buried mineralization in arid regions. Researchers claim that from the analysis of willow twigs they can delineate Pb–Zn stratabound deposits beneath more than 100 m of loess and over 100 m of redbeds. Few publications appear to come from China, and when last consulted a few years ago, the Chinese considered that their biogeochemical studies are still in the experimental stage.

In Australia, too, biogeochemical studies are enjoying renewed investigation. A perceived problem in the past has been the complexities that arise in dealing with the multitude of species comprising Australia's two main genera of plant – the eucalypts and the acacias. In Australia alone, there are about than 850 species of eucalyptus (family Myrtaceae) and more than 1000 species of acacia (family Mimosaceae). As yet, it has not been established if there are significant differences in metal uptake and accumulation among all the species of each of these genera. If there are, then indeed, there are problems in establishing the baseline information that is desirable for determining which might be the preferred species for sampling. However, if differences are quite minor among members of a genus or perhaps other grouping of species, then it should prove possible to mix data from similar species without compromising a survey. Furthermore, if an area has a particular species that is dominant (e.g., red river gum, *Eucalyptus camaldulensis* – a species investigated by Karen Hulme at the University of Adelaide), then that species can be used since it is the *spatial* relationships of

element concentrations, rather than the absolute concentrations, that are the overriding dominant factors of value to biogeochemical exploration.

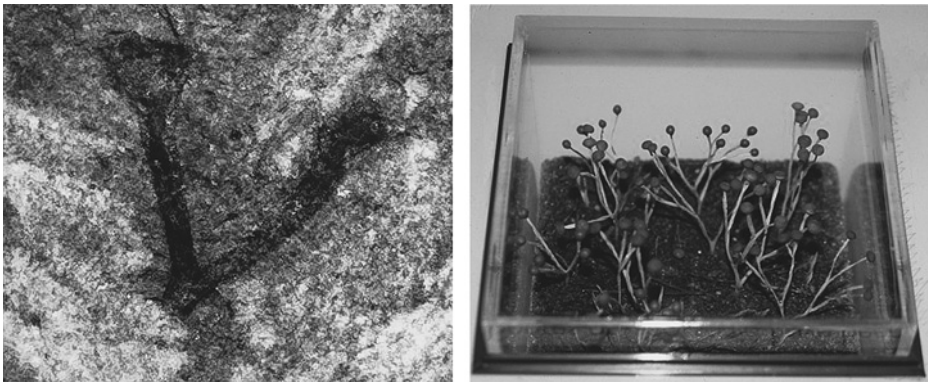
The Association of Exploration Geochemists (now renamed the Association of Applied Geochemists) has been instrumental in disseminating biogeochemical exploration methods and data through sponsoring short courses and publishing the course notes (Parduhn and Smith, 1991; Dunn et al., 1992c [which includes many references to Clark Smith's studies]; Dunn et al., 1993a; Dunn et al., 1995a).

In recent years, journal papers by many authors have provided case histories and overviews of procedures and various sample media from a full range of environments from around the world. Some of the best sources of information on the application of biogeochemical methods to mineral exploration are the 'Journal of Geochemical Exploration', 'Applied Geochemistry' and 'Geochemistry: Exploration, Environment, Analysis'.

PLANT EVOLUTION AND CHEMISTRY

At about the end of the early Silurian Period, the first vascular land plants appeared. *Cooksonia* was a small fragile plant, just a few centimetres tall, comprising a slender bifurcating stem topped with small spheres (sporangia) in which the reproductive spores were formed (Fig. 1-2).

Over the ensuing 50–100 million years, this was the type of plant that grew in suitably moist conditions while evolving into more complex entities. By the Carboniferous (~360–300 million years ago), lush plant growth flourished in equatorial regions and huge trees prevailed – such as the fern-like but seed-bearing pteridosperms, the huge green-stemmed lycopods (*Lepidodendron*, *Sigillaria*, up to 35 m tall), the giant *Calamites* (20 m) and the mangrove-rooted *Cordaites* (up to 45 m).



Cooksonia from the Lower Ludlow of Wales

Reconstruction of *Cooksonia* by C. Berry (Ludlow museum, UK)

Fig. 1-2. Earliest form of plant life (Silurian). Source: http://www.xs4all.nl/~steurh/engcook/ecooks2.html#reco, May 2005.

During their long period of evolution, plants have become sophisticated in adapting to their environments, both physical and chemical, and in order to survive they have developed a requirement for certain elements. In addition to hydrogen, oxygen and carbon there is general agreement that essential elements include nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, boron, chlorine, iron, manganese, zinc, copper, molybdenum and nickel. Other elements are considered 'essential' by some workers and/or 'beneficial' by others and as research continues the beneficial effects of an increasing number of elements, often in very small concentrations, are being recognized. Kabata-Pendias and Pendias (1992) provide many useful tables that summarize the role of metals in plants and the effects of too much (toxicity) or too little (deficiency). As a consequence of these requirements for metals, biogeochemical data should be viewed somewhat differently from those of soils and rocks.

Laboratory experiments have demonstrated that the most basic of life forms – bacteria and fungi – are capable of accumulating extraordinarily high concentrations of several metals (Table 1-I).

Studies in the natural environment have recorded metal concentrations in macrofungi, such as the edible mushrooms, that are significantly lower than in the laboratory tests. However, they are still extremely high compared to the soils in which they were growing that contained background concentrations of elements. This attests to the ability of fungi to preferentially scavenge some metals (Table 1-II).

The efficiency of roots depends on the distance of their depth of penetration into the soil and, more importantly, the surface area of roots that comes into contact with the ground. Mycorrhizal fungal hyphae vastly increase the volume of surface area to which roots have access. Not only do they absorb water and pass it into a plant, but,

TABLE 1-I

Concentrations of metals in primitive life forms (experimental data) – from Lepp (1992)

	Bacteria	Concentration (%)	Fungi	Concentration (%)
Ag	*Thiobacillus ferrooxidans*	35	*Rhizopus arrhizus*	5.4
Cd	*Zooglea ramigera*	40	*Rhizopus arrhizus*	3
Co	*Zooglea sp.*	25		
Cr			*Rhizopus arrhizus*	3.1
Cu	*Zooglea ramigera*	40	*Rhizopus arrhizus*	1.6
Hg			*Rhizopus arrhizus*	5.8
Ni	*Zooglea sp.*	13		
Pb	*Micrococcus luteus*	35	*Rhizopus arrhizus*	10.4
Zn			*Rhizopus arrhizus*	2

TABLE 1-II

Average concentrations of selected elements in common plants compared to highest concentrations recorded in macro-fungi growing in soils containing background levels of these elements – modified after Lepp (1992) with supplementary data

	Average plant (ppm)	Highest fungal concentration (ppm)	Genus	Common name
Ag	0.02	1253[1]	*Amanita*	Death-cap mushroom
As	0.1	427	*Amanita*	Death-cap mushroom
Au	0.2 ppb	2250 ppb[1]	*Lepiota*	'Shaggy-stalked parasol'
Cd	0.05	300	*Amanita*	*Death-cap mushroom*
Cu	5	469	*Amanita*	*Death-cap mushroom*
Hg	0.02	80	*Agaricus*	*Common edible mushroom*
Sb	0.1	1423[1]	*Chalciporus*	Peppery bolete
Se	0.02	55[1]	*Boletus*	Edible mushroom
V	0.5	700	*Amanita*	*Death-cap mushroom*

[1]From Borovička et al. (2006a,b, in prep.).

by excreting enzymes that assist in breaking down soil particles and microfauna, they are more efficient at extracting nutrients than the roots themselves.

Fungi play an important role in nutrient transfer into plants. About 300,000 species of plants have relationships with fungi that are endotrophic, indicating that the fungal hyphae penetrate and grow inside a plant's roots. However, the relationship with a tree is ectotrophic, meaning that a sheath of mycorrhizal fungi encases the roots. This mass of mycorrhizal fungi is estimated to connect trees with as much as one thousand times more soil area than the roots themselves (Luoma, 1999).

The root microenvironment can be highly corrosive, with acidity locally below pH 1 (Meyer et al., 1973) at the interface with the growing medium. The roots are as conservative as possible in the use of energy to acquire essential nutrients, thereby taking the path of least resistance by first accepting elements in gaseous form, then those in solution, and then seeking out additional requirements by selectively extracting labile elements loosely bonded to soil surfaces, such as the amorphous manganese and iron oxide coatings to which metals are known to be adsorbed. As a last resort, roots attack the fine-grained inorganic particles coating bedrock joints, and the crystalline phases of soils and bedrock.

The extent of root systems can be extraordinarily large. Dittmer (1937) estimated that a single rye plant (*Secale cereale*), 50 cm tall and with a clump of 80 shoots had a cumulative total root length of 380 miles (611 km) that included fourteen billion root hairs. Upon each of these roots, rootlets and root hairs there are myriads of mycorrhizal fungi continually accessing plant nutrients from the ground while passively tolerating other elements and passing this soup of material into the plant structures.

Magnesium is the element that gives chlorophyll its green colour. The remaining constituents of chlorophyll are the four basic elements of life – hydrogen, carbon, nitrogen and oxygen. In fact, magnesium is to plant 'juices' what iron is blood. There is close similarity between chlorophyll and haemoglobin; at the hub of every haemoglobin molecule is one atom of iron, while in chlorophyll it is one atom of magnesium (Peattie, 1991). Consequently, analysis of a green plant part can be expected to return a Mg concentration from 500 ppm up to several percent. Woody tissue, however, such as outer bark and twigs, typically contains only a few hundred ppm Mg. From an exploration point of view, this example illustrates that some knowledge of the essentiality of an element and 'what goes where' in a plant is useful in developing an understanding of the levels of elements that might be expected. Whereas the composition of a typical plant is substantially different from that of a rock, it is of use to bear in mind the analogy given by Kovalevsky (1987) that the ash of a plant (i.e., minus all its organic constituents) is similar in composition to a dolomite ($CaCO_3 \cdot MgCO_3$).

As to why and how trace elements become 'locked' into plant cells, it is sobering to consider the sequence of events described by Suzuki and Grady (2004) that takes place during photosynthesis:

> When a photon of sunlight hits a chloroplast, one electron is ejected from each molecule of chlorophyll; this energy excites the molecule which then uses that excitation to carry out a chemical reaction … the energy released by the ejected electron separates water into … hydrogen and oxygen … and carbon dioxide into its separate elements. Then the released carbon, hydrogen, and oxygen recombine to form carbonic acid, which is instantly changed into formic acid … this becomes formaldehyde and hydrogen peroxide, which immediately breaks down into water, oxygen and glucose.

The fact that carbonic acid, formic acid and hydrogen peroxide are involved serves to illustrate the complexity of the processes that permit the mobility and complexing of any trace elements that have been drawn up into the plant structure via the roots. It is also perhaps relevant that hydrogen peroxide is a strong oxidizing agent that is the basis of several methods of selectively leaching elements from soils. In effect, the plant conducts a 'selective leach'.

Attempts have been made by several researchers to define the average composition of plants, e.g., Salisbury and Ross (1969), Lisk (1972), Bollard (1983), Marschner (1988, 1995), Mengell and Kirkby (1987), Kabata-Pendias and Pendias (1992), Kabata-Pendias (2001), Markert (1992). Markert (1994) reviewed a large amount of

data and published a table listing element concentrations in a world 'Reference Plant' (Table 1-III). Over the past decade, there has been a wealth of new data obtained from low-cost multi-element ICP-MS analyses that have provided much lower detection limits than were readily available for some elements. In light of these new data, the stated concentrations by Markert have been modified for those elements marked with an asterisk.

Markert's very useful guide was an ambitious and difficult task because there are such wide variations in composition of the many plant species from around the globe. It has largely stood the test of time of more than a decade of new data, but the values should be considered for what the table represents – a broad guide to the world average composition of all parts of all plants. With the advent of ICP-MS improved estimates can be made for some elements (notably Au, Ag, Hg, PGEs, Re, Te, Tl) from many tens of thousands of analyses. These modifications are shown in the table in bold font and marked with an asterisk. It is of the utmost importance to realize, too, that element concentrations among individual tissues from a single plant are dramatically different. Just as the various components of a plant – wood, bark, twigs, foliage, flowers/cones, etc. – bear no physical resemblance to each other, nor do the chemical compositions of each type of tissue. There are chemical barriers to element translocations between tissues that are discussed in the next section.

Table 1-IV shows significant differences in the major element content of ash obtained by igniting conifer needles and twigs to 1000°C. This temperature was selected to remove both organic components and CO_2, and is considerably higher than the temperature usually set to reduce tissues to ash prior to analysis. Temperatures between 470°C and 500°C are used to remove only the organic components and conserve some potentially volatile elements. This table shows, too, that the residual ash from high temperature ignition is basically a carbonate-rich material (dominated by Ca) with substantial 'impurities'. There are substantial differences between conifer needles and twigs, with needles having much lower contents of SiO_2, Fe_2O_3 and commonly Al_2O_3, but much higher levels of K_2O, P_2O_5, and MnO. Every species of plant and every plant tissue has a different composition (e.g., grasses and horsetails have high-silica content at the expense of the carbonate) and, understandably, some uptake may be controlled by classic geochemical affinities of elements. Ranges in composition among species can be as great as the differences, for example, between rhyolite, dunite, sandstone and limestone.

These data serve to emphasize how important it is in any biogeochemical exploration survey to be consistent in the collection and analysis of plant tissues. This is because the geochemical exercise being performed is to compare the *relative compositions* within a survey area. Lack of consistency in sampling will give rise to a classic case of mixing 'apples with oranges'; this can result in a lot of apparently 'interesting' data that can be very misleading. It is akin to mixing A, B and C soil horizons and expecting to produce a meaningful element distribution map.

TABLE 1-III

Element abundances in plants (dry weight) – Summary of estimates of worldwide averages of all tissues from all plants (modified after Markert, 1994)

Element	Units	Concentration
Major elements (>0.1%)		
C	%	44.5
O	%	42.5
H	%	6.5
N	%	2.5
K	%	1.9
Ca	%	1
S	%	0.3
P	%	0.2
Mg	%	0.2
Cl	%	0.2
Si	%	0.1
Trace elements (<1000 ppm)		
Ag*	**ppb**	**20**
Al	ppm	80
As	**ppm**	**0.1**
Au*	**ppb**	0.2
B	ppm	40
Ba	ppm	40
Be	ppb	1
Bi	ppb	10
Br	ppm	4
Cd	ppb	50
Ce	ppm	0.5
Co	ppm	0.2
Cr	ppm	1.5
Cs	ppm	0.2
Cu	ppm	10
Dy	ppb	30
Er	ppb	20
Eu	ppb	8
F	ppm	2
Fe	ppm	150
Ga	ppm	0.1
Gd	ppb	40
Ge	ppb	10
Hf	ppb	50
Hg*	**ppb**	**20**
Ho	ppb	8
I	ppm	3

Continued

TABLE 1-III *Continued*

Element	Units	Concentration
In	ppb	1
Ir*	**ppb**	**0.01**
La	ppm	0.2
Li	ppm	0.2
Lu	ppb	3
Mn	ppm	200
Mo	ppm	0.5
Na	ppm	150
Nb	ppb	50
Nd	ppm	0.2
Ni	ppm	1.5
Os	ppb	0.0015
Pa	ppb	?
Pb	ppm	1
Pd*	**ppb**	**0.1**
Po	ppb	?
Pr	ppb	50
Pt	ppb	0.005
Ra	ppb	?
Rb	ppm	50
Re*	**ppb**	**0.1**
Rh*	**ppb**	**0.01**
Ru	ppb	0.1
Sb	ppm	0.1
Sc	ppb	20
Se	ppb	20
Sm	ppb	40
Sn	ppm	0.2
Sr	ppm	50
Ta	ppb	1
Tb	ppb	8
Te*	**ppb**	**20**
Th	ppb	5
Ti	ppm	5
Tl*	**ppb**	**20**
Tm	ppb	4
U	ppb	10
V	ppm	0.5
W	ppm	0.2
Y	ppm	0.2
Yb	ppb	20
Zn	ppm	50
Zr	ppm	0.1

TABLE 1-IV

Major element composition (%) of the ash of conifer needles and twigs ignited at 1000°C. LOI is the loss on ignition ash obtained at between 475°C and 1000°C

	Mtn. hemlock needles		Balsam fir needles	Jack Pine twigs		
	$n = 3$	$n = 3$	$n = 418$	$n = 3$	$n = 27$	$n = 4$
SiO_2	2.39	2.56	3.63	38.96	38.50	37.58
Al_2O_3	4.28	8.22	0.79	9.61	9.85	6.98
Fe_2O_3	0.55	0.77	0.33	2.90	3.03	2.85
MgO	3.74	3.51	4.37	3.82	4.40	4.27
CaO	25.21	27.17	30.16	21.85	21.85	23.01
Na_2O	0.08	0.08	–0.02	1.34	1.42	1.45
K_2O	20.65	16.69	19.50	3.27	3.58	3.82
TiO_2	0.05	0.05	0.01	0.31	0.30	0.34
P_2O_5	9.46	6.12	8.27	1.85	1.75	2.04
MnO	5.86	5.93	1.40	0.08	0.12	0.14
LOI (1000°C)	27.60	28.80	26.32	15.80	15.50	15.60

BARRIER MECHANISMS

Over 30 years ago, Alexander Kovalevsky emphasized the 'barrier concept' to element uptake by plants, and introduced the principle of 'barrier-free prospecting' (Kovalevsky, 1974). The concept states that plant species and different plant organs (his 'bio-objects') have varying degrees of resistance to the uptake by roots of elements present in the substrate. 'Non-barrier' plants are those that can accumulate an element in a constant plant-to-soil ratio regardless of the amount of that element in the ground. These are ideal species for biogeochemical exploration. At the other end of the scale are the 'barrier' plants that accumulate little or none of an element in underlying soil by establishing mechanisms at the root/soil interface to exclude certain elements from entering the plant. Such plants are of little use in exploration. Most plants (about 95%), however, fall somewhere between these two extremes and they are able to accumulate a certain amount of an element before there is an adverse affect on growth. When this concentration is reached the plant establishes a 'barrier' to further uptake by the roots. In addition to the barrier mechanism, many plants cope with concentrations of elements that are surplus to their requirements by storing them in a tissue (e.g., outer bark) where a plant's health will not be adversely affected. Examples of both amorphous and crystalline phases found within plant structures are given in Chapter 2.

The barrier mechanism is an important concept, and some texts have attempted to classify plants, plant tissues, and elements on this basis. However, so many factors may come into play (e.g., soil acidity, soil moisture, the presence of other elements to modulate the effects of toxicity, underlying lithology) that one can be misled into ignoring certain plant species because they have been classified in some texts as 'non-informative'. For example, the Russian literature states that plants establish barriers to uranium uptake and therefore they are of limited use in biogeochemical exploration for uranium (Kovalevsky, 1987). For many plants and environments this is true, but now that commercially available ICP-MS provides data for U at ppb levels with excellent precision and accuracy it has become apparent that slightly elevated levels of U commonly occur in association with many types of mineralization in many plant species. Also, there are major exceptions to the mantra that most plants establish barriers to U uptake. In northern Saskatchewan black spruce (*Picea mariana*) growing on the Athabasca Sandstone locally have more than 1000 times the normal background concentrations of U (Dunn, 1983a) indicating that it is a non- or practically non-barrier species. Additional species in this environment yield very high levels of U (e.g., Labrador tea [*Ledum groenlandicum*], leather-leaf [*Chamaedaphne calyculata*] and other boreal species).

It is necessary to be aware that, whereas one plant may not be responsive to a certain type of mineralization, another plant may give a strong response. Fortunately, many plant species may give similar *patterns* of element distribution with respect to mineralization, but the absolute concentrations may be dramatically different. This emphasizes that wherever possible a single plant species should be used for a survey. On occasion a normalization factor can be applied to allow for the different absorption characteristics of plant species and tissue types. This possibility is discussed later.

To reiterate what was stated earlier, a basic premise to be held in mind is that 'plants do not always provide the same geochemical information as soils'. In an exploration programme for mineral deposits a soil sample collected for analysis is usually only a handful of a specific soil horizon. This soil is usually sieved to obtain a few grams of a specific size fraction (e.g., –80 mesh aperture) for analysis. By contrast, a plant sample, because of its typically extensive root system, may represent an integrated signature of several cubic metres of all soil horizons and sometimes bedrock. Furthermore, roots may extract metals directly from migrating groundwater and accumulate them in their tissues, whereas little or none of that metal may be adsorbed by the soil. Table 1-V is an extreme example of this situation, showing a wide range in uranium concentrations in spruce twigs yet no significant variation in the underlying soils. From the soil survey results, significant uranium mineralization (world-class Athabasca U deposits) would be missed, whereas from the biogeochemical data it is clear that the area contains high enrichments of uranium.

Robert Brooks succinctly summed up the context within which biogeochemical methods of exploration should be viewed:

TABLE 1-V

Concentrations of U in black spruce (*Picea mariana*) twigs, and in the Bf-horizon soil underlying each tree, Athabasca Sandstone, Saskatchewan, Canada

Site	U (ppm) in ash of twigs	U (ppm) – equivalent in dried twigs	U (ppm) in Bf horizon soil
1	5	0.1	1.9
2	8	0.16	2.4
3	20	0.4	2.5
4	26	0.5	2.0
5	113	2.3	1.9
6	226	4.6	1.8
7	303	6	1.8
8	408	8.2	1.8
9	486	9.7	2.0
10	886	17.7	1.9

> In the treatment of biogeochemistry, it is not claimed that the method is a universal panacea for the search for minerals, and indeed under some conditions, it may not be wise to expect too much from the method. However, if used in the right place by suitably trained personnel well versed in its potential and application, it will prove to be a valuable auxiliary or even primary method in the continuing search for minerals throughout the world. (Brooks 1983, p. 111)

Almost a quarter of a century has passed since that judgement was made. In the interim extensive research has been conducted and there has been an exponential increase in the amount of data that is now available to place newly acquired data in context with newly acquired survey results. To quote another statement of Robert Brooks (Brooks et al., 1995, p. 239):

> potential pitfalls are now better understood, and methods have been streamlined. Biogeochemical prospecting for minerals is not difficult provided a number of simple steps and precautions are followed. It is a young science requiring a great deal more knowledge before its enormous potential can be fulfilled.

Progress continues to be made on an ever-expanding world stage.

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Chapter 2

PLANT FUNCTION, CHEMISTRY AND MINERALOGY

In this chapter a brief synopsis is provided of a vast amount of literature on the essential chemical make-up of plants and the intricate processes that take place in moving elements into, within and out of plants. These processes can be considered as 'Biogeochemistry' in the classical sense, and it is a topic dealt with in considerable detail by Schlesinger (2005).

For the exploration geologist interested in using plants to locate concealed mineralization, the elements of particular relevance are mostly the non-essential elements. Ideally, the exploration geochemist should have a basic grounding in the classic aspects of biogeochemistry, but to effectively run a biogeochemical survey for minerals this is not a requirement. The intention of this short chapter is to fill the gap between the classical knowledge of the geologist/geochemist and that of the biologist/botanist/plant chemist with some salient information. References are provided for those wishing to delve deeper into the complexities of the plant world and its classical biogeochemical studies.

PLANT REQUIREMENTS

There is a distinction that needs to be made as to what different disciplines mean by the term 'mineral'. To the geologist a mineral is a natural compound formed by geological process. A more precise definition is 'a naturally occurring homogeneous solid, inorganically formed, with a definite chemical composition and an ordered atomic arrangement' (Berry and Mason, 1959). To the plant chemist the term 'mineral' is often used more loosely when referring to a single chemical element. A plant chemist will indicate that 'Copper is an essential mineral for plant metabolism', meaning simply that it is an essential element. Throughout this book, the term 'mineral' refers to its strict geological sense.

In botanical terminology, there are two main groups of elements: (1) Essential, or nutritional elements and (2) Trace, or non-essential elements. Essential elements are divided into macronutrients and micronutrients. The macronutrients are further divided into primary and secondary. There is also the recognition of a group of elements that are 'beneficial'. They have not been proven to be essential, but experimentation has found that plant growth may be enhanced by their presence at certain concentrations. Sodium is an element that falls into this category.

Recently, the concept of a 'functional plant nutrient' has been introduced (Subbarao et al., 2003). In their paper they note that the concept of 'essentiality' was defined by Arnon and Stout (1939) as

> those elements necessary to complete the life cycle of a plant. A few other elements such as Na have an ubiquitous presence in soils and waters and are widely taken up and utilized by plants, but are not considered as plant nutrients because they do not meet the strict definition of 'essentiality'. Sodium has a very specific function in the concentration of carbon dioxide in a limited number of C4 plants and thus is essential to these plants, but this in itself is insufficient to generalize that Na is essential for higher plants. The unique set of roles that Na can play in plant metabolism suggests that the basic concept of what comprises a plant nutrient should be re-examined. We contend that the class of plant mineral nutrients should be comprised not only of those elements necessary for completing the life cycle, but also those elements which promote maximal biomass yield and/or which reduce the requirement (critical level) of an essential element. We suggest that nutrients functioning in this latter manner should be termed 'functional nutrients.' Thus plant mineral nutrients would be comprised of two major groups, 'essential nutrients' and 'functional nutrients.' … other elements such as Si and Se may also confirm to the proposed category of 'functional nutrients.'

All 90 naturally occurring elements are found in most plant tissues, but with many at extremely low concentrations. In addition to C, H and O, only 16 elements are truly essential for growth of all plants (Table 2-I, first column). An additional 16 elements shown in the remaining columns of Table 2-I are essential to some plants and/or beneficial to the metabolism of others. The remaining 55 elements are mostly taken up passively in small quantities (traces or ultra-traces) as plants absorb the nutrient elements that they need for growth and reproduction. At this time they are not known to play an essential role in plants, but at ultra-trace levels more elements may prove to be essential. This large group of trace elements can be *tolerated* by most plants, with some species tolerating higher concentrations than others.

In extreme cases 'hyperaccumulation' of a non-essential element, such as Tl, can take place although elements that can hyperaccumulate are mostly those that are essential to many plants (e.g., Ni, Cu, Zn, Co, Mn). Hyperaccumulation of metals (e.g., 1% Ni in *Alyssum bertolonii*) was first recognized by Minguzzi and Vergnano in 1948, but it was not until 1977 that the term was introduced by Robert Brooks and his co-workers (Brooks et al., 1977). Brooks (1998) summarized the numbers of plants that are known to be hyperaccumulators of eight elements (Table 2-II). Since that publication appeared there have been additional plants recognized (e.g., a second genus that can hyperaccumulate Tl).

Some elements that are considered non-essential can, in some situations, substitute for an essential element and perform a surrogate 'essential' role. Molybdenum

TABLE 2-I

Essential elements

The biogeochemistry of life – essential elements				
Essential to all animals and plants	Essential to several classes of animals and plants	Essential to a wide variety of species in one class	Essential to only a few species	Recent work indicates essentiality, but of unknown function
Hydrogen(H) Carbon (C) Nitrogen (N) Oxygen (O) Sodium (Na) Magnesium(Mg) Phosphorus(P) Sulfur(S) **Chlorine (Cl)** Potassium (K) Calcium (Ca) **Manganese (Mn)** **Iron (Fe)** **Copper (Cu)** **Zinc (Zn)** **Selenium (Se)**	Silicon (Si) **Vanadium (V)** **Cobalt (Co)** **Molybdenum(Mo)** **Iodine (I)**	**Boron (B)** **Fluorine (F)** **Chromium (Cr)** **Bromine (Br)**	**Lithium (Li)** **Aluminium(Al)** **Nickel (Ni)** **Strontium (Sr)** **Barium (Ba)**	**Rubidium (Rb)** **Tin (Sn)**

Note: Elements in bold type are generally considered to be trace elements in plants.
Source: The United States Geological Survey website http://geology.er.usgs.gov/eastern/environment/environ.html#1 (8th October 2006).

and W are examples. A primary function of Mo is in nitrate reductase, an important enzyme that converts nitrate to ammonium. Tungsten can substitute for Mo in the synthesis of nitrate reductase with reduced effectiveness, but still maintaining some function. Similarly, Br can substitute for Cl in some plants.

It is advantageous to have some knowledge of these plant requirements when it comes to interpreting biogeochemical data with respect to mineral exploration. For example, concentrations of Zn in plants normally appear quite high when compared to other elements or to Zn concentrations in soils. Part of this enrichment is because Zn is an essential element and, therefore, will be present at moderate concentrations even when there is no Zn mineralization in the substrate. As a result, the geochemically similar element Cd is likely to be a better 'pathfinder' element for Zn mineralization than Zn itself. However, if there is substantial sphalerite mineralization near surface it is highly likely that it will be reflected as enhanced concentrations of Zn in tissues of trees that are rooted within the geochemical envelope of Zn enrichment emanating from the mineralization.

TABLE 2-II

Plant hyperaccumulators of eight elements and the families in which they are most often found (after Brooks, 1998)

Element	No. of species	Family
Cadmium	1	Brassicaceae
Cobalt	26	Lamiaceae, Scrophulariaceae
Copper	24	Cyperaceae, Lamiaceae, Poaceae, Scrophulariaceae
Manganese	11	Apocynaceae, Cunoniaceae, Protaceae
Nickel	290	Brassicaceae, Cunoniaceae, Euphorbiaceae, Flacourtiaceae, Violaceae
Selenium	19	Fabaceae
Thallium	1	Brassicaceae
Zinc	16	Brassicaceae, Violaceae

ELEMENT UPTAKE AND FUNCTION

The uptake of elements by plants is dependent upon ionic size and charge, and the microscopic structure of root surfaces.

- some elements can go in and out at will,
- some elements are physically excluded,
- some elements are actively pulled through the root walls (i.e., osmosis), and
- some elements are actively excluded.

An element can 'plug' a root wall aperture, and subsequently exclude other elements from passing through the wall or it may change the charge of the wall. This is the 'barrier' mechanism described in the Russian literature (e.g., Kovalevsky, 1987). Furthermore, the size of the apertures depends upon the temperature and availability of water, and can change during the course of the year, giving rise to seasonal variability in plant composition.

The role of various elements in plant metabolism is summarized in Table 2-III. As noted above, many elements play a role in plant metabolism yet others may have no function. Gold, for example, is not known to be of any use to plants, yet in plants growing over mineralization it can be absorbed and concentrated a thousand-fold over its usual background levels.

Additional information on the forms and principal functions of trace elements essential for plants is provided by, amongst others, Kabata-Pendias and Pendias (1992), Kabata-Pendias (2001), and by Chapin and Eviner (2005).

Photosynthesis is one of the most metal-sensitive processes of plant metabolism, hence when a plant absorbs unusually large amounts of some metals (e.g., Cu) photosynthesis can be affected, such that a plant may show stress, yellowing of leaf

TABLE 2-III

The roles of essential and beneficial elements in vascular plants ('S' = structural; 'E' = enzymatic). Modified after compilation by Scagel (in Dunn et al., 1993a)

Element	Class	Use
Al	E	Colloidal properties of cells
B	S, E	Carbohydrate metabolism, flavinoid synthesis
Ca	S	Cell walls, N-metabolism
Cl	E	Enzymes, osmotic functions, stomatal movement
Co	E	Coenzyme
Cu	S, E	Enzymes, coenzymes, phosphorylation
F	E	Respiration
Fe	S, E	Chloroplasts, respiratory enzymes, phosphorylation
I	E	Protein function
K	E	Enzyme activity, osmotic regulation, stomatal movement
Li	E	Salt metabolism
Mg	S, E	Central element in chlorophyll molecule, enzymes, phosphorylation
Mn	E	Chlorophyll synthesis, enzyme activator
Mo	E	Nitrogen metabolism, enzymes
N	S	Amino acids, proteins, cell membranes, enzymes, chlorophyll synthesis, protein synthesis
Ni	E	Translocation of N
P	S, E	Nucleoproteins, phospholipids, high-energy phosphate bonds, energy transfer, phosphorylation
Rb	E	Partial analogue for K
S	S	Amino acids, proteins, coenzymes, phosphorylation
Si	S	Cell walls
Sr	S	Partial analogue for Ca
Ti	E	Nitrogen fixation
V	E	Nitrogen metabolism
Zn	E	Enzymes, hormone synthesis

tips (chlorosis), or subtle changes in spectral reflectance that hyperspectral imagery may be able to differentiate.

Uptake of an excess of trace elements can result in the induction of deficiencies of other essential elements. These interactions trigger secondary responses, involving enzymes, which either protect membranes against further damage or may partially bypass metal-sensitive reactions. As a result the physiological state of the cell may be altered and the plant may become more metal-tolerant.

Within a plant, elements may be variously mobile. The mobility determines where deficiency or toxic symptoms will be expressed and how elements will accumulate with increasing age. Table 2-IV lists the uptake mechanisms and relative mobility in

TABLE 2-IV

Classification of some chemical elements found in plants according to their uptake, transport and mobility in the phloem (after Bukovac and Wittwer, 1957)

Element	Quantities	Uptake transport	Phloem mobility
Ag	Trace	Passive	
Al	Micro	Active	
B	Micro	Active	Low mobility
Ba	Trace	Passive	Low mobility
Ca	Macro	Active	Low mobility
Cl	Micro	Passive	Mobile
Co	Micro	Passive	
Cr	Trace	Passive	
Cu	Micro	Active	Intermediate
Fe	Micro	Active	Intermediate
I	Trace	Passive	
K	Macro	Active	Mobile
Li	Trace	Passive	Low mobility
Mg	Macro	Active	Mobile
Mn	Micro	Active	Intermediate
Mo	Micro	Active	Intermediate
N	Macro	Active	Mobile
Na	Trace	Passive	Mobile
Ni	Micro	Active	
P	Macro	Active	Mobile
Rb	Trace	Passive	Mobile
REE	Trace	Passive	
S	Macro	Active	Mobile
Se	Trace	Passive	
Si	Micro	Active	
Sr	Trace	Passive	Low mobility
Ti	Trace	Passive	
U	Trace	Passive	
V	Micro	Passive	
Zn	Micro	Active	Intermediate

the phloem (downward movement) of essential and trace elements in plants. These results (Bukovac and Wittwer, 1957) are complicated by the fact that the mobility of elements classified as 'intermediate' varies with species, the stage of growth and the concentration in the plant (Loneragan, 1975). Consequently, this classification should be viewed as only a general indication of the relative ability of element movements. Elements that are mobile will express deficiency symptoms in older plant parts and toxic symptoms in the youngest parts. Low-mobility elements will

express deficiency symptoms in the youngest plant parts and toxic symptoms in the oldest plant parts.

ROOT FORM AND CONTROLS ON ELEMENT UPTAKE

Roots are complex structures that are highly variable in their morphology and in their lateral extent and depth of penetration. Reviews of roots and chemical interactions in their rhizosphere are given by Richards (1986), Jones (1998) and Arienzo (2005). The combined length of roots, rootlets and their mycorrhizal fungi (considered to be essential for tree survival) can be immense. Roots have been reported at a depth of 53 m (Phillips, 1963) and there are anecdotal reports of roots penetrating to greater depths from their presence in the roofs of mine galleries. It has been estimated that a single rye plant, just over 1 m tall can have a combined length of these roots, rootlets and mycorrhizae totalling over 600 km, and through millions of microscopic apertures a root system constantly extracts from the soil and groundwater those nutrients that the plant requires along with other elements that the plant is able to tolerate. The additional roles that bacteria play are discussed in Chapter 12. Thus, the root system of a single plant can integrate the geochemical signature of many cubic metres of soil, groundwater and sometimes the precipitated oxides that coat the surfaces of joints and faults in bedrock, and mineral surfaces. Locally, the extraordinary physical power of roots can be observed in road-cuts or cliff faces revealing the way the root system of a tree strives to firmly anchor itself, sometimes by wedging apart and propagating rock fractures while seeking out sufficient water and nourishment for its very existence. Furthermore, on a hot sunny day 100–150 l of water (with its dissolved elements) may be transported through the roots and stem to the leaves of a large tree (Kramer and Kozlowski, 1979). Given these sobering statistics it is evident that a tree can be considered as an efficient integrator of the chemistry of a large volume of soil, groundwater and sometimes bedrock, and thereby reflects a comprehensive geochemical signature of the substrate.

Practically all of the movement of elements from roots to foliage occurs in the xylem sap, carried by mass flow in the transpiration stream with subsequent flow down the phloem to complete the fluid cycle. Not all elements move in the xylem at the same rate, with some slowed by adsorption on cell walls during xylem flow. Thus, some elements get to the growing parts of the plant more quickly than others, thereby accounting for seasonal fluctuations in concentrations of some elements. In the autumn, soon before the leaves fall, some elements return to twigs where they are stored over the winter months before they are required for the next year's growth cycle.

There are a number of environmental factors that can modify the capabilities of roots to absorb elements. The pH immediately around the roots is generally a microenvironment that can be considerably more acidic than the surrounding rooting environment of the soil, and values as low as pH 1 have been reported. The non-woody roots and root tips are surrounded by a mucilage sheath that is acidic and

may modify element fluxes. Many trace elements are relatively stable at slightly acidic pH due to the stability of the ligand chelates with which they are associated (Pb > Cu > Ni > Co > Zn > Cd > Mn). Seasonal variation in pH influences the availability of several elements (e.g., Cu, Zn, Pb, Cd, Cr, Ni) thereby further accounting for some of the seasonal variations in metal uptake and emphasizing the importance of conducting a survey using live tissues in as short a time frame as possible (few weeks). The relative mobility of elements is shown in Table 2-V.

The salt content of a groundwater solution determines what elements are taken up or not in competing for active sites on the roots. In particular Na is readily taken up by many plants, but blocks the subsequent uptake of K. Notes should be kept of sites from near alkaline and seaside locations because the uptake of some elements may be modified by such conditions. Along major roadways in northern climates, the salt content of plants may be elevated because of the application of salt to road surfaces in winter months to dissolve ice.

Redox potential determines the availability of many elements to plants. For instance, arsenic becomes readily available under low redox conditions. Elements that are particularly affected are those that require active uptake. Plant root respiration cannot occur at low redox potentials and elements cannot be actively transported. Redox potential also determines the form (i.e., chemical species) of an element in the soil solution.

Decaying organic matter tends to bind trace elements and remove them from soil solution, thereby preventing their uptake by plants. Consequently, consideration should be given to the environmental conditions of a survey area, because by combining samples from trees growing on organic-rich and inorganic soils, there may be differences in distribution patterns of some elements that are largely attributable to local ground conditions.

Several factors can influence root development and subsequent metal uptake.

- Slope and aspect can modify thermal conditions increasing the length of the growing season and regulating transpiration rates.
- Moisture levels that can regulate the length of the growing season and degree of soil aeration.
- History – stand age and tree age. Young trees have a wider lateral to depth ratio of roots than older trees.
- Soil depth and texture.
- Pathology – insects and fungi.
- Climatic events – drought, winter desiccation and frost.

SUMMARY COMMENTS ON CHEMICAL REQUIREMENTS OF PLANTS

There is a formidable amount of literature on plant physiology, nutritional requirements and plant chemistry in general. Clearly, controlled pot and greenhouse tests have done much to determine the conditions under which metals can accumulate

TABLE 2-V

Relative mobility of different trace elements in relation to pH. Immobilizing factors: Fe/Mn = Fe/Mn oxides; OM = organic matter; C = clay; Red. = reducing conditions; Ca = calcium carbonate (modified after compilation by Scagel, in Dunn et al., 1993a)

Element	Acidic (<pH 5.5)	Weakly acidic to neutral (pH 5.5–7.0)	Alkaline (pH > 7.0)	Immobilizing factors
Ag	High	Medium–low	Very low	Fe/Mn, OM, Red.
As	Medium	Medium	Medium	Fe/Mn, C
Au	Low mobility	Low mobility	Low mobility	
Ba	Low	Low	Low	Red., Ca, C
Be	Low	Low	Low	Fe/Mn; OM; C
Bi	Low	Low	Low	Fe/Mn, Red.
Cd	Medium	Medium	Medium	Red., OM
Ce	Poorly soluble	Poorly soluble	Poorly soluble	
Co	High	Medium–low	Very low	
Cu	High	Medium–low	Very low	Fe/Mn, OM
F	High	High	High	
Hg	Medium	Low	Low	Fe/Mn
Li	Low	Low	Low	Fe/Mn, C
Mo	High	High	High	Fe/Mn, Ca, Red.
Nb	Poorly soluble	Poorly soluble	Poorly soluble	
Ni	High	Medium–low	Very low	
Pb	Low	Low	Low	Ca, Red.
Pt	Poorly soluble	Poorly soluble	Poorly soluble	
Ra	High	High	High	Fe/Mn, OM
Rb	Very high	Very high	Very high	
Sb	Low	Low	Low	Fe/Mn, Red.
Se	High	High	Very high	Fe/Mn, Red.
Sn	Poorly soluble	Poorly soluble	Poorly soluble	
Ta	Poorly soluble	Poorly soluble	Poorly soluble	
Te	Very. low	Very low	Very low	
Th	Very low	Very low	Very low	C
U	Low–medium	High	Very high	Fe/Mn, OM, Red.
V	High	High	High	Red.
W	Poorly soluble	Poorly soluble	Poorly soluble	Fe/Mn
Zn	High	High–medium	Low–Very low	Fe/Mn, OM, Ca

in plants and the levels that can occur prior to the onset of toxicity. However, experiments in the natural forest are far less common, and of necessity they need to be extremely long term – tens if not hundreds of years.

It appears that in natural forest, over a number of years plants establish equilibrium with their environment. Many factors can interact to govern element uptake,

but there is sufficient stability in plant chemistry for the biogeochemical method to be a robust approach to mineral exploration provided consistency in collection, preparation and analysis of tissues is maintained. An awareness of the factors that can modify element uptake needs to be constantly maintained, and there should be a general appreciation of the complexities of the natural environment. Consequently, brief notes on field conditions should be made and, if possible, broadly quantified in order to assist in data interpretation. Quantification of field parameters need only be a simple broad categorization. For example, aspect can be recorded as 'south facing' and degree of slope can be simply recorded as steep, moderate, gentle or flat. Thus a field notation of 1S would indicate a gentle south-facing slope, whereas 3N would denote a steep north-facing slope. By recording parameters in this manner they can be related to chemical factors (by simple observation or statistically) during interpretation of the subsequent elemental data.

MINERALOGY OF PLANTS

Skinner and Jahren (2005) gives a comprehensive overview of biomineralization in plants and animals, eloquently describing those phases related primarily to sulphur, iron, silica, phosphorus and carbonates. It has long been established that inorganic phases, such as silica phytoliths, develop on plant surfaces, giving grasses and horsetails (*Equisetum*) their roughness. Figure 2-1 shows examples of some forms of phytolith associated with the cultivar 'Einkorn wheat', from the Gramineae Family, and provides clear evidence of why cereals and grasses can be harsh to the touch.

The scanning electron microscope (SEM) is required for detailed observation of plants for crystalline and other metal phases, because they are rarely more than 100 μm in size. Figure 2-2 shows the development of a manganese phosphate phase, about 100 μm × 10 μm, which developed within the inner trunk wood of the coniferous tree 'mountain hemlock' (*Tsuga mertensiana*) from Mt. Washington on Vancouver Island. This phase appears to be non-crystalline, and is wedged between the longitudinal fibres.

There are more than 100 families of tropical plants that contain silica and 215 families that contain calcium oxalates (Skinner and Jahren, 2005). The most frequently occurring mineral phases observed in plant structures are crystals of calcium oxalate (CaC_2O_4). They are commonly most concentrated in the bark (Fig. 2-3a, 3b), although they occur, too, within twigs and foliage (Fig. 2-3c, 3d). Whereas Ca oxalate is a simple mixture of Ca, O and C, it is not absolutely pure. Pharmaceutical analyses indicate that it typically has 0.5% Ba and 0.4% 'Poorly soluble residues'. These poorly soluble residues are likely to be primarily Si, Al, Fe, K, Mg and Na (all of which can form independent oxalates), but in addition minor and trace elements may be located within the crystal lattices of Ca oxalates in plants. Those reported

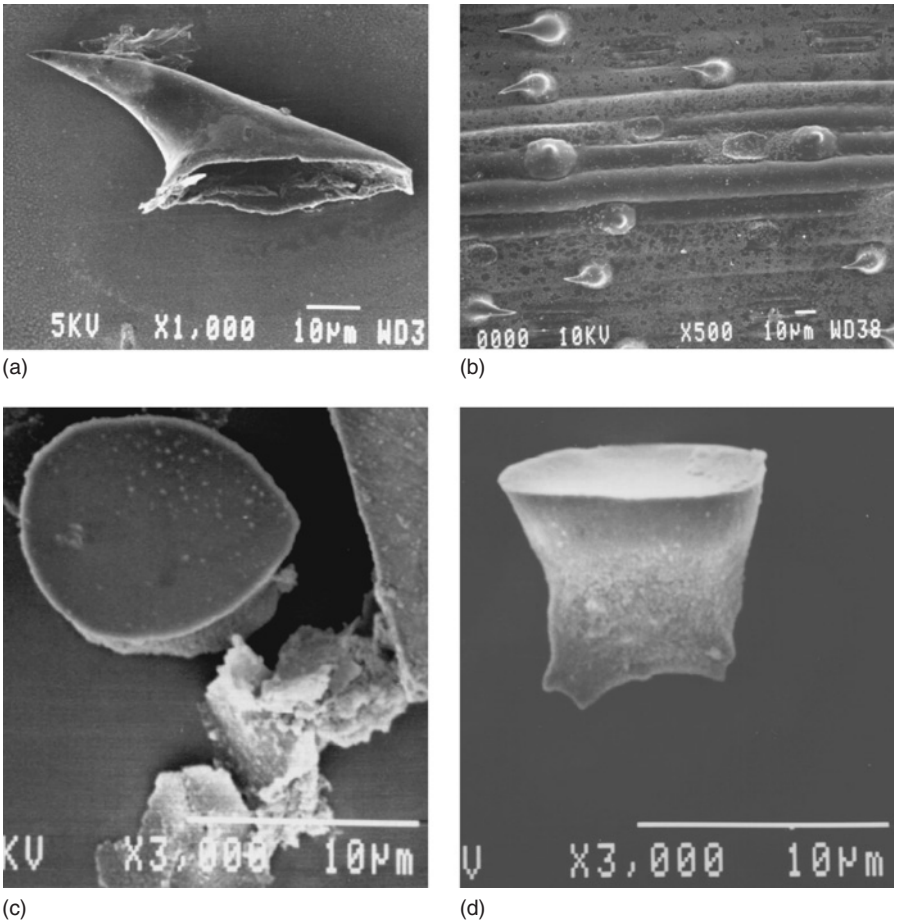


Fig. 2-1. Scanning electron micrographs (SEM) of silica phytoliths on einkorn wheat (*Triticum monococcum*) – (a) hair cell; (b) lamina – abaxial section; (c) and (d) inflorescence, top and side views.
Source: http://home.byu.net/~tbb/tball/index2.html (Terry Ball, Brigham Young University), Feb. 2004.

include Sr, Rb, REE, S, F, Cl and Br. To date, no multi-element ICP-MS analysis of Ca oxalate residues from plants appears to have been published, so it is quite feasible that many additional trace and ultra-trace elements may be sequestered within the Ca oxalate crystal structures.

On most modern SEMs the practical resolution is an object approximately 0.1 µm in diameter. Examination of plant structures under the SEM quite commonly reveals metal phases that occur as crystals or amorphous nucleations that are <2 µm in diameter. Whereas some mineral phases seen on plant surfaces are not definitively

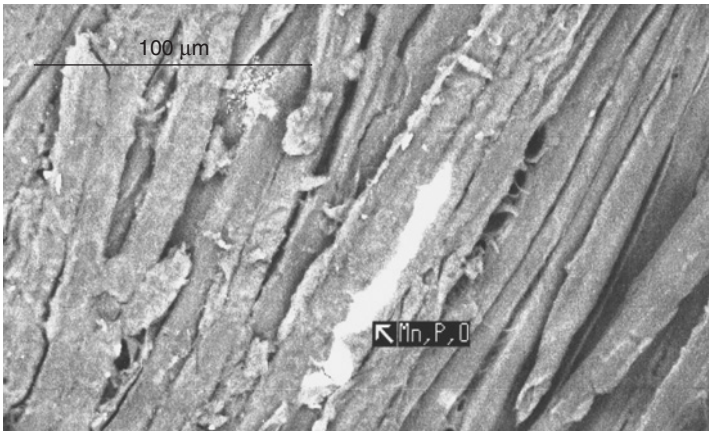


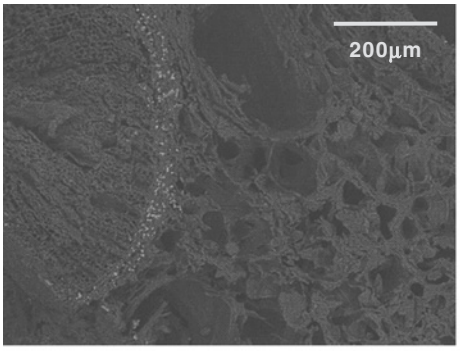
Fig. 2-2. Linear white area is manganese phosphate phase within conifer trunk wood.

formed in the plant and may be airborne particulates, crystalline phases of metals can sometimes be observed within or attached to plant cells. Figure 2-4 shows some heavy metal phases that have nucleated to form well-defined crystal structures. Figure 2-4a shows a 2 µm Zn + S crystal (presumably sphalerite) within a twig of western hemlock from the Carolin (Ladner Creek) Au mine in British Columbia; Figure 2-4b shows two larger elongate Zn + Mn + O crystals (~10 µm) in a needle of lodgepole pine from the vicinity of the Sullivan Pb-Zn mine at Kimberley in southern British Columbia.

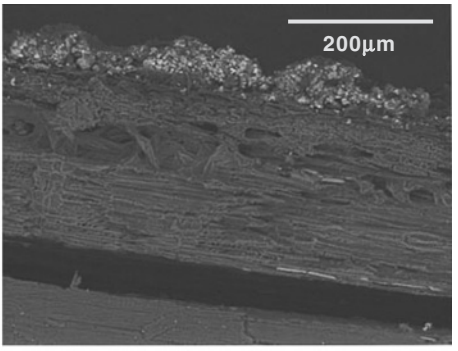
Under the SEM, backscatter images are useful for viewing heavy metals because they appear as bright spots against a darker background. The higher the atomic number of an element, the greater the backscatter image contrast, such that very heavy elements like Au, Hg and U appear as the brightest objects on those rare occasions when they have nucleated to a sufficient degree to be resolved by the SEM. Once located by this technique, their composition can be determined from X-ray dispersive analytical instrumentation attached to the SEM.

Of particular note are the photomicrographs shown in Fig. 2-4c, 4d. They depict the same fields of view, with 4c showing a reflected light image, and 4d a backscatter image. The photomicrographs are close-up views of the inside of bark collected from mountain hemlock (*Tsuga mertensiana*) that was growing on an unexploited gold deposit at Mt. Washington on Vancouver Island. The deposit has not been developed as a mine because it sits on a popular ski-hill, and so there has been little ground disturbance by exploration activities. Each photomicrograph shows a well-formed trigonal crystal about 1×0.5 µm, which, when the X-ray beam was focused on the object, proved to be gold. This appears to have crystallized out of the sap within the tree.

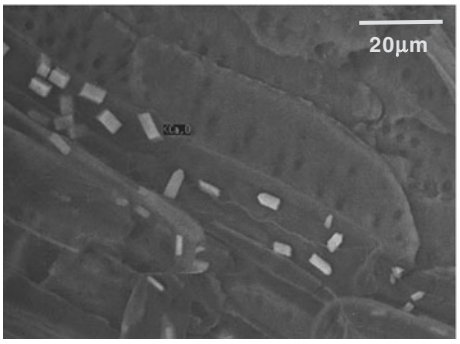
Not all metal phases are crystalline. In fact, the majority appear as poorly defined irregular aggregations that are not readily apparent until examined in backscatter mode. Figure 2-5 shows a selection of these in trees from several locations.



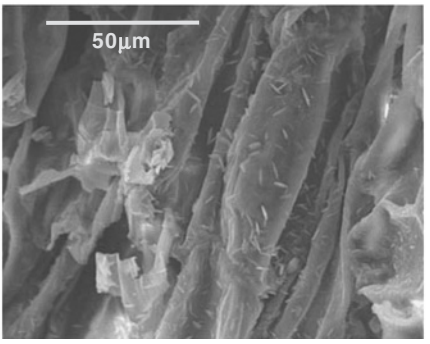
a: Cross Section of twig from lodgepole pine (*Pinus contorta*). Crystals concentrated where bark is forming around new growth



b: Cross section of twig of western hemlock (*Tsuga heterophylla*). Bark at top is crowded with crystals



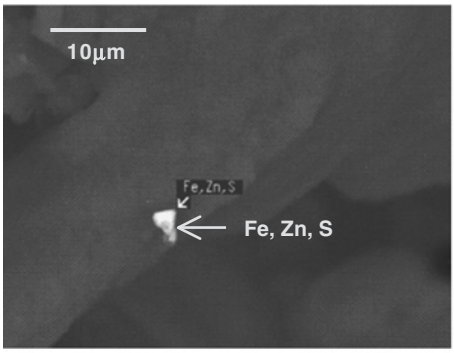
c: Ca oxalate crystals (5-10µm) formed in twig of black spruce (*Picea mariana*)



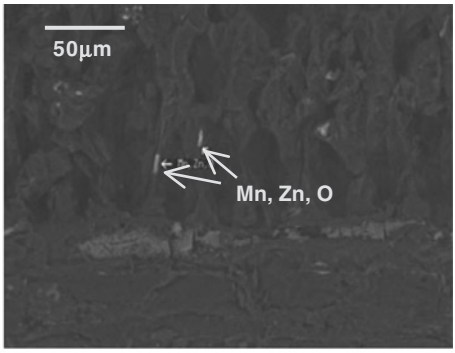
d: Ca oxalate crystals (5-10µm) within needle from western hemlock (*Tsuga heterophylla*)

Fig. 2-3. Development of calcium oxalate crystals within plant tissues – (a) Cross section of twig from lodgepole pine (*Pinus contorta*). Crystals concentrated where bark is forming around new growth; (b) Cross section of twig of western hemlock (*Tsuga heterophylla*). Bark at top is crowded with crystals; (c) Ca oxalate crystals (5–10 µm) formed in twig of black spruce (*Picea mariana*); (d) Ca oxalate crystals (5–10 µm) within needle from western hemlock (*Tsuga heterophylla*).

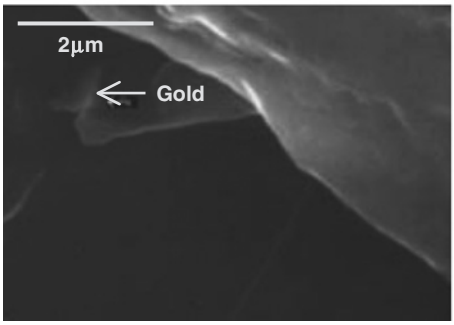
- Au–Ni phase. This is of interest, because mineralogy texts do not indicate any such phase as having been recognized in rocks. Consequently, it appears that this phase has nucleated from the organic acids that coursed through the plant's xylem and phloem cells.
- Bi phase. The X-ray analysis did not indicate any other element associated with the Bi. ICP-MS analysis of the bark revealed Bi concentrations well above usual background values of 20 ppb Bi.
- Linear smear of Fe oxide (with a trace of Cl), about 100 µm long in a twig of mountain hemlock. The smear may be the result of cutting with a diamond knife, but other FeO phases (notably spherules) within the twig indicate that the mineral phase formed within the twig.



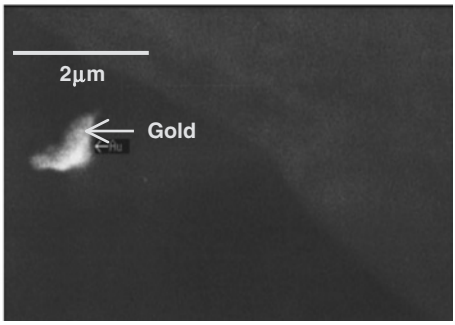
4a: Crystalline Fe+Zn sulphide phase in twig of western hemlock (*Tsuga heterophylla*)



4b: Crystalline Zn+Mn oxide in needle of lodgepole pine (*Pinus contorta*)



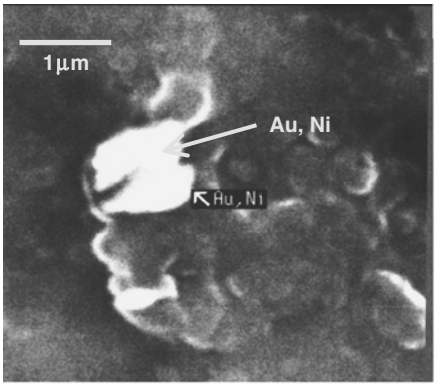
4c:Crystalline gold within mountain hemlock bark (*Tsuga mertensiana*)



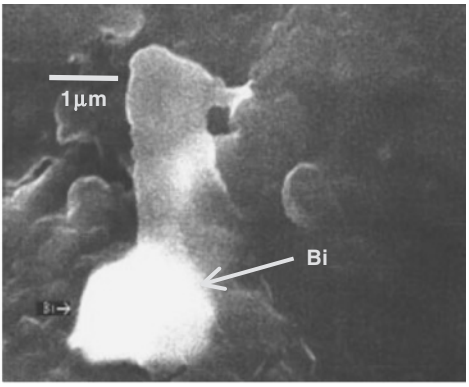
4d: Crystalline gold within mountain hemlock bark – backscatter image. Same field of view as 4c.

Fig. 2-4. SEM photomicrographs of crystalline metal mineral phases within plant tissues – (a) Crystalline Fe + Zn sulphide phase in twig of western hemlock (*Tsuga heterophylla*); (b) Crystalline Zn + Mn oxide in needle of lodgepole pine (*Pinus contorta*); (c) Crystalline gold within mountain hemlock bark (*Tsuga mertensiana*); (d) Crystalline gold within mountain hemlock bark – backscatter image. Same field of view as 4c (modified from Dunn, 1995b).

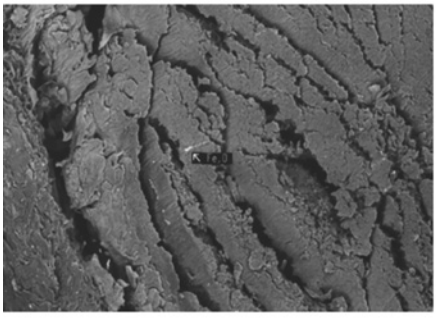
- Irregular 3 µm diameter patch of Cu, Fe oxide in mountain hemlock bark.
- As, Fe, S phase (arsenopyrite?) located in the phyllogen, close to the cambium, of a sagebrush twig from the near the Nickel Plate (Au skarn) mine, Hedley, British Columbia. The same specimen contained Fe spheroids in the radial parenchyma (not shown here).
- Ta, Sn phase with peculiar protuberances of different composition from near the Bernic Lake rare metal pegmatite mine (Li, Ta, Nb, Be, Cs, Rb), Manitoba. A backscatter image is shown on the right half of the photomicrograph. At other localities nebulous aggregations of Sn oxide and Sn chloride have been identified – for example in a sagebrush twig from near the Nickel Plate mine.



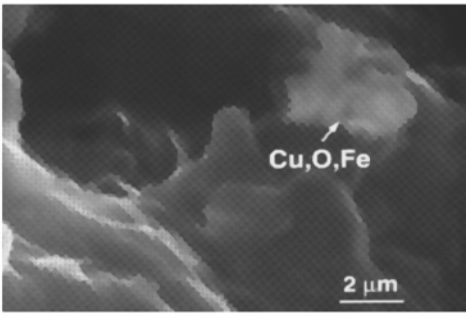
5a: Au-Ni phase in bark of mountain hemlock (Dunn, 1992)



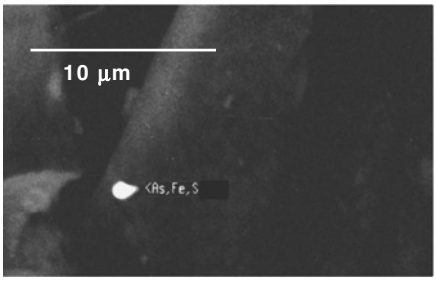
5b: Bi phase in bark of mountain hemlock



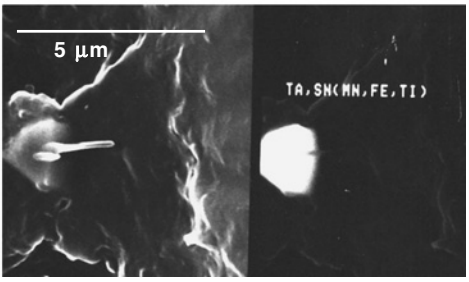
5c: Cross section of conifer twig. Bright streak is smeared iron oxide phase ~100 μm (Dunn, 1995)



5d: Cu, Fe oxide in bark of mountain hemlock



5e: As, Fe, S phase in sagebrush (*Artemisia tridentata*)



5f: Ta, Sn phase in alder twig (*Alnus crispa*)

Fig. 2-5. Heavy metal phases in plant structures – (a) Au-Ni phase in bark of mountain hemlock (Dunn, 1992); (b) Bi phase in bark of mountain hemlock; (c) Cross section of conifer twig. Bright streak is smeared iron oxide phase ~100 μm (Dunn, 1995); (d) Cu, Fe oxide in bark of mountain hemlock; (e) As, Fe, S phase in sagebrush (*Artemisia tridentata*); (f) Ta, Sn phase in alder twig (*Alnus crispa*).

Other phases that have been observed include the following:

- Cu–Al oxide phase (0.75 μm in diameter) within the cortex of a mountain hemlock needle,
- Iron oxide spherules in twigs and needles of mountain hemlock,
- Calcium phosphate in mountain hemlock twigs,
- Barium sulphate phase ~8 μm in diameter in bark of mountain hemlock and also in needles of Douglas-fir,
- Oxides of Ni, Sn and Zn in the bark of mountain hemlock (all <2 μm),
- P and Bi chlorides in the trunk wood of mountain hemlock,
- Zinc oxide in trunk wood of mountain hemlock,
- Cesium silicate in the stomata of a western hemlock needle,
- Ni, Fe and As phases in twigs of sagebrush, and
- Crystalline Zn (sulphide?) in a sagebrush twig.

There is a wealth of information yet to be obtained from SEM studies of heavy metal phases concentrated within plant structures, and there appears to be little published research on these phases. The important point to note is that in addition to the chemical evidence of metal concentrations in plant tissues, there is an abundance of visual evidence when tissues are viewed and analysed with instrumentation that can resolve micron-sized features. There is compelling evidence that minerals nucleate within plant tissues.

Chapter 3

FIELD GUIDE 1: CLIMATIC AND GEOGRAPHIC ZONES

SELECTION OF PLANT SPECIES

This section summarizes the common plants to be found throughout various climatic regions of the world. Many, but by no means all, of those listed have been tested for their effectiveness as biogeochemical exploration sampling media. The lists are far from exhaustive; they are intended as a general guide to what to look for in each region, and provide a starting point for conducting a survey in any given area. Since this book is prepared primarily for those involved in mineral exploration and therefore those with more of a geological background than a botanical or environmental training, the plants are referred to by common names with the Latin binomials added for those seeking more comprehensive information. For many geologists this is the easiest way to learn about the plants, and field assistants generally relate better to simple descriptions and common names. Nowadays, thanks to the internet, it is a speedy process to use a search engine to find a wealth of information about any of the plants listed below, including plates in full colour. Addresses of key web sites are provided that should assist in finding detailed information on the plants that are described.

Work carried out in the former Soviet regime included some extensive compilations of analytical data (mostly by emission spectroscopy) on a wide variety of plant species from the Siberian tundra southward through the various vegetation zones to the Caspian Sea. The emphasis, however, was on the boreal forests of Siberia. Although in many cases details of relationships to specific types of mineralization were not always obvious (lack of scale and location, because of the perceived political sensitivity of the information at the time), extensive lists of species relevant to biogeochemical exploration were published (e.g., Kovalevsky, 1987, 1995b, 2001; Kovalevsky and Kovalevskaya, 1989). Typically, such lists were organized by plant species and tissue types (or 'bio-objects' as they were sometimes referred to) according to their considered sensitivity to mineralization. The lists are sorted mostly from non-barrier species (plants that can pick up high concentrations of metals, generally in proportion to concentrations in the ground – see Chapter 1) to high-barrier (plants that establish barriers at their roots to the uptake of metals). These lists provide useful guides, but they are restricted to plants found throughout the Russian states, and on occasion a plant listed as 'high-barrier' (i.e., non-informative) has been found to be quite informative in locations outside of the Russian environments studied by Kovalevsky and others. This is probably because of

different environmental and/or geological conditions that have permitted the passage of elements into plant structures classified as, for example, non-informative in Russia, yet have proved to be informative elsewhere.

An example of this situation is that of uranium. Out of 65 plant organs studied by Kovalevsky (1987), 97% were classified as exhibiting strong barriers to U uptake, and none fell into his 'non-barrier' category. However, other studies from around the world have reported high concentrations of U in plant tissues (summarized in Dunn et al., 1985), demonstrating that barriers to the translocation of U from soils to roots, twigs, and, to a lesser degree, into foliage can quite commonly be minor. In northern Saskatchewan, Canada, the U-rich Athabasca Sandstone has an extensive cover of pine and spruce. Twigs of black spruce (*Picea mariana*) have been found to accumulate high levels of U over deeply buried U deposits, even though the soils in which they grow have only background levels of U (Dunn, 1983b). However, concentrations in needles growing on these twigs are substantially lower – usually by an order of magnitude – and so the needles establish a high barrier to U translocation.

The topic of 'relative' uptake of metals by different tissues and species is discussed later, but it is of relevance to point out that in the case of U and the barriers discussed here, the *patterns* of relative uptake remain quite constant. Figure 3-3 compares concentrations in the ash of twigs and needles, each from a single tree, collected along an 8-km traverse near the eastern margin of the uraniferous Athabasca Sandstone Group in northern Saskatchewan. Although there are substantially different concentrations in the twigs versus the needles from those twigs, the profiles are very similar.

This example leads to a concept of fundamental importance to biogeochemical exploration, involving pattern recognition.

- Similar distribution patterns of a single element may be derived from sampling different sample media provided sampling procedures remain consistent for each medium.

To elaborate, the close correspondence between the analysis of twigs and needles illustrated in Fig. 3-1 confirms that provided a set of samples is collected consistently, and data from different species and/or tissues are not combined into a single dataset (unless normalized), then areas of anomalous concentrations can be outlined regardless of whether twigs or needles are collected. This attests to the robustness of the biogeochemical method for mapping element distributions in many environments.

The recognition of spatial relationships of different elements to mineralization, usually from the analysis of a single species, is a key step in the interpretation of biogeochemical data. This point will be discussed later.

Orientation

The first step in preparing to conduct a biogeochemical survey is to look for the most widespread species within the area of interest, then determine from published

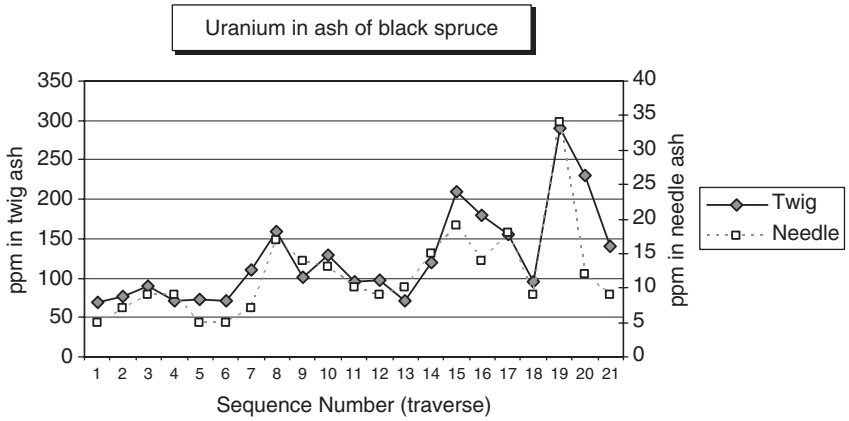


Fig. 3-1. Uranium in ash of black spruce (*Picea mariana*) twigs (10 years growth) and needles along a traverse near the eastern edge of the Athabasca Sandstone Group, Saskatchewan, Canada (modified from Dunn, 1983c). Analysis by INAA.

information if that species is likely to be informative (i.e., do the easy-to-reach parts accumulate the elements of interest, and have they been shown elsewhere to outline the presence of mineralization?). If published information is not available, and if time is not too critical (and assuming that the survey area can be easily accessed), it is advisable to conduct an orientation survey to collect a few samples of common species and submit them for multi-element analysis. This will provide preliminary and valuable insight to the levels of metals present, and help the geologist/geochemist fine-tune the analytical package that should be requested for a larger survey. This knowledge is not a prerequisite, but it will serve to optimise the geochemical contrast that can be provided by selecting the best sample medium. In the case of the U example shown in Fig. 3-1, if needles had been selected as the prime sample medium instead of the twigs, it would not have made a significant difference to the over all patterns of element distribution, even though U concentrations were much lower in the needles. The danger here, though, is that because of the lower concentrations in the needles, some samples from a broader survey area might have yielded values below the limit of detection, whereas the twigs on which the needles were growing might yield useful geochemical relief in the data because of their ability to concentrate U.

Sometimes one species is not sufficiently widespread to sample at every survey station. It is not uncommon for a proposed survey area to extend through several vegetation zones. In the temperate forests of North America there may be, for example, Douglas-fir at low elevations, with lodgepole pine on surrounding slopes, and subalpine fir at higher elevations. Similarly, in the boreal forest there may be a transition from black spruce-dominated forest to jack pine. In these situations it is necessary to establish overlap zones where two species grow, so that samples of both species can be collected at a few sites. By establishing the relative element

concentrations of pairs of species it is possible to normalize the data to a common benchmark. However, because of the differences in element requirements and tolerances among species (and more so among genera), this normalization commonly proves to be a rather imprecise exercise.

As a basic premise, for a given survey the same type of plant tissue should be collected from the same species of tree or shrub, unless there is prior knowledge that no significant chemical differences occur between two or more species. For some plant *species* there may be a negligible difference in the element uptake characteristics. Such is the case for black spruce (*Picea mariana*) versus red spruce (*Picea rubens*), and for some species of *Acacia*. However, for others (e.g., black spruce versus white spruce (*Picea glauca*)) there are some substantial elemental differences.

In a test to compare the chemistry of outer bark from white spruce with that from black spruce, samples were collected from adjacent trees at 12 sites in northern Ontario. Table 3-I shows the average concentration ratios. The data clearly indicate that most heavy elements are more concentrated in the black spruce, notably Bi, Cd, Hg, Ni and Pb. For some elements there is little or no difference (e.g., ratios of 0.9–1.1 for Cu, Na, P, Re, S, Ta). The elements that are consistently more concentrated in the bark of the white spruce include elements essential for plant nutrition (B, Ca, K, Mg and Zn) with associated trace elements that show typical geochemical affinities for these elements – Ba and Sr with Ca; Cs and Rb with K; and Sn, Te and Tl with less obvious associations.

Substantial differences between *species* seem to be the exception rather than the rule, although they must be considered and there are some sobering examples of extreme differences. In the 1950s, Helen Cannon and her co-workers examined the U and Se uptake of a wide range of plants, including various species of poison vetch/locoweed (*Astragalus*) from the Colorado Plateau (Cannon, 1952, 1964). Among species of the same genus (*Astragalus*) they found substantially greater differences in Se uptake than the relatively subtle differences illustrated in Table 3-I.

Differences among many common plant species are usually more subtle than these two examples. However, in light of differences that might occur between other pairs of species, unless the exploration geologist has a firm handle on elemental similarities and differences among species, it is better to play it safe and collect only samples of a single species. Where it is not possible to obtain a systematic coverage of a survey area, a few comparative tests of the sort shown (Table 3-I) can be undertaken and similar ratios established between species. When these ratios are available, it is then possible to normalize the datasets to a common species and establish a meaningful plot of element distribution patterns. Bear in mind, though, that this procedure is not always fully satisfactory, and, because of different barriers to element uptake, may simply not be possible for some pairs of species. Data should be carefully evaluated before jumping to conclusions and merging data from two species into a single dataset.

Among plant *genera* there are commonly substantial chemical differences. In that there are some fundamental morphological and chemical differences between plant

TABLE 3-I

Ratios of elements in outer bark of black spruce (*Picea mariana*) compared to white spruce (*Picea glauca*) – pairs of trees from 12 sites in northern Ontario. Analysis by ICP-MS on an aqua regia digestion

	BS:WS Outer Bark Ave for same site comparisons		BS:WS Outer Bark Ave for same site comparisons
	$n = 12$		$n = 12$
Ag	1.4	Na	0.9
Al	1.3	Nb	1.5
As	1.3	Ni	1.9
Au	1.2	P	1.1
B	0.8	Pb	1.8
Ba	0.8	Rb	0.5
Bi	1.8	Re	1.0
Ca	0.6	S	1.0
Cd	2.1	Sb	1.4
Ce	1.6	Sc	1.6
Co	1.4	Se	1.5
Cr	1.2	Sn	0.4
Cs	0.8	Sr	0.5
Cu	0.9	Ta	1.0
Fe	1.6	Te	0.7
Hf	1.2	Th	1.5
Hg	1.9	Ti	1.5
In	1.2	Tl	0.7
K	0.5	U	1.6
La	1.6	V	1.6
Li	1.5	W	1.5
Mg	0.8	Y	1.6
Mn	1.3	Zn	0.7
Mo	1.5	Zr	1.4

genera, the normalization process described above may be less robust than for species. This is because a particular genus may establish a more rigorous barrier to the uptake of certain elements than another genus of the same family. Thus, the relationship may not be linear, and so a normalization process may have to involve regression or a more complex mathematical normalization. Clearly, the validity of patterns derived from such processes is increasingly tenuous on moving up the hierarchy of plants: species compared with species may well be possible; genus compared to genus is less robust.

Boreal (northern) forest

This section elaborates on some features of plants that are common in northern forests, and introduces some details on considerations that should be made during the collection of a few of the most common species from this environment. More details are given in the next chapter.

Large tracts of the northern latitudes are covered with boreal forest. Fortunately, there are only a few common tree and shrub species that are likely to occur in sufficient abundance to be of use for a biogeochemical survey. This makes the boreal forest one of the most favourable regimes for biogeochemical prospecting. The most common conifers are spruce, pine, fir and tamarack. Birch and aspen are the dominant deciduous trees and the most common shrubs are alder, willow and Labrador tea. Discussions in this section focus on species present in North America, but a similar range of trees and shrubs occurs across vast tracts of Siberia, Russia and Scandinavia. For detailed lists of species the reader is referred to the extensive publications by Kovalevsky, of which much of the key information is summarized in English in Kovalevsky 1987 and 1995.

For those contemplating a biogeochemical survey as an exploration option in the boreal forest, and who have not much knowledge of tree identification, a useful first step is to learn to differentiate between the three principal conifers found in the northern forests – spruce, pine and fir. They do have characteristic profiles (e.g., a bulbous top to black spruce, and a 'layered branch' appearance to balsam fir), but these are not fully diagnostic and serve as a first pass to zero in on the desired species. Closer examination of twigs and needles reveals some marked differences. Figure 3-2 shows typical examples of the three genera, from left to right fir (*Abies*), pine (*Pinus*) and spruce (*Picea*).

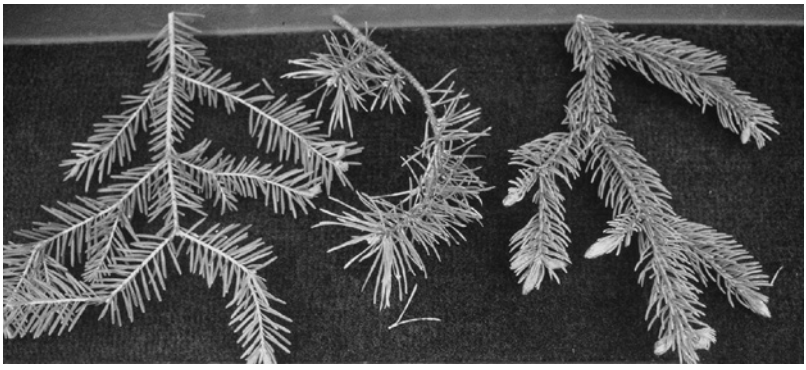


Fig. 3-2. The three principal conifers of the northern forests: fir (left), pine (centre) and spruce (right).

- *Fir* (left) has flat, soft needles. The photo shows 5 years of growth, with a new pair of branches each year.
- *Pine* (centre) has long sharp needles. The number of years of growth is more difficult to discern. This sample is about 3 years old.
- *Spruce* (right) has short, abundant and usually spiky needles (especially white spruce; black spruce needles are softer). The photo shows 4 years of growth.

A short list of the most favourable species for biogeochemical prospecting in the boreal forests of North America includes the following.

- Black spruce (*Picea mariana*)
- White spruce (*Picea glauca*)
- Red spruce (*Picea rubens*) [only present in eastern North America, notably in the Maritime Provinces where it is the Provincial tree of Nova Scotia; chemically, it is very similar to black spruce with which it is thought to hybridise]
- Jack pine (*Pinus banksiana*)
- Tamarack (*Larix laricina*)
- Balsam fir (*Abies balsamea*)
- Paper birch (*Betula papyrifera*)
- Mountain alder (*Alnus crispa*)
- Speckled (river) alder (*Alnus rugosa*)
- Labrador tea (*Ledum groenlandicum*)
- Aspen (*Populus tremuloides*)

Out of the above, the black spruce, balsam fir or mountain alder are the preferred species, and aspen is the least useful. Jack pine can also be very informative (as can all of the above to lesser or greater degrees), but in many areas it is less widespread than the spruce or alder.

In the boreal forest of Sweden, the principal biogeochemical sample medium recognized and utilized by the Geological Survey of Sweden (SGU) is stream peat – organic material consisting of aquatic mosses and roots of aquatic higher plants. They are barrier-free with respect to trace metal uptake and integrate the long-term geochemical signature of stream water (Brundin and Nairis, 1972; Brundin et al., 1987; Selinus 1988; Lax and Selinus, 2005). The biogeochemical database at SGU contains analyses of more than 30 elements in 35,262 samples (Lax and Selinus, 2005). Most samples are composed of (in descending order) roots of sedge (*Carex* L.), willow moss (the bryophyte *Fontinalis antipyretica*) and the roots of meadowsweet (*Filipendula ulmaria*). The last mentioned is a member of the rose family and, like other members of this family, it contains cyanogenic glycosides. It is important to be aware that plants containing these compounds should not be reduced to ash if accurate concentrations of Au are required. This is because during the dry ashing process, Au may combine with the cyanogenic compounds (of which

there are many) to form Au cyanide that volatilizes long before the usual ashing temperature of about 475°C is reached (Girling et al., 1979).

Black spruce

This tree is extremely widespread in the boreal forests and has proved to be one of the most responsive species to metal enrichments in the substrate, commonly accumulating higher levels of many elements than most other species. It tends to accumulate a wide range of elements in its tissues, and can therefore be considered the 'biotite' of the botanical world, because by analogy biotite can incorporate in its crystal lattice the 'left-over' elements from a rock-forming liquid.

Black spruce is dealt with here in some detail and serves as an example of how to go about collecting a sample and why certain recommendations for collection are made. This sets the scene on the thought process that needs to be employed in biogeochemical sample selection and collection (see Chapter 4).

Black spruce occurs in the boreal forests of North America and in stunted form in the tundra. A field method to help in identifying this tree is to use a $\times 10$ magnification hand lens to examine the twig surface. If it appears hairy, then it is a black spruce (red spruce is similar, but only occurs in eastern North America); if it is smooth, with no hairs, it is a white spruce.

Figure 3-3 shows scanning electron micrographs, at two magnifications, of the surface of a black spruce twig. In the left hand photomicrograph the tentacle-like protuberances are the hairs; the ultra-fine anastomosing web is a network of fungal hyphae. Fungal hyphae, only $\sim 2\,\mu m$ in diameter, extend continuously at a rate that can be extremely rapid – up to $40\,\mu m$ per minute under optimal laboratory conditions. Each hypha is supported by the continuous movement of materials into the tip from older regions of the hyphae. So a fungal hypha constitutes a continuously moving mass of protoplasm in a continuously extending tube, which, on a micron scale, is continuously redistributing the elemental composition of the twig surface. In Chapter 1 it was shown that macro-fungi are capable of concentrating

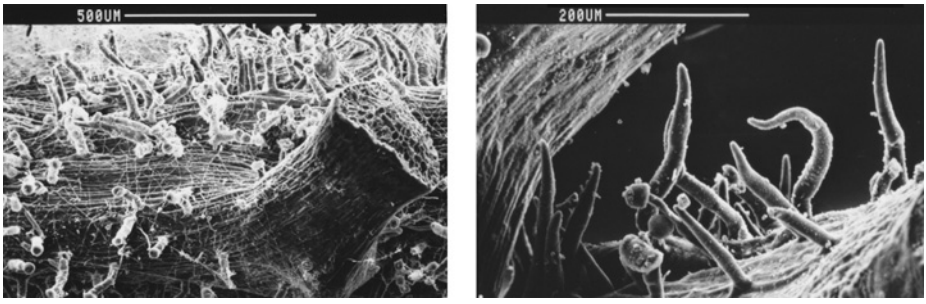


Fig. 3-3. Scanning electron micrographs of the surface of a black spruce twig. Left: stalk to which a needle attaches, and fine hairs that are characteristic of black spruce twigs (~0.2 mm), with ultra-fine web-like fungal hyphae. Right: close up of the 0.2 mm hairs.

extraordinarily high levels of many metals. It is to be expected, therefore, that in the micro-environment of a twig surface there is a constantly evolving chemical composition and metals are being drawn from the twig surface. If, therefore, a sample is washed prior to analysis, a considerable portion of the fungal filaments may be removed, thereby removing some of the natural geochemical signature of the twig. This factor needs to be considered when deciding whether or not to wash a sample, and is discussed in detail later (Chapter 6).

In the right hand close-up photomicrograph of Fig. 3-3 the fine hairs, mostly 0.1–0.2 mm in length, give the effect of a surreal landscape and show that these little hairs have even finer protuberances.

In most environments of the boreal forest, a 25–30 cm length of black spruce twig comprises ~10 years of growth. This is an appropriate amount to collect, because variations in the geochemical signature over 10 years are integrated. Figure 3-4 shows U concentrations in dry black spruce twigs, plotted against the age of the twig.

These variations may be caused, for example, partly by a relatively wet and/or hot summer, but more importantly the changing ratio of twig wood to twig bark as the trees grow. For those elements, such as U, that concentrate in the bark, the older the twig the higher the proportion of wood (which has low U concentrations) compared to bark (high U concentrations).

A definitive study to demonstrate the location of U in the plant structure was undertaken by Apps et al. (1987), using fission track mapping of black spruce twigs. One of the samples tested was a U-rich twig that was provided to the authors from a 1981 collection made by the Saskatchewan Geological Survey. The tree from which the sample was collected was growing in virgin forest near the eastern margin of the Athabasca Sandstone, some 10 km from the Rabbit Lake U mine. At the time of collection Rabbit Lake was the only mining operation within a radius of 250 km. Chemical analysis of that sample had yielded 1260 ppm U in ash (approximately

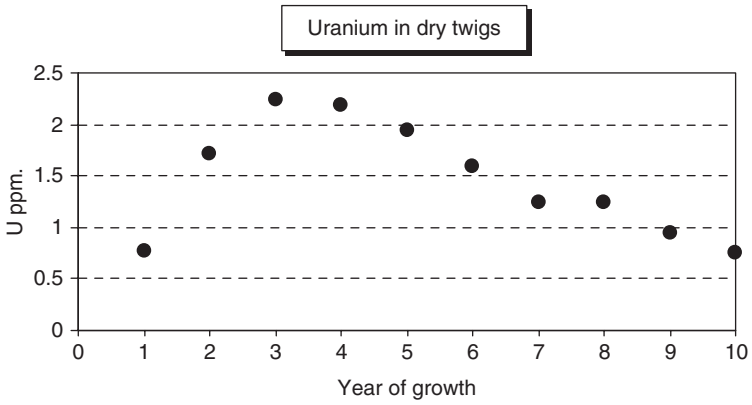


Fig. 3-4. Uranium in dry twigs, dissected into years of growth. Black spruce from the Athabasca Sandstone, northern Saskatchewan.

25 ppm U in dry tissue) and so it was sufficiently enriched to be a good candidate for the fission track analysis.

The basic procedure involved placing Lexan polycarbonate plastic in contact with a clean flat surface of twig and irradiating in a SlowPoke nuclear reactor for 4 h. Full details are given in Apps et al. (1987). The images (Fig. 3-5) show on the left a photograph of the twig cross section. The rings define approximately 10 years of growth, marginal to which are the cork cambium (pale grey with no obvious structure) and the dark outer bark (the rhytidome). The bark is defined as all tissue outside the vascular cambium – a very thin layer (comprising only 2–4 cells) separating it from the inner woody xylem (i.e., growth rings). The dark area at the bottom of the cross section is an emerging small branch. The figure on the right shows the fission track image of the same field of view. The dark areas show the greatest irradiation etching, and from this it is evident that the great majority of the U (>90% from the authors' calculations) occurs in the outer bark. Even the bark forming around the small twig that is branching at the bottom of the photograph is defined by the intensity of the irradiation damage.

This example provides compelling evidence that the U is not concentrated in the woody tissue and, as the authors point out, the pattern shown in the previous figure

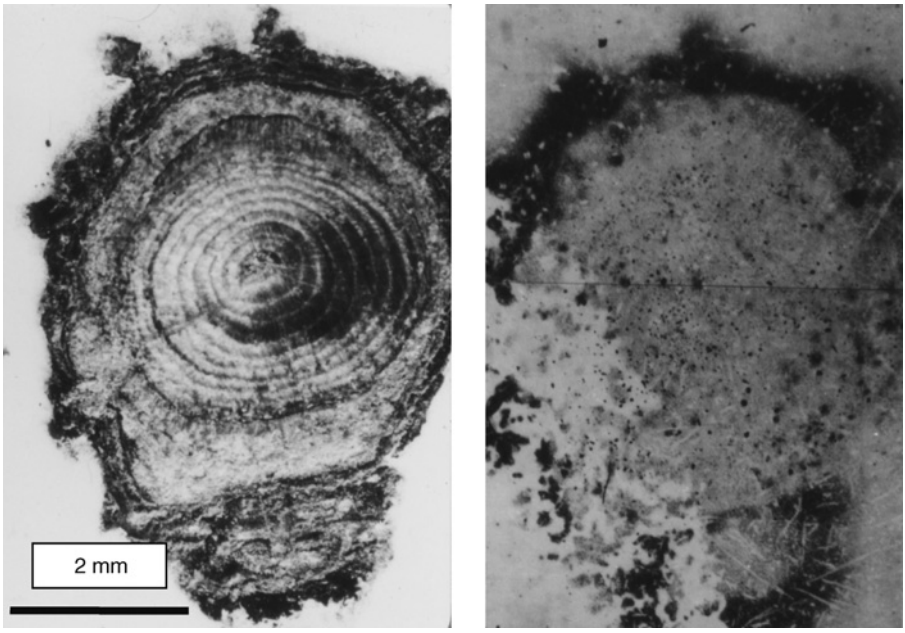


Fig. 3-5. Cross section of black spruce twig rich in uranium. Left: photograph to show plant structure; right: fission track image showing areas (black) of radioactive damage from the U content of the tissues. Annotated from photographs courtesy of Dr. Mike Apps.

(Fig. 3-4) of decreasing U concentration with increasing age can be explained by the steadily increasing biomass with age – i.e., as the twig grows the ratio of bark to woody tissue increases. Given this phenomenon, it is evident why it is of importance in a biogeochemical survey to maintain a constant thickness and length of twig at each sample station. In a given survey using twigs, the length and thickness of twig that is collected is not critical provided a consistent sample is obtained at all sample stations. Some surveys have mistakenly collected only the thick part of a twig. Although this does not optimize the element concentrations, it gives a level playing field in that the distribution patterns can be plotted with confidence that a consistent sample medium has been collected. The collection should be viewed in the same way as the collection of soils: there is no point in comparing the analysis of a −80 mesh fraction from one sample station with a –250 mesh fraction from another.

Depending upon how vigorous the black spruce twig growth is, five to seven live twigs from around the circumference of a tree usually provides more than enough representative material. Dead twigs should be avoided, because they may have partially shed their bark and, as noted, there are substantial differences between the chemistry of the bark and that of the twig wood. In areas of particularly vigorous growth (southern boreal forests) or of stunted growth (dwarfed trees of the tundra) it may be practical to collect a lesser or greater number of years of growth, respectively. Needles do not need to be removed from the twigs in the field, because once they are dried in the laboratory they readily fall off. As an alternative the outer bark can be collected as a suitable sample medium. More details on the selection of plant tissues are given in the next chapter.

A question often asked is, should more than one tree be sampled and the twigs pooled into one sample to give a more representative evaluation of the ground chemistry? Ideally, the answer is yes; however, in the real world some complications may arise. Firstly, by pooling several twigs from several trees quite a large sample needs to be processed, and so costs are increased. Secondly, whereas at one sample station there may be several appropriate trees for sampling, at another there may be only a single tree. This would introduce a bias to the sampling. Studies have indicated that there is little or nothing to be gained from sampling more than one tree. The key factor is to be consistent.

Balsam fir

This coniferous tree (*Abies balsamea*) is common eastward from north-eastern British Columbia, across the northern forests to the Maritime Provinces, and into the north-central United States. A related species, the subalpine fir (*Abies lasiocarpa*) from central and western British Columbia, is also sometimes referred to as balsam fir. Reconnaissance surveys have been conducted covering some 20,000 square kilometres of Nova Scotia using twigs of balsam fir as the sample medium (e.g., Dunn et al., 1989, 1994a, 1996a,b).

Unlike the spruce in which many elements are significantly more concentrated in twigs than needles, the difference between these two types of tissue in balsam fir (and firs in general) is less pronounced. Cohen et al. (1987) found dry balsam fir needles to comprise an effective medium for delineating Au mineralization at Hemlo in Ontario. Similarly, surveys in the Bathurst Camp of New Brunswick have made effective use of balsam fir needles. Fir samples need to be thoroughly dried before attempting to separate the needles, because there is a tenacious tissue (the abscission tissue) that holds needles to the twig. Once dried, they separate quite easily. The thin, smooth bark, with its many blisters of sticky sap (the yellow, oily resinous 'Canada balsam' that is used for mounting petrographic thin sections) is not a suitable sample medium, because it is both messy and poorly informative.

Alder

In the boreal forests of North America there are two species of this large shrub (typically 2–3 m, but it can be up to 5 m tall). To the non-botanically minded field geologist it can be recognized as the common, tall shrub that is frustrating to try and walk through, because of the springiness and moderate density of the stems which impede progress.

Mountain, sitka or green alder (*Alnus crispa*) is common to relatively dry areas, whereas river or speckled alder (*Alnus rugosa*) occurs in wet areas. The mountain alder is known by various other botanical names, but the consensus among taxonomists is generally the species name *crispa*.

Mountain alder is usually the more widespread of the two species and, although they have only minor chemical differences (more Co in twigs of the river alder) it is preferable to collect just one species. A twig length of ~30 cm is commonly 3 years of growth. For most exploration purposes it is best to collect only this newer growth (shiny reddish surface), because that is where many trace metals of interest (e.g., Au and notably Mo) are concentrated. Older growth has grey bark and contains relatively high concentrations of Zn, Ca, Ba and REEs. Either twigs or leaves can be used, but the chemistry of leaves changes quite rapidly early in the growing season so a survey at that time should be conducted in as short a time as possible – preferably no more than 2 weeks, once the leaves have grown to their full size.

Ways for the non-botanist to quickly differentiate between the two species in the field are as follows:

1. New growth of mountain alder has reddish twigs; twigs of river alder are green.
2. Leaves of mountain alder have a finely serrated margin; leaves of river alder are much more jagged.
3. Examination of a snipped cross section of an alder twig will usually reveal a small brownish triangle in the middle of the river alder; this is white in the mountain alder.

Temperate coniferous forest

Temperate rain forests only occur in seven regions around the world: the Pacific Northwest, the Valdivian forests of southern Chile, southern New Zealand and Tasmania, northwest Europe, southern Japan and between the Black Sea and the Caspian Sea.

In most temperate coniferous forests, evergreen conifers predominate, while some are a mix of conifers and broadleaf evergreen trees and/or broadleaf deciduous trees. Temperate evergreen forests are common in the coastal areas of regions that have mild winters and heavy rainfall, or inland in drier climates or mountain areas. Many species of trees inhabit these forests including cedar, fir, spruce, pine, hemlock and Douglas-fir, with lesser numbers of juniper, cypress, redwood and yew. In the southern hemisphere dominant trees are pine, kauri and podocarpus. The understory also contains a wide variety of herbaceous and shrub species, such as salal, buffaloberry ('soopalallie') and alder in western North America.

The largest area of temperate forest is western North America, extending from northern California to southern Alaska. The diversity of flora is greater than that of the boreal forest, making this a more complex area within which to conduct biogeochemical surveys. The dominant north–south trend of the mountain ranges and valleys in western North America gives rise to different climatic zones within which there are considerable differences in rainfall and temperature. The result is a series of north–south-trending zones of vegetation that have been defined as biogeoclimatic zones – i.e., an ecological classification based on vegetation, soils and climate (Pojar et al., 1987). Each zone has its characteristic flora, and locally, within a distance of only a few hundred metres, because of change in elevation or aspect there may be a different assemblage of trees. However, on a property-scale there are a number of common tree species that, according to the biogeoclimatic zone, can be of considerable use to a geochemical survey.

- Lodgepole pine — *Pinus contorta*
- Ponderosa pine — *Pinus ponderosa*
- Pacific silver fir — *Abies amabilis*
- Sub-alpine fir — *Abies lasiocarpa*
- Western hemlock — *Tsuga heterophylla*
- Mountain hemlock — *Tsuga mertensiana*
- Engelmann spruce — *Picea engelmannii*
- White spruce — *Picea glauca*
- Douglas-fir — *Pseudotsuga menziesii*
- Western redcedar — *Thuja plicata*
- Western larch — *Larix occidentalis*

In different climatic settings there occur other species of pine, spruce, fir, larch, yew and cedar. Of the many deciduous species that may occur, red alder, birch, maple,

willow and poplar are the most common. The choice of sample medium is guided by temperature and moisture regime, and the elevation. In coastal regions at moderate to low elevations Douglas-fir, western redcedar and western hemlock are three of the most common and useful tree species. Farther inland and at higher elevations there is lodgepole pine (one of the most useful trees), Pacific silver fir, mountain hemlock, subalpine fir, Engelmann spruce and white spruce. In the drier interior there is Ponderosa pine, characterized by its soft, thick orange to grey bark that falls off in large scales. For most of these species either the twigs with foliage (maintaining a consistent number of years of growth) or the outer bark can be sampled.

The Scandinavian coastal conifer forest is made up mostly of Norway spruce (*Picea abies*) and, to a somewhat lesser extent, Scots pine (*Pinus sylvestris*). Juniper (*Juniperus spp.*) is also common. There is a rich understory of mosses and ferns, and there are also broad leaf trees scattered in this forest, including birch, willow, poplar and alder.

The Sierra Juárez and San Pedro Mártir pine-oak forests is a small ecoregion that covers the higher elevations of these ranges of the northern Baja California Peninsula in Mexico, near the border with California. The forests are predominantly pine (10 species), juniper, fir and oak (5 species).

In South America, the Valdivian and Magellanic temperate rain forests are the second largest in the world, after the Pacific temperate rain forests of North America. They share many plant families with the temperate rain forests of New Zealand, Tasmania and Australia, and vary from dominantly deciduous to evergreen forest (notably southern beech, *Nothofagus betuloides*), to coniferous with the monkey-puzzle (*Araucaria*) and *Podocarpus nubigena*.

In the temperate forests of southern Australasia, pine and southern beech are two genera of plant that are among those most studied for their biogeochemical exploration potential. Timperley et al. (1970, 1972) provide discussion and details of various species from New Zealand. Their findings are summarized in Brooks (1983).

Temperate deciduous forest

Most temperate deciduous forests are located in the eastern United States, Canada, Europe, China, Japan and parts of Russia. Numerous studies have been conducted in these areas, commonly with an environmental bent, and results include a wealth of information from many European countries that can be of use to the explorationist.

Biogeochemical surveys in deciduous forests are commonly more difficult to conduct than those in the coniferous forests. Typically, the deciduous forest has a diverse flora and inconsistent distributions of species such that the desired species may be absent from many of the proposed sample stations. Commonly, tree bark is smooth, branches are out of reach and growth increments are irregular and more difficult to discern than those of the conifers. Furthermore, many tree species

(poplar, maple, oak, elm, beech and red alder) take up relatively low concentrations of the elements that are of interest for locating mineral deposits. Exceptions are willow, birch, beech and sometimes oak. As a broad generalization, the trees of the temperate deciduous forest generate biogeochemical anomalies that are of relatively low magnitude, and therefore more difficult to detect. Leaves may be more informative than the twigs (especially for Hg), but surveys should be conducted within a short time frame because of the rapid changes in leaf chemistry that take place.

Growing amongst the trees, there is commonly an understory of shrubs and bushes that may be both more informative than the trees and more practical to collect. Shrubs that have been used with some success include beaked hazelnut (*Corylus cornuta*) and red-osier dogwood (*Cornus stolonifera*).

Locally, there may be many other possible choices of shrub that could be used. Since comprehensive databases on many plant species are not available, when confronted with a field situation where data are lacking for a widespread species, an orientation survey to sample that species (or several species) should be conducted. Provided appropriate precautions are taken to collect similar growth from the same species at each test site, the sensitivity of a plant to mineralization or to different substrates and environmental conditions can be determined. Although there are some plant species that are better than others for biogeochemical surveys, there are few (or, perhaps, none) that are completely uninformative when chemically analysed.

In temperate forests of Australia and New Zealand some of the more important species of use are

- Monterey pine — *Pinus radiata* (especially the outer bark)
- Blackwood — *Acacia melanoxylon*
- 'Cauliflower bush' — *Cassinia aculeate*
- Southern beech — *Nothofagus* (especially leaves)

Tundra

The shrubby groundcover of the tundra is dominated by very slow-growing dwarf spruce, birch and willow. Species that are commonly the most useful are the following:

- Black spruce — *Picea mariana*
- Dwarf birch — *Betula glandulosa*
- Labrador tea — *Ledum groenlandicum, L. palustre, L .decumbens*
- Black crowberry — *Empetrum nigrum*
- Leather leaf — *Chamaedaphne calyculata*
- Bearberry — *Arctostaphylos alpina*
- Bog blueberry — *Vaccinium uliginosum*
- Willow — *Salix lanata and other willow species*

Many other genera occur, but they are likely to be only of local use. In general, collect all of the above-ground (i.e., aerial) portions of the small shrubs. Separation of tissues can best be undertaken in the laboratory. Data on these species are provided in Reading et al. (1987) and Coker et al. (1991).

Arid and semi-arid environments – general comments

The root systems of many plants from arid regions penetrate deep into the substrate. Live roots have been found in mine excavations 60 m beneath the surface. The volume of ground from which they extract metals is therefore enormous, and plant composition represents an integration of the geochemical signature of many cubic metres of soil, rock and groundwater. Consequently, with the exception of the rare extreme environments such as the Atacama Desert that are too arid to support even small shrubs, biogeochemical methods have wide application in arid environments. Many species have tough thorns, so it is advisable to wear strong leather gloves.

North America – arid and semi-arid

In the arid and semi-arid regions of western North America some of the more common species that have been used include the following:

Northern basin and range

• Sagebrush	*Artemisia tridentata*
• Shadscale	*Atriplex confertifolia*
• Greasewood	*Sarcobatus vermiculatus*
• Rabbit brush	*Chrysothamnus nauseosus*
• Hopsage	*Grayia spinosa*
• Horse brush	*Tetradymia glabrata*
• Mountain mahogany	*Cerocarpus betuloides*

A long list of additional plants that have been used in U exploration in this environment is given in Cannon (1952, 1964).

Southern basin and range, and desert of the southwestern USA and Mexico

• Mesquite	*Prospopis juliflora*
• Creosote	*Larrea divaricata*
• Acacia ('catclaw')	*Acacia greggii*
• Match brush	*Gutierrezia lucida*
• Palo verde	*Cercidium microphyllum and C. floridum*

Central America

In Central America the Monterey pine (*Pinus radiata*) is a useful sample medium (Honduras). In Costa Rica, high concentrations of Au are recorded in the tree 'chaparro' (locally known as 'raspa' – *Curatella americana*) – Siegel and Pagagua (1991). Chaparro has a large geographic range that includes Cuba, Hispaniola, southern Mexico, Honduras, Belize, Nicaragua, Costa Rica, Panama, Guatemala, El Salvador, French Guiana, Suriname, Guyana, Trinidad, Venezuela, Colombia, Peru, Brazil and Bolivia.

North Africa-arid – southern Morocco into the Sahara Desert

Many of the species common to these areas (pines, figs, palms, oleanders, rock rose [*Cistus spp.*]) extend throughout the Balkans and into the Middle East where acacia becomes one of the most common trees.

The following short lists are for species sampled in Morocco for which biogeochemical data are available (Brooks et al., 1995; Dunn et al., 1996c). They were found to be the most widespread species in the areas studied, and they are reported to be present in many countries bordering the Mediterranean and in the Middle East.

- *Anvillea garcinii* (Arabian oxeye)
- *Convolvulus trabutianus* (morning glory)
- *Ononis natrix* (goat root)
- *Lavandula spp.* (lavender). There are several species, of which the most common is *L. multifida* (fern leaf lavender) and considered a weed because of its ability to grow in a wide range of environments. This ubiquitous feature is advantageous for a biogeochemical sample medium
- *Artemisia herba-alba* (sagebrush or wormwood)
- *Haloxylon* (salt tree)

Mediterranean coast

Lavandula spp. (lavender). The *L. multifida* (described above) and *L. stoechas* (French lavender) both occur. *L. stoechas* is the more common, and grows throughout the Mediterranean region. It is found, also, in Australia where it has been declared a 'noxious weed' in Victoria

- *Erica arborea* (heath tree), found throughout the Mediterranean
- *Pistacia terebinthus* (terebinth or turpentine tree)
- *Tetraclinis articulata* (sandarac [national tree of Malta])
- *Quercus ilex* (holly oak)
- *Cistus* and the similar genus *Halimium* (both known as rock rose)

Middle East – from Turkey and Armenia, through Saudi Arabia to Pakistan, and Africa east of the Sahara

A short list of common plants that could be used for biogeochemical exploration includes *Acacia tortilis* and *Acacia negrii* (and other Acacia species), *Juniperus excelsa* (juniper), *Olea europaea* (olive tree), *Cleome* (spider flower), *Dodonaea viscosa* (hopbush), *Pulicaria crispa* (with the appearance of a small sun-flower or large daisy), *Rumex limoniastrum* (a type of dock or sorrel; this is a weed with sour leaves that are unpalatable to most animals, so it is quite common), *Astragalus atropilosus* (poison vetch) and *Achillea biebersteinii* (similar to the common yarrow of North America).

Armenia has a particularly rich flora that ranges from wet forest to desert steppe with more than 3500 plant species representing more than half of the 6000 contained within the entire Transcaucasus region. There are 17 vegetation zones ranging from desert plants to oak, beech and pine forests, wet marshland and sub-tropic plants to alpine meadows. For the arid regions, *Astragalus spp.* and *Achillea* would be good starting points.

Central, West and South Africa

Some of the earliest geobotanical and biogeochemical studies were conducted in Central Africa (Rose et al., 1979), and in recent years there has been a resurgence of interest in the use of vegetation for exploration in this area. Much of this new information is currently proprietary in nature, so suffice to say that there have been some interesting elemental concentrations discovered, and the biogeochemical method has been achieving considerable success in locating mineralization.

Among the plants for which non-confidential information is available there are various species of acacia including the following:

- *Acacia mellifera* (Black Thorn), widespread throughout much of sub-Saharan Africa and north-eastward into Egypt. One potential problem as a biogeochemical sample medium is that goats are very fond of the young leaves!
- *A. seyal*, a useful strongly gregarious tree species that is tolerant of periodically inundated heavy clays in countries at the southern edge of the Sahara Desert, especially Mali, Chad and Sudan, and southward into Tanzania. The word *seyal* derives from an Arabic word for 'torrent' and denotes association with water courses.
- *A. polyacantha* ('White thorn', 'Falcon's claw' or 'catechu tree') is one of the most attractive species of acacias in Africa, occurring from Ethiopia southward through Kenya, Tanzania and Rwanda.
- *Blepharis* (a wildflower, considered a weed, with no common name), a common species throughout tropical and South Africa (Nkoane et al., 2005).
- *Boswellia* (frankincense) in dry areas of Nigeria (Okujeni, 1987).
- *Aformosia* in Nigeria and Namibia.

- *Brachystegia spp.* (Prince of Wales feathers) common tree in eastern and southern Africa. Nitrogen-fixing.
- *Combretum* (large leaved forest bushwillow), a common plant of tropical and sub-tropical Africa.

Acacia, as in many parts of the world (Fig. 3-6), is widely present throughout Africa, and many species have been tested for their potential use in biogeochemical prospecting. Extensive lists of plants are given in Brooks and Malaisse (1985) and Brooks (1987). The chemistry of common plant species growing over kimberlites in Central and South Africa has been examined, and is worth further consideration. At present, details are proprietary.

India

The sub-continent of India has many climatic regimes and a wide diversity of flora. Details of a number of local biogeochemical studies using a diverse array of local plants have been reported in the literature. Among the most common that are encountered across the country are mesquite (*Prosopis juliflora*) and acacia. In northern India *Berberis* (barberry) has been found to accumulate U. Elsewhere, *Impatiens* (busy-lizzy) and *Lindenbergia* are reported to accumulate high concentrations of Zn. The liana *Combretum decandrum* is reported to accumulate high levels of Ni. A website by the Botanical Survey of India presents a summary of common plants of India along with some excellent coloured illustrations: http://envfor.nic.in/report/0001/chap02.html.

Summaries of most of the more important studies can be found in the *Journal of Geochemical Exploration* (abstracts on the CD in the back pocket) and publications of the Indian Academy of Science.

Fig. 3-6. Worldwide distribution of the genus *Acacia*.

China, Mongolia and Japan

The spectrum of climatic regimes across China ranges from desert in the northwest, through several steppe zones extending from Tibet to Mongolia, and southward into a forest zone. There are few published biogeochemical studies from China. For those interested in conducting biogeochemical surveys there are several useful websites for summaries of the flora of China.

http://flora.huh.harvard.edu/china/

http://www.icimod.org/focus/biodiversity/chibio.htm

http://www.fao.org/ag/agl/swlwpnr/reports/y_ea/z_cn/en/text_e/d3.htm

The last gives succinct accounts of what to find in each climatic region and from these summaries it is evident that there are many species similar to elsewhere in the world. For more detailed information there is the recent publication of the 1:1,000,000 'Vegetation Map of China' (Xueyu Hou, 2001). It augments the monograph 'Vegetation of China' (1980). This new publication comprises 60 separate 1:1,000,000 sheets; it covers the whole of China and illustrates the geographical distributions of 11 groups of vegetation types, 796 formations and many smaller categories. It is a huge publication, weighing 5.8 kg and is in Chinese, but with English captions to the illustrations. Coupland (1993) provides more focused information on grasslands, not only in China, but across Europe and the rest of Asia. Other books in the 30-volume series entitled 'Ecosystems of the World' (series editor D.W. Goodall, published by Elsevier) cover the full spectrum of the world's ecosystems.

The forests of China can be divided into boreal coniferous, temperate, subtropical and tropical coniferous forests. There are also deciduous broadleaved forests, evergreen broadleaved forests, tropical seasonal rain forests, rain forests and mangrove forests. Although there are many *species* endemic to China, the many genera are common to other forested regions of the world – e.g., the conifers pine (of which seven species are endemic), spruce, fir, larch, hemlock; deciduous forest dominated by birch, beech or oak. Given the general paucity of information available on the biogeochemistry of China, an orientation survey would be advisable. However, there are many genera from other locales around the world for which some biogeochemical information is available, and so information published on similar species would provide some focus for a survey.

The more arid areas of China and Mongolia have plants common to many cold to temperate arid zones, such as the sagebrush (*Artemisia*). In the extensive grasslands of Inner Mongolia there are not many species to choose from, and at first sight there appear to be virtually nothing other than grasses. However, closer examination does reveal a number of quite common plants that are not immediately eaten by grazing animals – including sagebrush, caragana, Russian thistle (tumbleweed, *Salsola spp.*), and poison vetch (*Astragalus spp.*). *Potaninia mongolica* (a member of the rose family) has been reported to be effective at concentrating Cu (Kong Ling-shao et al., 1992). Other bushes and shrubs used in exploration include *Anabasis* (a succulent halophyte) and species of the onion genus *Allium*.

In Mongolia there are two principal vegetation zones; the Siberian taiga in the north, dominated by cedar and larch forests, and the Central Asian steppe-desert in south. The prevailing types of steppe plants are various kinds of *Miscanthus* (feather or Japanese silver grass), *Artemisia* (sagebrush) and *Potentilla* (cinquefoil). Most important in Mongolia's desert steppes are *Piptanthus mongolicus* (a leguminous shrub); *Convolvulus* ('morning glory', of which a related species was found to be of use in southern Morocco (Dunn et al., 1996b)); and *Oxytropis* (one of the so-called 'locoweeds', because when eaten, the poisonous alkaloids that they contain cause peculiar behaviour in animals, or even death). *Carex* (sedge) is widespread and could be of use for, in particular, base metal exploration. Although there are no known case histories from Mongolia, *Carex* roots are traditional sample media in Sweden (Brundin et al., 1987) and Kabata-Pendias (2001) reports that most species seem to be barrier-free with respect to the translocation of Pb, and exhibit low-barrier characteristics for Ni, Cr and Mo.

The long chain of islands comprising the Japanese archipelago encompasses a wide range of vegetation zones. In the south of Japan the Japanese beautyberry (*Callicarpa mollis*) was found to be an effective indicator of Au mineralization (Yoshiyuki Kita et al., 1992). Other species of use were the fern *Arachnioides aristata*, the fig (*Ficus erecta*) and the artichoke (*Gleichenia japonica*). The leaves of pepperbush (*Clethra barbinervis*) are known to accumulate certain heavy metals, and bulk samples of this species have been collected by the Japanese National Institute of Environmental Sciences for use as a Standard Reference Material (SRM-NIES-1). Elsewhere in Japan, because of the lack of published studies it would be advisable to conduct an orientation survey before launching into a sampling programme.

Australia

Of the total Australian landmass, 75% falls into four classes of vegetation: Open Forest (4.6%, mostly eucalypt); Woodlands (13.9%, mostly eucalypt and acacia); Open Woodlands (25.8%, eucalypt, acacia and cleared grazing land); and Tall Shrublands (31.1%, mallee, mulga and arid acacias).

There are more than 800 species of eucalypts. Much more detailed research is required on the chemistry of these species and their sensitivities to underlying mineralization. However, it is perhaps not as daunting a problem as it might first appear, because the vast majority of species are aggregated into groups of related species, based on the widely held assumption that *Eucalyptus* began as a more or less single evolutionary event and that all extant species are descendants. In fact the Australian National Herbarium divides the 800 or so species of *Eucalyptus* into just 13 subgenera, 2 of which are the ghost gums (subgenus *Blakella*) and bloodwoods (subgenus *Corymbia*) that constitute the single genus *Corymbia*. So as far as the true eucalypts are concerned there are really only eleven subgenera that need careful evaluation and comparison.

Worldwide there are more than 1350 species of acacia. About 1000 acacia species occur in Australia, and of these, about 955 belong to the subgenus Phyllodineae – the familiar wattles. Wattle is an important plant in Australia, such that the national floral emblem is *Acacia pycnantha*, the Golden Wattle.

Plant genera in Australia for which biogeochemical exploration data are available include the following:

- Eucalypts ('gum') — Various species – e.g., Red River Gum (*Eucalyptus camaldulensis*)
- Acacia — e.g., *Acacia aneura* ('mulga'). Note: a mature tree is usually >100 years old, which is unusually long-living for acacias
- Saltbush — *Atriplex*
- Poverty bush — *Eremophila*
- Paperbark — *Melaleuca*
- She-oak — *Casuarina*
- Bottlebrush — *Callistemon*

A comprehensive website for obtaining detailed information on Australian plants can be found at http://www.anbg.gov.au.

South America (excluding the Amazon)

The principal widespread plants of potential value for biogeochemical exploration in the arid regions of South America include the following:

- *Baccharis spp.* (Thola bush of the Andean highlands, or Carqueja of southern Brazil) is one of the most common plants from the drier areas of South America (Viladevall and Queralt – see Chapter 11 – case history for Au). In southern Brazil some local studies have examined a number of species, including *Baccharis trimera* (Lima e Cunha et al., 1997). This genus is probably the most studied from a biogeochemical exploration perspective, and, because of its widespread occurrence and proven ability to accumulate metals it is a high priority biogeochemical sample medium.
- *Larrea spp.* is another candidate for biogeochemical exploration from this environment. There are five species of this evergreen shrub, represented in North America by the Creosote bush *Larrea tridentata*, and by the 'Jarillas' of South America where species are closely related. It is sufficiently common to be classified as a weed.
- *Parkinsonia spp.* (formerly *Cercidium spp.*, 'Palo verde') occurs in Argentina, Mexico, the southern USA, as well as semi-desert areas of Africa. It has been tested in a few studies and shown to yield high levels of some trace metals, notably Au, Ba and Br.

- *Bulnesia spp.* (Palo santo, locally known as *ibiocai*) is a tree that occurs on the Gran Chaco of southern Bolivia and Paraguay. Results have indicated, mostly, quite low levels of trace metals although modest concentrations of molybdenum.

Humid tropics – Amazon, West Africa, Indonesia

The great diversity of flora in the tropics poses a significant initial problem for conducting biogeochemical surveys. Interestingly, the earliest report of a biogeochemical study, anywhere in the world, was in tropical forest, using wood from the Baromalli (*Catostemma fragrans*) to look for gold in Guyana (Lungwitz, 1900).

In the tropics especially, an effective approach to setting up a biogeochemical programme in a region of diverse flora is to conduct some preliminary investigations by visiting botanical gardens, and talking to local botanists at museums and universities. Also, the use of local guides familiar with the jungle can be of immense value to a sampling programme – they usually have a fundamental knowledge of the flora and can point toward species that are commonly encountered. No special equipment is required – however, the machete commonly used in jungle environments can be a valuable tool.

In the Tapajos area of the Amazon (Pará State) common species that have proved of value include the following (Dunn and Angelica, 2000).

Imbauba (foliage and bark)	*Cecropia sp.* (there are several species, identified by leaf shape and fruits)
Vassoura de Botão (twigs and foliage)	*Borreria verticillata*
Boa macaca (foliage)	*Parkia ulea*
Banana braba (foliage)	*Ravenala guianensis*

The outer bark from the Brazil nut (Castanha-do-pará) and bark from other large trees can be of use.

Tree ferns (*Cyathea*) from the western Amazon have provided good responses to Au mineralization and Mo porphyries. Other species, such as the liana *Clusia spp.* were less informative. Surveys using bamboo-like plants (*Chusquea spp.*) from the same general area have met with limited success in that they proved to be the least sensitive of several plants collected at the same sample stations. In a study that included another bamboo-like plant, *Ischnosiphon*, collected near the Igarapé Bahia deposit in Pará State results were mixed (Machesky et al., 1993).

In the tropics there are many trees that have distinctive shapes or vein patterns to their leaves, assisting the non-botanist in identifying the frequency of plants in a given survey area. In Papua New Guinea and throughout Melanesia there is the tree *Astronidium paluense* that has a very distinctive leaf and has been of use in locating Au/As mineralization by the biogeochemical method (McInnes et al., 1996).

SUMMARY NOTES

Although there are millions of plant species, the world can be divided up into zones with discrete plant assemblages. Fortunately for the biogeochemical method, there are commonly only a few common plant species growing within an area of interest to an exploration programme that are of sufficient biomass for sampling. Almost invariably, after an hour or two of reconnoitring a survey area while focusing on the vegetation, the realization dawns that certain species are dotted around at intervals that render them suitable for conducting a survey.

In the northern forests the task of finding a suitable sample medium is relatively simple, with only a handful of common trees, most of which are distinct in appearance and easy for the non-botanist to identify after only a short period of instruction. In arid terrains there are also only a few common species within the limited geographic area that is usually the extent of a mineral claim, and most are quite easy to recognize once the key features have been explained.

The temperate rain forests have greater diversity and present a greater challenge. A day or so with a botanist, or a local worker familiar with an area can be invaluable. Similarly, in the tropics where there is a great diversity of plants, a little advance planning by talking to a local botanist at a university or a museum before going into the field, can provide considerable focus for what to expect. Simple publications of museum guides to local flora are an excellent investment, and so is time spent in the field with local guides who commonly have amazing in-depth knowledge of the local flora. Not only are they able to provide local names (which can be checked later in a library to find a botanical name if this is required), but they also have an intimate knowledge of where certain plants grow and what their uses might be – either as timber, foodstuffs or for medicinal use. Such information can give the field geologist clues that can be very useful for a field survey even if they sometimes seem a little obtuse. As an example, one of the pioneers of exploration biogeochemistry, the late professor Robert Brooks, quotes in his 1983 book an account from Pagliuchi (1925) of a bird that the locals described as 'el minero' (the miner).

> this bird is always found in the vicinity of gold mines or wherever gold is abundant. Thereafter, as I rode through the country, wherever I hear the cry of 'el minero' either I saw a quartz ledge extending across the trail or was able to find one by looking about a little. Apparently the natives were correct … Finally a miner told me that if I wanted gold I must always look for the mora [1]tree … the solution of the puzzle dawned on me. The mora tree grows and thrives only on siliceous ground, and 'el minero' feeds on berries of this tree; hence, whenever his cry is heard, one is assured of finding a quartz vein in the vicinity. Of

[1]The mora tree described by Pagliuchi is probably the large shrub *Rubus glaucus* – the Andes Berry (known locally as 'mora') and not the tree *Mora excelsa* that Brooks assumed it to be, because the Andes Berry fits better with the fact that it has berries that 'el minero' feeds on. *Mora excelsa* has large woody pods, and no berries.

> course, the quartz may or may not be auriferous, but when prospecting in a country known to be auriferous, it must be admitted that the bird is a helpful guide to the prospector.

In this chapter, lists of flora have been provided for most of the key climatic areas of the world where biogeochemical exploration might be contemplated. The lists of plants are, intentionally, short and not all plants for which data have been published are referenced. Exhaustive lists can be found in the literature, but they tend to be rather bewildering to the non-botanist. The exploration geologist has enough to consider with the physical environment and the rocks themselves, and so short lists of plants are more easily absorbed. The lists are intended to provide a focus – a starting point, because for many of the plants listed others have gone ahead and tested them for their biogeochemical exploration potential. In any given area other species may be present that would warrant testing. The science is sufficiently young that new surprises concerning element enrichments in plants are regularly being made, adding to the excitement for the field geologist and the exploration industry in general.

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Chapter 4

FIELD GUIDE 2: SAMPLE SELECTION AND COLLECTION

GENERAL CONSIDERATIONS

The rationale for applying biogeochemical methods to mineral exploration is that plants absorb metals present in the ground and transfer these metals via their root systems to the growing plant. Quite simply, the more of a metal that there is in the ground, the more it is likely to be taken up in solution by a plant and will be deposited within that plant. There are, however, processes that take place that prevent this from being a linear association.

Roots have to find water and dissolved nutrients in order to sustain a plant's life. A single large tree can pump more than a hundred litres of water from the ground every day and transport it via its xylem up to its extremities. Several processes interact to transfer a solution from the soil environment up into a tree. Osmosis explains the pull of water into the roots, and some minor movement can take place by capillary action. However, the principle movement through a tree is best explained by evaporation through stomata of its foliage. This evaporation creates a vacuum that then permits more water and its dissolved constituents to be drawn into the plant. Some volatile compounds escape with the water vapour, but most remain within the plant structure, in the phloem or deposited on cell walls, to be cycled back to the ground, as illustrated in Chapter 2.

Metals are absorbed from soil, from groundwater and locally from bedrock where roots penetrate faults, joints, cleavages and the interstices or boundaries between mineral grains. The significant advantage of applying plant chemistry to exploration is that the root system of a plant may penetrate through many cubic metres of the substrate, and therefore integrate the geochemical signature of a large volume of all soil horizons, the contained groundwater, gaseous emanations and bedrock where it is covered by only a few metres of overburden. The live roots occasionally found in mine galleries deep underground probably only occur where there is a very low water table in an arid environment. Intuitively, it would seem that roots need to probe deeply into the substrate in order to extract all the nutrients that they require. However, depth of root penetration is not critical for a biogeochemical response, because elements can migrate upward from considerable depth in solution, by diffusion, in electrochemical cells, and possibly by seismic pumping (i.e., release of metals due to earth tremors) to be accessed by root systems. Consequently, there is

commonly not a good correlation between plant and soil chemistry, especially in areas where there is exotic overburden.

The microscopic mycorrhizal fungi on root surfaces effectively transfer nutrients into plant structures. Discussion of these mechanisms is given in Chapter 1, where it is noted that the complex microenvironment surrounding roots can be highly corrosive and soil acidity derived from organic acids can be as low as pH 1. Furthermore, simply because plants minimize the energy output required to flourish, roots will take the path of least resistance and first accept elements in gaseous form, then those in solution, and then seek out additional requirements by selectively extracting labile elements that are loosely bonded to soil surfaces or rock fractures. Russian workers have indicated that gases are absorbed by plants 3000 times more readily than elements in solution, and the latter are absorbed 300 time more readily than elements locked in the crystal lattices of minerals comprising rocks or soils (Kovalevsky, 1974). Loosely bound elements are mostly adsorbed to soil coatings of amorphous manganese and iron oxide coatings. These coatings are the targets, also, of various soil selective extraction techniques used in exploration geochemistry. Consequently, the plant can be considered as a type of selective leach process. Once the sources of elements in gases, in solutions and adsorbed on surface coatings have been exhausted, further plant requirements are met by attacking the less labile components of the substrate – the crystalline phases of soils and bedrock.

Many texts suggest that for biogeochemical exploration to be successful there should be a high correlation between the metal content of the soil and that of the plant (Brooks, 1983). This is a valid concept for some parts of the world where there are residual soils. However, as noted in the first chapter, plants establish barriers to metal uptake in order to protect themselves from potential toxicity so that over a broad range of concentrations the metal content of a plant may not be proportional to the metal content of the soil (Kovalevsky, 1987, 1995a). Consequently, a good positive correlation between plant and soil chemistry does not always occur, especially where exotic overburden such as lacustrine clay, alluvial plain silt, glacially derived material, or wind-blown loess has been deposited on mineralized bedrock. This situation may be further complicated by elements that remain dissolved in groundwater and taken up directly by plant roots without precipitating in the soil medium. This is particularly true of highly soluble elements (e.g., halogens and some U complexes) that can remain in solution until intercepted by the rhizosphere (root zone) of a tree. Furthermore, although some elements may be absorbed directly from the interaction of their roots with the groundwater and/or the capillary fringe of the water table, others may be taken up in gaseous form (e.g., Hg and halogens). In summary, whereas the physicochemical environment of the soil may not be conducive to element adsorption from groundwater or gaseous phases, plant roots can absorb elements directly from these phases and concentrate them in the plant tissues.

For these reasons, whereas plant to soil coefficients can be established in laboratory experiments, the real world is rarely that simple. In attempting to determine the relationship between the chemistry of the soil and that of a tree, the usual

procedure is to collect a bag of soil and a bag of tree tissue. However, there arise some fundamental considerations.

- Which soil horizon should be collected?
- Which size fraction of the selected soil horizon should be analysed and by which analytical method?
- Which type of plant tissue (and from which species of plant and location on that plant – top or bottom, north or south) should be collected for comparison with that fraction of the underlying soil that has been selected for analysis?
- What are the effects of topography and ground conditions in determining the uptake by a plant of the various elements?

Typically, each soil horizon has a different metal content, as does each size fraction of that soil. Similarly, each vegetation tissue type has a different ability to collect and store metals; and concentrations in living tissue change with the seasons. The problem is compounded by the fact that a soil sample is usually no more than a handful of a single horizon, and as such represents a miniscule sample compared to the volume of material sampled by the root system of a large tree. Furthermore, the relationship between plant and soil chemistry improves with depth down the soil profile, so the tree is the 'natural drill' that collects metals from depth.

Table 4-I shows the relationship between Au and As in outer bark and the soils in which the trees were growing. The bark of both the Douglas-fir and Engelmann spruce has strongest correlations with the C horizon soils, indicating that this horizon is the primary source of these metals that were drawn into the trees. In effect, the trees have efficiently drawn up the Au and As and sequestered them in their bark, thereby providing a simple window to the chemistry of the C horizon, without having to dig a pit – which in this area would have required digging to a depth of up to 1 m.

TABLE 4-I

Correlation coefficients (r) between Au and As in bark ash and underlying soil horizons near the Nickel Plate past-producing mine (gold skarn) at Hedley, southern British Columbia

Soil horizon	Douglas-fir bark $n = 12$		Engelmann spruce bark $n = 13$	
	Au	As	Au	As
Forest litter	0.13 (ns)	0.10 (ns)	0.48 (ns)	0.58 (s)
A horizon	0.63 (s)	0.63 (s)	0.65 (s*)	0.65 (s*)
B horizon	0.60 (s)	0.55 (s)	0.79 (s**)	0.80 (s**)
C horizon	0.76 (s*)	0.64 (s)	0.90 (s**)	0.88 (s**)

Note: ns – not significant ($P < 0.05$); s – significant ($P > 0.05 - < 0.01$); s* – highly significant ($P > 0.01 - < 0.001$); s** – very highly significant ($P > 0.001$).

Plants are complex structures that apply extraordinarily sophisticated mechanisms to select those elements that they require for efficient metabolic function, while tolerating other elements and sequestering them out of harm's way, and excluding other elements that could have significant toxic effects. Each species of plant is unique in its chemical composition and, therefore, its value to biogeochemical exploration. For any given species for which there is no information available on its propensity to accumulate elements, an orientation survey is highly advisable in order to optimize the value of the biogeochemical method to exploration. It is as well to be aware that in many situations the information supplied by plant chemistry will be different from that derived from analysis of soils or other surficial deposits – each provides its own 'layer' of geochemical information in the same way that different geophysical measurements provide different types of information on the physics of the Earth.

Seasonal variations

In Chapter 2, there is a brief introduction to botanical aspects of seasonal variations in plant chemistry. This section now puts these complex interactions into a practical perspective and provides examples of variations and how to deal with them so that a biogeochemical survey is a practical proposition. An often-overlooked factor in soil sampling is that soils, too, exhibit seasonal variations in composition (Victoria et al., 1989; Kaiser et al., 2001), so geochemists should always be conscious of this phenomenon.

To reiterate what has previously been noted, numerous studies have established that throughout the year, the elemental composition of living plant tissues can vary considerably as a plant grows. Consequently, it is advisable to conduct a biogeochemical survey in as short a time frame as possible – preferably 2–3 weeks during the growing season. During the winter months a plant's metabolism is quite stable, and so a sample collection programme can be of longer duration. Dead tissues, such as outer bark, provide the exception, because with time there is little or no change in their composition, and therefore they represent valuable media to use for any surveys that are to be conducted over a long period (several months or even years).

Each plant species exhibits its own compositional variations throughout the year, such that for some, but not all, elements a tree or shrub sampled in the spring yields concentrations different from those in the same plant during the summer.

For biogeochemical exploration, the potential complexities created by seasonal variations in plant chemistry can be significant, but provided appropriate considerations are applied, they are manageable. The literature contains details of various studies that appear to offer some conflicting advice. For example, Brooks (1983) notes that 'it seems to be well established that the elemental content of many deciduous leaves rises to a maximum just before exfoliation', and he provides as evidence a series of plots from a study by Guha (1961). Scrutiny of Guha's plots

shows that this is true for some elements in some species (e.g., Mn and B in sycamore), but the opposite is true for Cu in the three species that were studied, since all yielded lowest concentrations just before exfoliation. In short, the patterns are not consistent.

Subsequent studies have provided more consistent results with the spring emerging as the period when maximum concentrations occur – especially of Au. Studies of Au in a variety of plant species from the Hemlo area of Ontario demonstrated that, in trees growing over mineralization, the highest Au values were recorded in the spring (Cohen et al., 1987). Similarly, in Colorado Stednick et al. (1987) and Stednick and Riese (1987) confirmed this pattern for Au, but found no time dependence for Cu and Zn. These findings are in accord with previous studies in the more northerly latitudes of the La Ronge Gold belt in Saskatchewan (Dunn, 1985), where 17 mountain alder shrubs (*Alnus crispa*) located approximately 250 m apart were revisited on four occasions from June 1984 to April 1985. Each shrub was of similar maturity and height (about 2 m), and on each occasion all 17 were sampled in the same day. The tissues selected for analysis comprised the most recent three years of twig growth, and leaves from these twigs were obtained during 1984. Alder is deciduous and so the leaves represented only the current year's growth, and in April 1985, when there was still snow on the ground, the leaves had not yet emerged.

At each of the 17 sample sites the changes in composition were consistent (Table 4-II) – i.e., in early August all of the shrubs recorded lower Au concentrations than in early June. Similarly, in September, all had higher concentrations than in the early August sampling. By the following April, with the first rise in sap, consistently and substantially higher Au levels were recorded (Table 4-II).

In this study the twigs were the first to be analysed, and it was only at a later date that the leaves were recovered from storage for analysis. Intuitively, it was considered likely that the losses of Au in the twigs from June to August might have been a result of the Au migrating from the twigs into the leaves resulting in higher concentrations in the leaves. However, this proved not to be the case and, if this process did take place, the Au was soon leached out of the leaf tissue. There are several possibilities that could explain this loss of Au.

- Au dissolved in the plant sap cycles through the plant and is eventually returned through the cells to the ground.
- Girling and Peterson (1978), from an experiment involving doping some soils with a radioactive Au isotope, demonstrated that Au has an acropetal tendency – i.e., it migrates to leaf tips.

During evapotranspiration, the formation of salts on leaf surfaces, and the spalling of tissues during hot summer days the Au could be released to the atmosphere from the microcosm that forms the entity of a plant. An SEM of the surface of a black spruce twig (Fig. 4-1) shows the flaking of cuticle (particles of about 5 μm in

TABLE 4-II

Gold in the ash of alder (*Alnus crispa*). Same 17 shrubs collected from northern Saskatchewan on four occasions during a one-year period

Site	Alder twigs gold (ppb) in ash				Alder leaves gold (ppb) in ash		
	1984			1985	1984		
	June	August	September	April	June	August	September
1	32	7	23	250	nd	nd	nd
2	53	6	17	47	nd	nd	nd
3	58	9	20	130	43	6	19
4	34	6	15	166	48	7	15
5	29	8	10	37	27	18	11
6	35	7	11	34	21	6	12
7	23	6	13	57	25	7	13
8	25	8	13	41	21	13	13
9	25	11	20	27	21	11	16
10	29	20	23	75	11	8	22
11	35	10	22	58	8	7	8
12	23	8	14	51	6	6	9
13	12	17	18	33	10	8	8
14	24	10	11	53	14	7	18
15	25	11	12	42	5	10	13
16	14	11	9	66	13	3	13
17	21	10	38	48	8	7	14
Average in ash	29	10	17	71	19	8	14
Average dry weight	0.6	0.2	0.34	1.4	1	0.4	0.7

Note: nd = not determined.

diameter) that would readily be removed by wind or rain. Since there is evidence that some elements migrate to plant extremities, then trace elements can be lost from a plant in this manner.

An alternative explanation of the acropetal tendency observed by Girling and Peterson (1978) is that the plants reacted to the radioactive isotope of Au in a manner different from the way that they react to naturally occurring gold (Au^{197}), by sequestering the more toxic radioactive form at the furthest extremities of its structure where it could do the least harm. Recent studies have dramatically illustrated that different chemical forms of an element are fixed in different locations in plant structures. A study of Se in the poison vetch *Astragalus*, using the laser light source synchrotron instrumentation, has clearly demonstrated that organoselenium occurs in different locations in leaves and twigs than Se complexed as a selenate (Pickering et al., 2000).

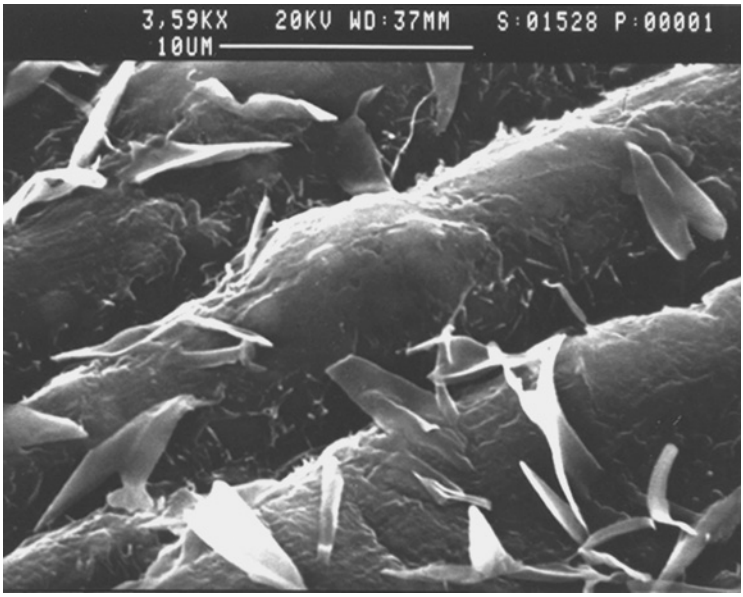

Fig. 4-1. SEM of the surface of a black spruce twig (*Picea mariana*) showing natural spalling of cuticle (Dunn, 1993, 1995).

Whereas Table 4-II shows that substantial seasonal variations in Au occurred in alder, for Mo these changes were not evident. Table 4-III shows data for the June and August collections, and includes also data for outer bark of black spruce. At most sites concentrations were similar and variations were within the precision levels of the analytical method.

From Table 4-II it is evident that the Au values from alder samples collected at different times of the year cannot be directly compared, although it might be possible to normalize the data from a regression equation. Conversely, Table 4-III shows that the Mo data from the different seasons could be merged without compromising the integrity of the survey. For alder, as a broad generalization, it seems that concentrations of most elements are reasonably consistent throughout the year and exhibit only minor seasonal differences (e.g., As, Ba, Ca, Cs, Mo, Rb, REE (rare-earth elements), Sb, Sr, Th, U, Zn). For a few elements there is substantial variation with highest concentrations occurring in association with the rising sap, then tailing off quite quickly as the summer progresses (e.g., Au, Co, Cr, Fe, Ni). The outer bark of the spruce is dead tissue, and so seasonal variations are not a consideration.

At an elevation of 1700 m in the northern Cordillera of the United States (Oregon), Ashton and Riese (1989) studied seasonal variations of As and Au in Ponderosa pine (*Pinus ponderosa*) and white fir (*Abies concolor*). In this environment they found minor seasonal differences in As, but substantially higher Au concentrations in the spring and fall than in the summer and winter.

TABLE 4-III

Molybdenum in the ash of alder (*Alnus crispa*) and black spruce (*Picea mariana*). Same 17 shrubs collected from northern Saskatchewan in spring and summer

Site	Alder twigs Mo (ppm) in ash		Alder leaves Mo (ppm) in ash		Spruce bark Mo (ppm) in ash	
	June	August	June	August	June	August
1	3	8	nd	nd	nd	nd
2	3	2	2	2	–1	1
3	7	4	nd	nd	nd	nd
4	2	4	5	4	1	–1
5	12	10	3	2	1	1
6	14	7	4	3	nd	nd
7	19	22	4	2	2	3
8	9	5	4	3	nd	nd
9	11	18	1	3	1	1
10	8	9	3	1	1	–1
11	5	9	2	1	1	1
12	23	14	1	1	1	2
13	2	2	4	1	–1	1
14	10	6	4	4	3	3
15	1	2	4	2	1	1
16	–1	4	1	–1	3	–1
17	–1	–1	4	–1	1	2

Note: nd = not determined.

In the hotter environment of central India seasonal variations of U were not clearly defined, although the common plant *Lagerstroemia parviflora* (myrtle family) in its dormant stage exhibited better background to anomaly contrast than other neighbouring species that were in a dynamic growth phase (Pande et al., 1993).

In a study of seasonal variation of 20 elements in first and second year needles of Norway spruce (*Picea abies*), the data fell into 3 groups (1) Ca, Sr, Ba and Mn; (2) Al, Br, Co, Fe, Hg, La, Sc, Sb and Zn; 3) K, Rb, Cs, P and Cl. Elements in groups 1 and 2 increased from spring to summer, while those from group 3 decreased (Wyttenbach and Tobler, 1988).

Another study monitored the variations in needles of balsam fir (*Abies balsamea*), collected from a single site every week for 6 weeks from early July to late August (Leybourne et al., 1999). Analytical determinations were undertaken in the laboratories of the Geological Survey of Canada in Ottawa using ICP-ES and ICP-MS on plant ash for 10 major elements and Ba, Be, Co, Cr, Cu, Ni, Sc, Sr, V, Zn, REE, Ag, Bi, Cd, Cs, Ga, Hf, In, Mo, Nb, Pb, Rb, Ta, Th, Tl, U and Zr. In general, variations were quite subtle (+/– a few percent), except for some trends during the sampling

period of increases in Ba, Ca, Mg, Mn, Sr and Zn by about 20%, and decreases of similar magnitude in Fe, K, P and Rb (Fig. 4-2). Except for Fe, these trends are in general accord with those noted above for Norway spruce needles.

For some elements the tree water flow-rate (related to evapotranspiration) has been shown to affect the seasonal variations of metal concentrations in tree crowns. Sailerova and Fedikow (2004) noted that the best correlation between seasonal

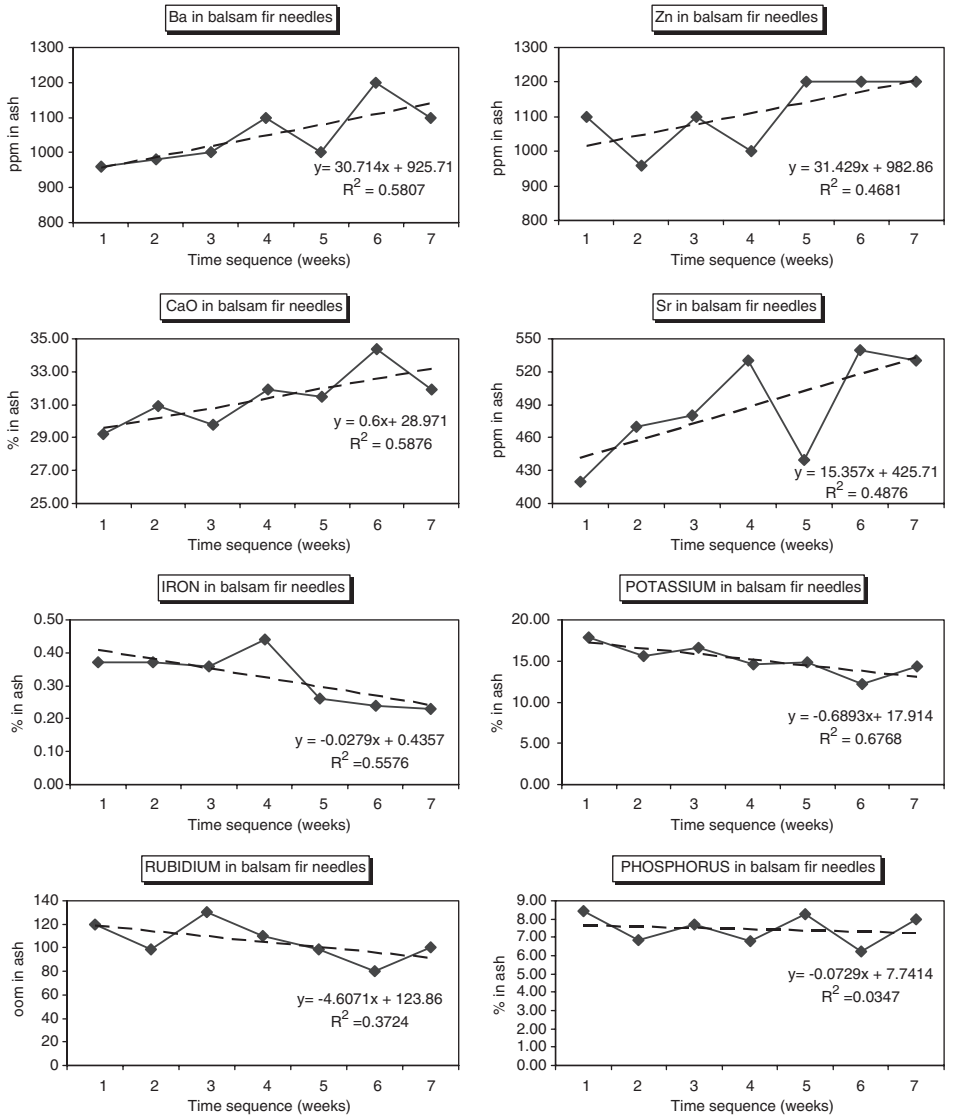

Fig. 4-2. Variations in element content of balsam fir needles (*Abies balsamea*) from New Brunswick, Canada, over a 6-week period. Concentrations in ash.

TABLE 4-IV

Data to demonstrate the flushing of some elements toward growing tips (collected in late spring) of Douglas-fir (*Pseudotsuga menziesii*) from the crown of a single 40 m tree on Vancouver Island. Concentration in ash analysed by ICP-MS on a nitric/aqua regia digestion

	Current year needles (ppm)	Previous year needles (ppm)
As	265	30
Cu	129	47
Ni	65	26
Zn	630	287

variability and tree water flow-rate was for the essential elements (Ca, K, Mg, Fe, Fe, P and Zn) and the least for the pH-sensitive elements (Ag, Co, Ni, Rb, Mn and Cu).

A study of first and second year Douglas-fir needles demonstrates the acropetal trend of As, Cu, Ni and Zn (Table 4-IV). Concentrations are in ash of needles from the crown of a single tree (40 m tall) on Vancouver Island.

Summary considerations of seasonal variations – a practical approach

1. In boreal and temperate climates, seasonal variations in plant chemistry can, and do occur for some elements, so a survey should be conducted in as short a time frame as is practically reasonable – preferably 2–3 weeks in the spring, or 4–6 weeks in the summer. A winter survey can extend over a 6-month period during plant dormancy.
2. Variations are restricted to living tissue – scaly outer bark is dead and therefore the only changes likely to occur in the short term are from minor leaching of elements during heavy rains.
3. Variations may be different for each plant species.
4. Variations are different for each element.
5. For those elements that do vary with the seasons, most variations are $<20\%$. However, data indicate that there can be substantial differences between current and previous year growth of conifer needles. To circumvent this problem, collect a consistent multi-year sample (e.g., most recent 5 years of growth) at each sample station; this integrates the long-term geochemical signatures and allows for both seasonal and annual variations in metal accumulation.
6. Highest background to anomaly contrast is likely to occur during the spring, but this is a period of dynamic chemical activity, so special care should be taken and sampling should be conducted within a short time frame – preferably no more than two weeks.
7. During the summer, when growth slows down, the plant chemistry is more stable so sampling can extend over a longer time period.

8. If a sampling programme is to last for several weeks it is a good precaution to repeatedly sample (e.g., once per week) a well developed tree or shrub of the same species as that selected for the survey in order to quantify seasonal trends in the plant chemistry. Given this information, a regression equation can be applied to the data for any element that might exhibit systematic trends in composition.
9. Any seasonal variations that may occur in the tropics have not been clearly defined. Xylem increment may continue to be added for most or all the year, hence many tropical trees lack growth rings or they are very indistinct (Kramer and Kozlowski, 1979). Although plants growing side-by-side may be in different stages of their life cycles, this steady year-round growth implies a steady influx of elements. However, it is advisable to err on the side of caution by trying to sample plants of similar maturity, avoiding very young fresh tissues.
10. Points to consistently question are: (a) Since there are or may be seasonal variations for the objectives of the survey, are these acceptable? (b) Are variations significant for the elements of particular interest? (c) Given that there may be some natural variations, are the data sufficiently consistent to be meaningful? – i.e., are they 'Fit for the Purpose' (Bettenay and Stanley (2001) and see discussion in Chapter 7)?
11. Consistency in sampling is of paramount importance.

SELECTION OF TISSUE TYPE

Once a plant has been selected as a potential candidate for use in biogeochemical prospecting – including having considered, in particular, its ubiquity in a proposed survey area and therefore assessed the likelihood that the plant will be present at all of the desired sample stations – the next decision to be made concerns which plant tissue should be sampled and submitted for analysis. The following criteria need to be considered.

- *What is the sensitivity of a certain plant tissue to the presence of the target metals in the substrate?* The answer to this question is not always available, but it is known, for instance, that Hg tends to be more enriched in leaves than twigs, and that in conifers U is more enriched in twigs than needles.
- *How practical is that tissue to sample?* Whereas leaves or twigs of shrubs may be readily available, some tree species have their limbs out of reach. In these cases the bark may be an option, or treetops are suitable targets. The latter would usually involve renting a helicopter and is, therefore, an expensive proposition, but as will be discussed later in this chapter it can still be an economical and expedient option based on a per sample basis.

For speed of sampling an operating rule in biogeochemical exploration is to collect material that is within easy reach. To achieve this goal it may require a compromise between the optimal sample medium when compared to another of

lesser sensitivity that would still prove to be very useful. For example, in a forest where the foliage or twigs of a particular species of pine are known to concentrate the metals of interest, there may not be sufficient light filtering through the canopy for there to be any growth within easy reach from the ground. In general it is impractical to carry a long pole pruner through dense forests in order to reach live growth. Bark, however, is easily accessible and a considerable amount of time and trouble can be saved by simply scraping outer bark scales and using these as the sample medium. Fortunately, bark is commonly a useful tissue type in that it concentrates many metals of economic interest and their 'pathfinder' elements.

Element differences among plant species

There are substantial differences in the uptake of metals by different species of plant from a single location, such that intermixing of plant species can generate an uninterpretable mixture of unrelated element concentrations. Table 4-V shows the variations that occur in trees rooted in the thin glacial drift cover that overlies Au mineralization on the west side of Harrison Lake, in southern British Columbia. Note in particular the wide range in concentrations of arsenic.

From Table 4-V it is evident that Douglas-fir (1600 ppm As in twig ash) is capable of actually scavenging As from the substrate, whereas alder (4 ppm As in twig ash) does not exhibit this tendency. It may be that Douglas-fir uses As to inhibit mould growth or deter invasive insects. Certainly the fresh young shoots are relatively enriched in arsenic (Table 4-VI), and intuitively it would seem that this harmful

TABLE 4-V

Element concentrations in the ash of tree tissues from a single location near Au mineralization at Harrison Lake, southern British Columbia

Common name	Species	Tissue	Au ppb	As ppm	Mo ppm	Sb ppm
Douglas-fir	*Pseudotsuga menziesii*	Twig	35	1600	<1	1
Douglas-fir	*Pseudotsuga menziesii*	Needle	23	130	<1	2
Douglas-fir	*Pseudotsuga menziesii*	Bark	53	250	<1	8
Western hemlock	*Tsuga heterophylla*	Twig	200	710	<1	8
Western redcedar	*Thuja plicata*	Twig	7	11	4	1
Western redcedar	*Thuja plicata*	Needle	5	6	<1	1
Western redcedar	*Thuja plicata*	Bark (all)	8	12	<1	1
Western redcedar	*Thuja plicata*	Bark (outer)	31	46	<1	11
Red alder	*Alnus rubrum*	Twig	14	4	57	0.5
Red alder	*Alnus rubrum*	Bark	<5	4	4	0.3
Douglas maple	*Acer glabrum*	Twig	12	6	4	1

TABLE 4-VI

Arsenic in dry Douglas-fir needles collected early in July 2001, from the crowns of four trees far from known mineralization at a site on southern Vancouver Island

	Arsenic (ppm) in dry Douglas-fir needles	
Site	Current growth	Non-current growth
1	5.1	0.35
2	5.3	1.45
3	7.7	1.27
4	17.3	2.79
Average	8.9	1.46

element might therefore protect the new growth from the ever-hungry creatures that seek out the tender nutrient-rich shoots.

Paradoxically, in the Harrison Lake example (Table 4-V) Mo in Douglas-fir is <1 ppm, yet it is concentrated to 57 ppm in alder twigs. The reasons for these differences are not always clear. For alder it has been suggested that Mo may enhance enzyme reactions that stimulate plant growth, and assist in N_2 fixation and NO_3^- reduction. Alders form extensive and rapidly growing fibrous root systems upon which nodules ('Frankia' nodules) form. Through a symbiotic association between the alder and the common bacterium 'actinomycetes', it seems that Mo may play an integral role in fixing nitrogen in the root nodules. The Mo is subsequently available to be translocated to the aerial parts of the plant.

Molybdenum is associated with many porphyry deposits; hence the selection of a sample medium that can provide a distinct Mo signature can be advantageous. A study that included Mo in common plants of the western Amazon serves as another good example of how Mo variations can vary substantially among plant species, yet the patterns of relative enrichment can be very similar. In this small-scale orientation study, samples of four tissues from three plant species were collected at about 30 sample stations (Fig. 4-3). The sample interval was at a spacing of 500 m, but it was adequate as the target was a large-scale porphyry system. The samples were foliage from an evergreen liana vine (*Clusia*, locally known as 'Churgun'); foliage from a type of bamboo (*Chusquea,* local name 'Suro'); and both foliage and spiny bark chunks from a tree fern (*Cyathea*, local name 'Chonta con espinas'). Samples were dried, milled to a powder, and the dry tissue analysed by ICP-MS after a nitric acid and aqua regia digestion.

Figure 4-3 shows plots of these four sample media. Each plot outlines a zone of considerable relative enrichment in the north central part of the survey area. Maximum values in the fern bark and fern foliage are 154 and 125 ppm Mo (in dry tissue), respectively. These are extraordinarily high values for Mo. Similar values occurred in

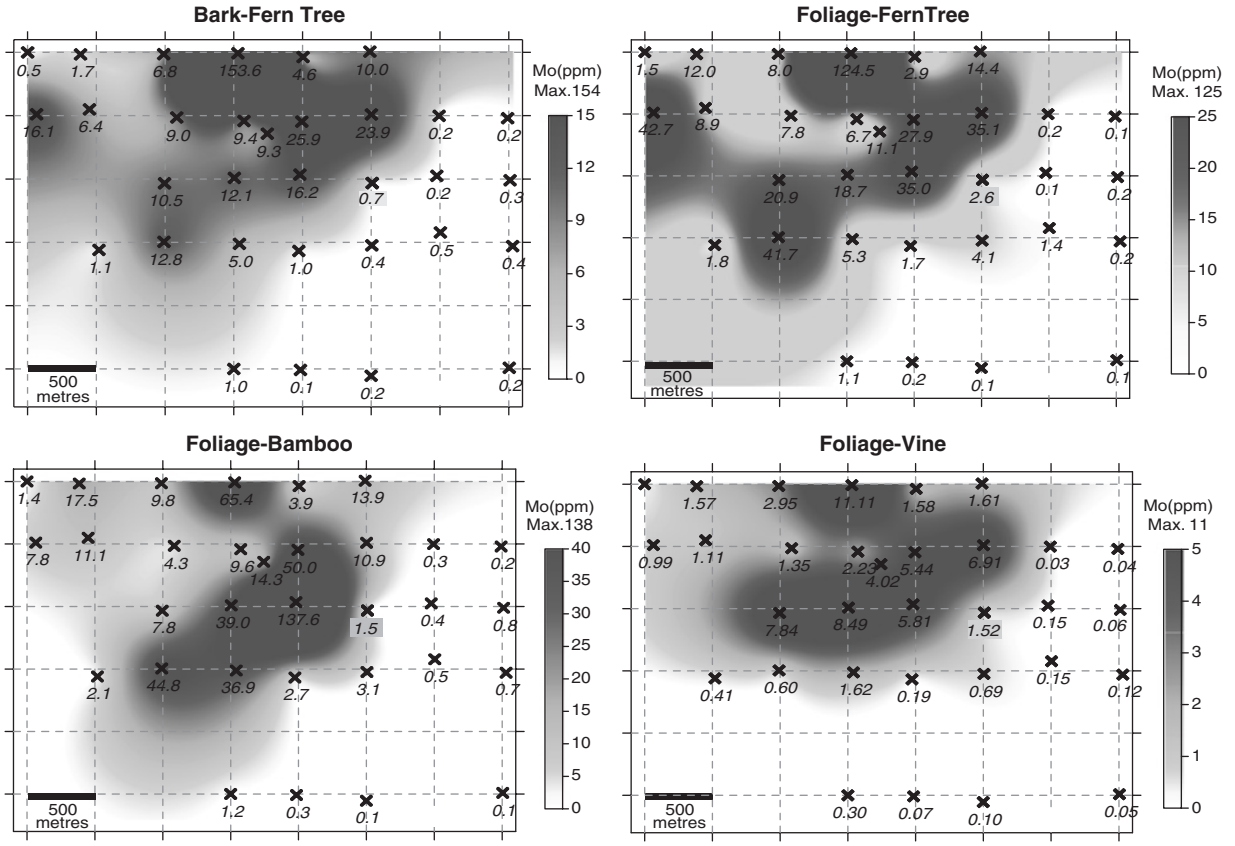

Fig. 4-3. Western Amazon – concentrations of Mo in dry tissues from common plant species growing in the vicinity of a Mo porphyry. Data courtesy of AngloGold Ashanti Ltd.

the bamboo foliage, but the liana foliage had concentrations that were an order of magnitude lower. In these datasets there is a particularly wide range of values, with sharp distinctions between the strong Mo anomalies (tens to more than a hundred ppm) compared to the background values of about 0.1 ppm Mo. However, it is striking how similar the Mo distribution patterns are – regardless of species and types of plant tissue. Two important outcomes of this survey are demonstrated and reiterated.

- Clearly, the data from different species/tissue types should not be mixed.
- The robustness of the biogeochemical method is evident, provided care is taken in consistently collecting the same material from the same species, and ensuring consistent preparation and analytical procedures – i.e., follow the same scientific rigour that should be applied to any geochemical sampling programme.

Another comparison of sample media, from the very different environment of the boreal forest, focused on precious metals and their pathfinder elements. Table 4-VII shows the biogeochemical variability in dry tissues from various trees and shrubs from the site of the rich, but small, abandoned Ni-PGE-Au mine at Rottenstone Lake in Saskatchewan.

The data from the suite of plants and tissues shown in Table 4-VII demonstrate that in the boreal forests the outer bark and twigs from black spruce yield the highest concentrations of the platinum group metals and several of the pathfinder elements, and are therefore the preferred sample media. Another point to note is that these concentrations are unusually high when compared with the background data shown in the last row.

These examples demonstrate the importance of identifying a species which concentrates the metal(s) of interest and thereby optimising anomaly to background contrast. However, even if no such prior knowledge is available for a particular survey area this does not preclude the use of the biogeochemical method, because the elemental spatial distribution patterns are usually similar for each plant species within a survey area. As is the case for exploration geochemical surveys in general, it is commonly accepted that the spatial relationships of element concentrations are usually of greater importance than the absolute concentrations. The objective is usually to provide focus for more detailed exploration rather than define specific drill targets.

The inhomogeneity of trees

In addition to great differences in the uptake of metals by different species of plant, within a single tree there are substantial differences in the element content of its various components. An example of this is given in Table 4-VIII that shows element variation within ashed components of a single tree from a site close to the now decommissioned world-class Sullivan Pb-Zn mine at Kimberley, southern

TABLE 4-VII

Element concentrations in dry plant tissues from the site of the Hall Deposit, Rottenstone Lake, Saskatchewan (modified from Dunn, 1988, in which the original data were expressed as concentrations in ash)

Sample medium	Pt (ppb)	Pd (ppb)	Rh (ppb)	Ir (ppb)	Ru (ppb)	Ni (ppm)	Co (ppm)	Cr (ppm)	Cu (ppm)	Zn (ppm)	As (ppm)	Se (ppb)	Te (ppb)	Bi (ppb)	Ash yield (%)
Black spruce twigs	17.6	31.7	1.58	1.36	6.1	15	0.8	12.1	28	19	0.11	242	37	90	2.2
Black spruce bark (outer)	45.1	63.2	2.17	2.05	12.8	29	1.6	53.2	60	17	0.19	342	76	182	3.8
Black spruce bark (inner)	4.3	7.2	0.20	<0.1	1.4	8	0.5	5.4	7	65	0.03	n.d.	n.d.	n.d.	3.4
Black spruce needles	3.3	9.8	0.24	<0.16	2.0	8	0.3	3.1	8	21	0.08	144	<12	20	4
Black spruce trunk wood	0.3	0.3	<0.01	<0.01	0.1	1	0.0	0.2	1	7	0.00	11	<1	1	0.37
Paper birch twigs	1.3	3.1	0.11	<0.05	0.6	13	1.1	0.6	6	89	0.02	51	8	7	1
Paper birch leaves	3.5	13.0	0.40	<0.16	2.2	24	1.3	3.4	12	100	0.06	260	16	28	4
Paper birch trunkwood	0.0	0.2	<0.01	<0.02	0.1	2	0.3	0.3	1	43	0.01	6	<1	1	0.47
Mountain alder twigs	0.7	1.9	0.14	<0.06	0.5	9	1.8	0.6	6	2	0.02	13	<3	4	1.1
Mountain alder leaves	1.5	7.0	0.13	<0.22	1.4	41	6.6	2.1	20	36	0.09	75	<13	13	4.4
Willow twigs	0.5	1.0	<0.03	<0.06	0.2	7	1.0	4.2	3	53	0.02	20	<4	3	1.4
Willow leaves	n.d	n.d	n.d	<0.24	0.7	30	4.2	1.1	n.d.	48	0.05	65	<18	18	5.9
Labrador tea twigs	3.3	7.4	0.28	0.30	1.4	26	0.9	3.4	27	9	0.06	180	18	32	2
Labrador tea leaves	10.9	19.5	1.09	0.59	4.1	33	0.9	7.4	45	14	0.12	203	31	70	3.9
Marsh grass	1.2	2.0	<0.03	<0.46	0.3	156	3.9	2.3	103	23	0.05	150	<20	13	6.5
Background[1]	0.005	<0.1	<0.01	<0.01	<0.1	1.5	0.2	1.5	10	50	0.1	20	20	10	

Note: n.d. = not determined.
[1]Background values are those shown in Table 1-III.

TABLE 4-VIII

Element concentrations in the ash of tissues from a single lodgepole pine (*Pinus contorta*) rooted in mineralized (Pb/Zn) tourmalinite, near the former Sullivan Pb-Zn mine, Kimberley, British Columbia

	Top Stem	Lower Twigs	Outer Bark	Roots
Ag (ppm)	1	3	13	77
As (ppm)	9	9	52	190
Au (ppb)	<5	<5	20	19
B (ppm)	1150	400	260	580
Ba (ppm)	48	310	1000	500
Cd (ppm)	52	95	143	135
Cr (ppm)	6	18	18	10
Cs (ppm)	110	9	5	38
Cu (ppm)	400	180	158	190
Mn (ppm)	13,000	27,000	4230	63,000
Ni (ppm)	180	22	14	24
Pb (ppm)	150	2950	4900	16,400
Sb (ppm)	2	3	13	5
Zn (ppm)	6100	7350	5700	12,800

British Columbia. Remarkably high concentrations of Pb, Zn, Cd, Ag and Mn occur in the roots. However, Ni, Cu, B and Cs are at their highest concentrations in the treetop. This substantial variation within a single plant should be appreciated before commencing a sampling programme – it emphasizes the importance of being consistent in collecting a similar amount of the same tissue type at each sample station.

A question that commonly arises concerns the long-term stability of biogeochemical data – especially in the vicinity of mining operations. The Sullivan Mine presents a rare opportunity to illustrate and comment on this question. In 1947, Harry Warren and Robert Delavault collected samples of twigs from lodgepole pine and willow at several localities close to the mine. Data were presented on the Zn content of these samples. Although the same trees were not sampled when a larger survey was undertaken in 1993, the data indicate that concentrations in the pine twig ash from samples collected near mineralized rocks of the Aldridge Formation some 45 years later (Table 4-VIII; 7350 ppm Zn) were similar to those found previously (Table 4-IX; 6300–14,000 ppm Zn).

A study of new and older growth from the crowns of Douglas-fir shows some of the significant flushing of metals to new growth, and underscores the importance of consistency in collecting a similar number of years of growth at all sample stations (Table 4-X). This example is from a single tree, but others yielded the same patterns of relative concentrations. Of note is that As, Cu, Ni and Zn are highly concentrated in the new needle growth compared to the second year growth. Conversely, B, Sr, Al,

TABLE 4-IX

Concentrations of Zn (ppm) in twigs of lodgepole pine and willow collected from around the Sullivan Mine in 1947 (from Warren and Delavault, 1949)

Location	Lodgepole pine twigs		Willow twigs	
	Dry	Ash	Dry	Ash
Aldridge Fm: near Sullivan #1 fault	60	3000	225	11,250
Diorite: near Sullivan #1 fault	90	4500	540	27,000
Aldridge Fm: near ore (no faulting)	195	**9750**	460	23,000
Aldridge Fm: near East fault (A zone)	126	**6300**	609	30,450
Aldridge Fm: near East fault (B zone)	280	**14,000**	732	36,600
Diorite: near Lois fault (no known mineralization)	55	2750	–	–

TABLE 4-X

Variations in the chemistry of needles and twigs from the crowns of mature (30–40 m) Douglas-fir (*Pseudotsuga menziesii*) – southern Vancouver Island. Samples collected from a helicopter. Concentrations are in ash; to calculate dry weight equivalent divide by 100 and multiply by the ash yield (last row)

	Needles 1st year	Needles 2nd year	Needles 1st + 2nd year	Twigs 3 years of growth
As (ppm)	337	40	83	2326
B (ppm)	297	370	363	487
Cu (ppm)	124	36	43	266
Ni (ppm)	100	39	47	92
Sr (ppm)	257	515	493	848
Zn (ppm)	660	297	342	1806
Al (%)	0.5	0.88	0.8	1.11
Ca (%)	9.1	17.2	16.2	20.6
Mn (%)	1.7	5.1	4.4	1.1
Ash yield (%)	2.9	4.2	3.7	2.6

Ca and Mn are more concentrated in the second year growth. All of these elements, except Ni and Mn, are enriched in the twigs demonstrating that the twigs act as a reservoir from which elements are flushed to new growth in varying proportions, according to the nutritional requirements and/or tolerances of the plant.

It is this sort of evidence that demonstrates the critical importance of consistency in collection of plant tissues, and reaffirms the situation shown for Au (Table 3-II) that optimal anomaly to background contrast is obtained from sampling in the spring when sap is rising and new growth is forming.

Thus, during the growing season there are constant fluxes of elements through a plant, such that the chemistry of a sample collected in the spring cannot be readily compared to that of another sample from the same plant that is collected later in the year. In addition to the natural cycling of plant saps, salts that accumulate on leaf surfaces and cuticle that dries and spalls in hot weather (Fig. 4-1) are steadily removed, along with their trace elements, by rain and wind. As foliage drops to the ground in the autumn, and as plants die, they return their metals to the ground to be picked up by the next generation of vegetation growth that, in turn, attains its own equilibrium as it establishes its metabolic requirements. It appears that chemical equilibrium of the microenvironment that surrounds a tree is soon established – perhaps within a few generations of growth.

Bark

Throughout the forests of North America, Europe and Russia and locally throughout the rest of the world, the sample medium that is commonly the most informative (and the easiest to obtain and process) is the scaly outer bark of conifers. Bark can be scraped from around the circumference of the tree, preferably at chest height (for both consistency and practical purposes). It is sometimes necessary to first snip off branches at the tree trunk in order to expedite bark scraping. Whereas a hunting knife can be used to scrape off bark scales directly into a paper bag (ensuring that the knife blade is pointing upward while scraping downward so that it does not dig into the inner bark), a hardened steel paint scraper and a dustpan with a semi-circle cut out (to rest against the curve of the tree trunk) is a more effective combination (Fig. 4-4). Bark scrapings can then be poured into an appropriate sample bag. Ragnar Bruaset, a prospector, from British Columbia, first suggested this innovative technique after he had grazed a few knuckles using the hunting knife method.

Some conifers (mostly the firs) have smooth barks that are impractical to collect, and are not particularly informative. Balsam fir, as an example, has a smooth bark studded with resin-filled blisters. The outer bark is difficult to shave off, the blisters yield messy smears of sticky resin, and furthermore the material yields low levels of most elements of economic interest.

Elsewhere in the world some mature deciduous trees develop bark textures that are sufficiently scaly for a few grams to be scraped off (e.g., Fig. 4-5). In a study of 23 species commonly found throughout Amazonia, most metals proved to be more concentrated in outer bark than in foliage (Dunn and Angelicá, 2000), with a notable exception being Hg which yielded considerably higher concentrations in the leaves (Table 4-XI).

There are usually substantial differences between the compositions of the outer bark ('rhytidome') and the inner bark ('bast'), so with rare exceptions it is important to separate the two tissues. This should be undertaken in the field, because if a complete bark profile is collected and allowed to dry, it becomes difficult to separate

Fig. 4-4. Collection of outer bark scales from typical conifer. A paint scraper (hardened steel) of the type shown is an effective tool for this purpose.

Fig. 4-5. Felled *Clarisia racemosa* ('oity') from the Amazon, showing scraped bark and a sample of leaves from the same tree.

TABLE 4-XI

Element concentrations in dry samples of outer bark (grey scales on a bright red inner bark – Fig. 4-5) and leaves from a single tree of *Clarisia racemosa* rooted in gold-bearing rocks at a 'garimpo' trenched site in Pará State, Brazil

	Bark	Leaves		Bark	Leaves
Au (ppb)	22.1	0.9	Na (ppm)	56.2	53
As (ppm)	0.12	−0.02	Ni (ppm)	−2	−2
Ba (ppm)	9	−5	Rb (ppm)	3	12
Br (ppm)	20	17	Sb (ppm)	0.01	0.012
Ca (%)	0.1	0.26	Sc (ppm)	0.17	−0.01
Ce (ppm)	2.5	−0.1	Se (ppm)	0.4	0.2
C (ppm)r	1	−0.3	Sr (ppm)	19	21
Fe (%)	0.063	−0.005	Ta (ppm)	−0.05	−0.05
Hf (ppn)	0.24	−0.05	Th (ppm)	0.9	−0.1
Hg (ppb)	70	150	U (ppm)	0.08	−0.01
K (%)	0.057	0.5	W (ppm)	−0.05	−0.05
La (ppm)	1.1	0.02	Zn (ppm)	6	13
Mo (ppm)	0.08	−0.05			

TABLE 4-XII

Concentrations (in ash) of elements in inner and outer bark from two red spruce trees, central Nova Scotia (ash yield approximately 2%). Analysis by INAA

	Tree A		Tree B	
	Inner bark	Outer bark	Inner bark	Outer bark
Au (ppb)	<5	51	9	126
As (ppm)	2	56	93	300
Sb (ppm)	0.1	10	0.7	3.5
Cr (ppm)	1	41	7	18
Fe (ppm)	500	16,000	2200	16,000
La (ppm)	0.5	16	3	18
Ba (ppm)	3600	1500	5100	2500
Zn (ppm)	3300	1600	9200	3900
Ca (%)	30	18	32	28

the two bark layers. Most elements of economic significance are concentrated in the outer bark, as was shown earlier with regard to PGE in black spruce (Table 4-VII). This is an important fact to recognize when conducting a biogeochemical survey. Table 4-XII shows that in an area of Au mineralization in eastern Canada, the

concentrations of Au, As, Sb, Cr, Fe and La (and the other REE) were significantly higher in the outer than in the inner bark of red spruce. However, the reverse was true for Ba, Zn and Ca, all of which concentrate in woody tissue. This pattern is similar for most tree species.

Mature Douglas-fir have very thick development of their outer bark (the 'rhytidome'), up to several centimetres in thickness. A question that might arise is should a chunk representing the entire outer bark be sampled, or is a simple scraping of the outermost material going to provide similar information? Table 4-XIII provides some answers to this question by comparing samples from two trees. The data show that for Au, As, Co, Mo, Rb and Zn the concentrations are similar; Ca is much higher in the entire rhytidome probably because of high concentrations of Ca-oxalate crystals; the remaining elements in the list (Br, Cr, Fe, K, La, Sb) are higher in the outer bark scrapings. The higher Br in the outer bark may be more a function of salt spray from proximity to the ocean than natural variations due to the trees' metabolism.

In the case of paper birch (*Betula papyrifera*) there are more than two layers of bark (Table 4-XIV). The tree sampled was rooted in a fault zone that extended from an open pit from which Au and Cu had once been extracted. In this example the middle bark layer contained the highest levels of Au, As, Ba, La and Zn, with lowest levels in the bast of the inner bark. Rubidium was quite evenly distributed across the bark layers.

TABLE 4-XIII

Douglas-fir bark – Bowen Island, Howe Sound, southern British Columbia. Concentrations are in ash determined by INAA

	Site 1		Site 2	
	Bark surface	Entire rhytidome	Bark surface	Entire rhytidome
Au (ppb)	56	64	81	78
As (ppm)	53	40	39	34
Ba (ppm)	2100	3300	2200	2100
Br (ppm)	140	28	84	16
Ca (%)	18	27.5	12.2	20.5
Co (ppm)	10	7	9	7
Cr (ppm)	44	26	51	39
Fe (%)	1.76	0.79	1.87	1.25
K (%)	8.06	4.31	3.41	2.26
La (ppm)	39	20	51	35
Mo (ppm)	9	4	6	7
Rb (ppm)	64	73	59	45
Sb (ppm)	63	34	62	26
Zn (ppm)	2200	2500	1200	1400

TABLE 4-XIV

Birch bark (*Betula papyrifera*) – concentrations (in ash determined by INAA) of bark layers. Anglo-Rouyn mine area, northern Saskatchewan

	Outer	Middle	Inner
Au (ppb)	108	153	10
As (ppm)	22	18	0.9
Ba (ppm)	450	870	1700
Ca (%)	5.2	12.2	28.7
Cr (ppm)	38	14	3
Fe (%)	2.76	0.71	0.05
La (ppm)	20	7	2
Na (ppm)	12,000	3410	506
Rb (ppm)	120	160	190
Zn (ppm)	3000	16,000	8800

Layers of bark can be readily peeled from paper birch, and the implication from these data is that a combined collection of the outer and middle bark would comprise the optimal sampling medium. This is fortunate, because collection of the inner bark requires a somewhat tedious process of cutting with a knife to remove a segment of the spongy material that it comprises, and it leaves a dark scar on the tree that can last for many years until new bark is formed. Of course, complete stripping of the inner bark from around the tree should be avoided as it would result in its death, because the conduit for the rising sap would be lost.

Twigs

In Chapter 3, the variations in the chemistry of individual twigs were discussed. It was demonstrated why surveys using twigs should comprise a similar number of years of growth; in particular, because the chemistry of a twig varies along its length and the highest concentrations of heaviest and toxic metals occur toward the tips. This is probably because the ratio of twig bark (containing most of the metals) to twig wood increases as the twig diameter decreases. Consequently, if 3 years of growth is collected at one site and 10 years growth from another, a comparison of the element concentrations would give misleading results because of the differences in composition of twigs of differing ages.

In the boreal forest, 10 years of growth is commonly 25–30 cm in length, with a maximum twig diameter of 3–5 mm. A similar length and diameter of twig from temperate or tropical climates will usually represent far fewer years of growth. A length of 25–30 cm is a practical size to collect, and 7–10 twigs of this size are usually more than sufficient for most analytical programmes.

TABLE 4-XV

Variations in the distribution of selected elements along branches of a single western hemlock at the Ladner Creek Au deposit, near Hope, southern British Columbia. Concentrations in ash determined by INAA (approximately 2% ash yield) (from Dunn and Ray, 1995)

	Thick (oldest)	Medium	Thin (youngest)
	(> 10 mm diameter)	(5–10 mm diameter)	(< 5 mm diameter)
As (ppm)	22	31	82
Au (ppb)	530	650	1590
Br (ppm)	19	18	18
Ca (%)	29	24	14
Co (ppm)	11	12	21
Cr (ppm)	32	26	84
Cs (ppm)	2	2	2
Fe (%)	0.8	1.1	2.3
La (ppm)	2	3	6
Na (%)	0.4	0.4	1.1
Sr (ppm)	430	480	450
Zn (ppm)	1500	1400	1900

To elaborate on the variations of chemistry with years of growth, data (Table 4-XV) illustrate changes in composition along a branch of western hemlock (*Tsuga heterophylla*) from above Au mineralization at the Ladner Creek Gold deposit (formerly the Carolin Mine) in British Columbia. The differences in Au, As and Cr distributions are particularly striking, with each being most concentrated toward the twig ends. As noted in the case of inner and outer bark, not all elements follow the same trend. Calcium is more enriched in the thick part of the branch, whereas Sr and Zn are homogeneously distributed.

Foliage

Whereas metal concentrations in the needles of conifers are generally lower than concentrations in twigs, metals accumulate in the leaves of many deciduous and evergreen species and, on a dry weight basis, the concentrations are commonly greater than the concentrations in twigs. This seems especially true of plants from the tropics. Table 4-XVI shows results from the analysis of dry tissues of *Acacia* from the vicinity of the North Mara Au mine, situated in the Lake Victoria Greenstone Belt in northern Tanzania. With few exceptions, there are substantially higher concentrations in the leaves than the stems; in particular Hg, Sb, REEs, Nb and Ag.

TABLE 4-XVI

Comparison of element concentrations in leaves and stems of *Acacia spp*. from northern Tanzania. Determinations of dry tissue by ICP-MS (nitric acid followed by aqua regia digestion). Data from Paul Taufen, courtesy of Placer Dome Tanzania

	Stems $n = 37$	Leaves $n = 37$	Relative concentration Leaf: stem
Ag (ppb)	3	23	8.4
Al (%)	0.006	0.037	6.0
As (ppm)	0.07	0.20	2.7
Au (ppb)	0.4	1.7	4.3
B (ppm)	16	48	3.0
Ba (ppm)	35	52	1.5
Be (ppm)	0.05	0.07	1.5
Bi (ppm)	0.010	0.015	1.5
Ca (%)	1.667	2.178	1.3
Cd (ppm)	0.009	0.013	1.4
Ce (ppm)	0.23	1.95	8.7
Co (ppm)	0.05	0.25	5.1
Cr (ppm)	2.6	3.2	1.3
Cs (ppm)	0.04	0.09	2.3
Cu (ppm)	9.1	8.4	0.9
Fe (%)	0.006	0.041	6.7
Ga (ppm)	0.050	0.149	3.0
Ge (ppm)	0.009	0.011	1.2
Hf (ppm)	0.004	0.028	7.5
Hg (ppb)	1.3	15.4	11.7
In (ppm)	<0.01	<0.01	
K (%)	1.007	1.214	1.2
La (ppm)	0.22	1.51	6.8
Li (ppm)	0.22	0.37	1.7
Mg (%)	0.185	0.286	1.5
Mn (%)	20	61	3.0
Mo (ppm)	3.2	2.8	0.9
Na (%)	0.011	0.026	2.3
Nb (ppm)	0.04	0.40	10.0
Ni (ppm)	1.3	1.4	1.1
P (%)	0.102	0.180	1.8
Pb (ppm)	0.06	0.425	6.7
Rb (ppm)	15	20	1.3
Re (ppb)	3	20	6.2
S (%)	0.114	0.221	1.9
Sb (ppm)	0.08	0.79	10.2
Sc (ppm)	0.09	0.13	1.3

Continued

TABLE 4-XVI *Continued*

	Stems $n = 37$	Leaves $n = 37$	Relative concentration Leaf: stem
Se (ppm)	0.23	0.43	1.9
Sn (ppm)	0.13	0.09	0.7
Sr (ppm)	243	264	1.1
Ta (ppm)	0.001	0.005	4.0
Te (ppm)	0.010	0.012	1.1
Th (ppm)	0.009	0.064	7.4
Ti (ppm)	3.6	14.1	4.0
Tl (ppm)	0.013	0.020	1.6
U (ppm)	0.005	0.026	5.1
V (ppm)	<1	<1	
W (ppm)	<0.1	<0.1	
Y (ppm)	0.05	0.6	11.3
Zn (ppm)	15	21	1.4
Zr (ppm)	0.16	1.32	8.3

In the case of Acacia, there is not a great difference between the ash yield of stems (commonly 5–7%) and that of the foliage, so the leaves would be the preferred medium for a number of reasons.

- They are readily removed from the stems after drying.
- They are easy to handle (no thorns) and are easy to mill to a fine powder.
- They contain significantly higher concentrations than stems of most elements.

However, if analysis is to be performed on the ash of some species (notably the conifers), then the lower ash yield of the twigs/stems results in concentrations of elements in twig ash that may be higher than in foliage – i.e., 2% ash yield from twigs represents a 50-fold concentration of elements compared to the dry tissue, whereas the 5% ash yield from some types of foliage represents concentrations of only 20-fold. Such factors need to be taken into account when designing a sampling programme and deciding upon the appropriate analytical protocols.

One more consideration is that seasonal changes in the chemistry of leaves are typically more rapid than for twigs – especially for deciduous species. The chemical requirements for leaf cell growth change as the plant enters its growth cycle. Horticulturalists are keenly aware that there are evolving needs for both major and trace elements during the growing season, and appropriate fertilization is required to optimize growth. The chemistry of newly unfurling leaves is different from that of mature leaves, later in the year. There is a complex chemical balance between what a plant requires at any one time, and what it is able to tolerate. By analogy, a small child is less tolerant to poisons entering the body than is an adult. For trees and shrubs chemical changes are less intense for the twigs than the leaves, because twigs integrate changes

in plant chemistry over a number of years, whereas deciduous leaves are short-lived. This explains why the recommendation for twig collection is to obtain several years of growth. A contributing factor to the more pronounced seasonal changes in deciduous leaves compared to twig chemistry is that in hot weather there is appreciable transpiration through the leaf stomata, with the result that there is nucleation of salts that are readily washed from leaves during rains or blown away with spalling cuticle.

These processes further explain why a biogeochemical survey that uses deciduous leaves should be conducted within a short time frame (a week or two), and any heavy rains, hot days or high winds should be noted. Note that throughout this last discussion, 'leaves' have been consistently prefixed by 'deciduous'. For evergreen leaves, it seems that, because of their multi-year existence, their chemistry is more stable, although it is wise not to mix newly unfurled pale green leaves with those of darker foliage that has been present for several years.

Trunk wood

Concentrations of most elements of exploration significance are substantially lower in trunk wood than elsewhere within tree structures. An exception is Ag in conifer trunk wood, since 10 ppm Ag in ash is a common background concentration, whereas twigs have <2 ppm Ag. This difference is less obvious in the analysis of dry tissues, because the ash yield of conifer wood is usually about 0.5%, whereas that of twigs is close to 2%. When these values are considered on a dry weight (DW) basis, 10 ppm Ag in wood is 50 ppb Ag DW and 2 ppm Ag in twigs is 40 ppb Ag. For many other elements the relative concentrations in wood compared to twigs are much lower.

If there is uncertainty as to whether twigs or bark might be contaminated with airborne dust, an analysis of wood ash might be a viable approach and help to resolve this problem. Table 4-XVII shows concentrations of Au, Ag and As in lodgepole pine from the vicinity of the former Nickel Plate mine. Typical background levels in the ash of these tissues are <10 ppb Au, <5 ppm As and variable levels of Ag with <2 ppm in both inner and outer bark, but about 10 ppm in trunk wood. The data demonstrate unusual enrichment of metals on the outside and inside of the trees, indicating absorption through the roots rather than airborne contamination.

In areas remote from any possibility of airborne contamination, anomalous concentrations in trunk wood can usually be related to underlying ore bodies (Walker, 1979; Dunn, 1981). However, in some cases such enrichments may not be entirely due to natural enrichment of these metals in the ground. If, from mining operations, there is a constant flux of metalliferous dust, when it is deposited on the ground it may be partially dissolved by rainfall and the solution absorbed by the root systems. Near extensive mine workings or smelters this 'secondary biogeochemical enrichment' is a conundrum and a complication that should be considered as a possible mechanism to create biogeochemical anomalies when interpreting biogeochemical data.

TABLE 4-XVII

Gold, As and Ag in the ash of pine tissues (analysis by INAA). Three lodgepole pines located about 1 km from the former Nickel Plate mine, Hedley, British Columbia

	Outer bark	Inner bark	Trunk wood
	Gold (ppb) in ash		
Site 1	420	114	128
Site 2	308	28	56
Site 3	238	32	36
	Arsenic (ppm) in ash		
	Outer bark	Inner bark	Trunk wood
Site 1	220	25	59
Site 2	160	22	41
Site 3	150	20	33
	Silver (ppm) in ash		
	Outer bark	Inner bark	Trunk wood
Site 1	1.5	<2	18
Site 2	0.9	<2	20
Site 3	<2	<2	20

In general, concentrations of many trace elements in dry wood are below detection levels, even when analyzed by the sensitive ICP-MS instrumentation. However, since the ash yield of conifer trunk wood is very low, by reducing wood to ash elements may be concentrated 100- to 400-fold, depending on the tree species. Wood ash from hardwood species has higher ash yields, higher levels of macronutrients than conifers and the silica content is frequently lower. Some biogeochemical surveys using conifer trunk wood ash have been undertaken in areas prior to any mine developments and have yielded some interesting anomalies, e.g., Au at the Red Mountain Stockwork, Idaho (Erdman et al., 1985) and U in Saskatchewan at Key Lake (Walker, 1979) and McClean Lake (Dunn, 1981). In the tropics wood ash was the medium used in the first recorded biogeochemical survey for Au noted in Chapter 1 (Baromalli and 'ironwood', Lungwitz, 1900).

Treetops

Studies have established that many elements can migrate to the tops of trees – e.g., Table 4-VIII. This phenomenon is illustrated further in some data from an undisturbed area of the Yukon, in the vicinity of the Brewery Creek Au mine, prior to its development (Table 4-XVIII).

TABLE 4-XVIII

Concentrations of selected elements in ash of tissues (top, twigs and bark at chest height) from black spruce (*Picea mariana*), Brewery Creek Au mine, Yukon. Analysis by INAA (ICP-ES for Cu and Ni)

	Site 1			Site 2			Site 3		Site 4	
	Top	Twig	Bark	Top	Twig	Bark	Top	Twig	Top	Twig
Au (ppb)	57	85	22	120	335	134	7	12	<5	10
As (ppm)	70	130	29	150	420	210	3.9	11	2.1	6.3
Ba (ppm)	13,000	24,000	17,000	12,000	14,000	11,000	480	2100	1400	3000
Cr (ppm)	33	37	17	50	49	30	24	24	26	24
Cs (ppm)	11	6.9	3.1	7	4.9	2.9	26	6.6	1.7	1.9
Cu (ppm)	155	144	117	133	117	88	162	209	137	162
Ni (ppm)	132	103	28	91	56	33	5	11	21	16
Sb (ppm)	130	200	47	300	710	290	1.9	5	1.8	3

Sites 1 and 2 were close to known Au mineralization comprising the 'Canadian Zone' prior to its development – it was just a trenched occurrence at the time that sampling took place. Sites 3 and 4 were from loess-covered northwest-facing slopes with permafrost and no known mineralization.

The data in Table 4-XVIII indicate that although the highest levels of some elements may not always occur in the crowns of trees, concentrations in the crowns are of significant magnitude that variations in the chemistry of the underlying terrain can be recognized.

Whereas in some forested areas the tops of scrawny trees can be quite readily sampled by simply bending them over to collect the upper branches (Fig. 4-6), in mature forest where trees are well developed this is not an option.

In order to obtain samples from the tops of mature trees a technique has been developed to hover in a helicopter over the crown and remove the top (or preferably the uppermost lateral branches) of the tree (Dunn and Scagel, 1989). From subsequent experience to achieve maximum utilization of the helicopter time it has been found that clipping treetops with shears is usually avoided, because the act of maintaining balance on the helicopter skid (even though a harness is worn) and wielding clippers at the same time is not a reliably safe procedure. It has proved faster and safer to simply break off the uppermost lateral branches or top of the chosen sample tree. Helicopter sampling is a very fast procedure, yielding up to 50 samples on a 250 m × 250 m grid in an hour, but it is demanding in that the procedure requires constant and rapid navigation and sampling decisions, while paying close attention to safety issues. There is very little time for any in-flight sample processing besides simply bagging the sample. For safety reasons, stand dominants are always the

Fig. 4-6. Collection of spruce twigs from upper branches – an appropriate sampling method in some environments.

preferred trees to sample. Furthermore the stand dominants are usually the most mature trees, therefore probably represent the best integrated signal of the local geochemical environment, and by always collecting the tallest and most vigorous of the trees in a forest stand they provide a consistent sampling medium.

A first step is to spend an hour or so conducting an orientation flight over the entire survey area to assess the architecture of the forest, the density of the desired species, and to appraise the ground conditions in general – e.g., noting any newly burnt areas, cleared areas and sites of potential contamination such as drill pads.

A typical survey procedure for the main survey is to snap a half metre or less from one or two lateral upper branches or the top of a single tree at each sample location. Continual communication must be maintained by the three-member crew (sampler, pilot and the assistant [who undertakes the bagging and GPS entry at pre-designated waypoints]). This is essential for both safety and efficiency. While in the air the assistant can on occasion undertake some preliminary culling of excess tissue, but there is no time for effective reduction of the sample material to the size and consistency required for analysis; time demands that the sample is quickly stuffed into a pre-numbered cloth sample bag (a polypropylene bag of about $10'' \times 17''$ [25×42 cm] is suitable) while the helicopter is positioning for the next sample collection. The bag must be tough to withstand a quick thrust of the plant stem without ripping, and so polypropylene bags of the type described in the next section are ideal. Sample trimming to a consistent composition is undertaken by ground personnel immediately after each flight. Figure 4-7 shows a typical procedure; although for illustration purposes a somewhat larger sample is shown than is usually required.

Fig. 4-7. Collection of treetop from a helicopter.

The two-person sampling crew undertakes a flight run to collect treetops, typically from about 50 trees before the crews need a short break and the helicopter needs to be unloaded at a pre-arranged helicopter staging/refuelling location. Distance between sample stations would normally be 250 or 500 m, although some reconnaissance surveys have been spaced on 2×2 km grids. Decisions on the size of the sampling grid should be made by considering the nature of the target mineralization. For example, a kimberlite target (e.g., 250×250 m) would require closer sample spacing than a large porphyry system (several square kilometres). The size of the grid actually makes little difference to the overall collection time, because of the speed of the helicopter. Once procedures have been streamlined, and clear communications have been established among the pilot and the samplers, it takes little more than one minute per sample station – i.e., from leaving one sample station, flying to

the next (predetermined using waypoints programmed into a GPS), positioning the helicopter over the spire of a tree, and reaching out to collect and bag the sample.

Since the helicopter crew only has time to do the sample collection, at least one person is fully occupied on the ground at the staging post culling the samples to an appropriate volume and consistency of material for subsequent laboratory preparation. This person assists in unloading the helicopter, organizing and passing on the next set of bags, and receiving a verbal update on the sampling conditions, any features or problems of immediate relevance to the sampling programme, and a general comment on the nature of the forest stand.

The following account uses black spruce as an example. Figure 4-8 shows a typical field base set-up for a survey conducted in winter to early spring in the boreal forest. It requires a large clean working top, large pruning shears for trimming the thick stem (1–2 cm diameter) that is buried deeply in the ball of cones, and smaller pruning shears for use as required to further trim the samples.

As a guide to the amount of material to collect, the following are approximate dimensions, weights and yields, which vary by the latitude of a survey area and the moisture conditions – i.e., the long-term degree of saturation of the ground such that it is dry (xeric), intermediate (mesic) or boggy. These factors affect growth rates and they can affect the tree chemistry (e.g., black spruce absorb more Mn in boggy areas than xeric or mesic sites). A typical amount of material to collect for analysis would be (a) for black spruce, with its dense top cluster of needles and cones, a single length of about 30 cm (the length to which it should be trimmed back at the base camp) or (b) for most other conifers two or three lateral branches of 30–50 cm (because they do not have the same dense ball of tissues at their tops). This amount of tissue has an average fresh weight of 400–500 g. The moisture content is 30–50%, so that a 500 g

Fig. 4-8. Boreal forest winter/spring field base set up for sample consolidation.

sample weighs less than 250 g when dry. This dry weight comprises 30% needle, 25% twig and 45% cones.

Given this breakdown of tissue types, the original sample of black spruce weighing 500 g yields approximately 100 g of dry twig tissue (including the thick stem comprising the spire of the tree, which can represent half of this twig weight). This is plenty of material if the analytical programme calls for analysis of dry tissue, because only 1 g is the usual requirement for most multi-element analytical procedures. However, some survey programmes, such as those designed to determine the very low concentrations of PGEs that are present in plant tissue, require preconcentration of elements by reduction to ash. The ash yield of dry spruce twigs is 1.5–2%, so that of the original large ball of fresh treetop material weighing 500 g, the ash yield of all the twigs and stems is <2 g of ash. Also, since the stem should be discarded, there is barely 1 g of twig ash available. Fortunately, most multi-element analytical programmes require only 0.25 g of ash, or 0.5 g at the most, so there is sufficient ash available.

This example demonstrates two important points of relevance to biogeochemical exploration.

- Although from a 500 g ball of fresh black spruce top there would appear to be ample material for analysis, it is sobering to realise how very little ash ($\sim$1 g) that there is available from that part of the treetop where most of the elements of economic significance are sequestered – namely in the thin twigs.
- Secondly, the often-high concentrations (compared to soils) that are reported from the ash of plant tissues are a result of the localization of elements in specific plant organs, and their further concentration by reducing the dry matter to ash.

A final point for treetop sampling is that the cost of collection is not as high as might at first be thought. If an exploration programme has a helicopter on site for other activities, and the flying cost is, for simplicity of calculations, \$1000 /h, once a sampling crew is up to speed, about 50 samples can be collected in 1 h, for an average cost of \$20 of helicopter time per sample. Large areas can be covered in a short period of time, regardless of terrain and ground conditions. In the northern forests the ground is frozen and snow-covered for half the year, thereby precluding the easy collection of soil samples (a power augur would be required), yet in no way inhibiting the collection of treetops. Even at $-20\,^{\circ}$C an appropriately dressed crew is still able to function quite efficiently, although at temperatures above zero productivity does improve. Furthermore, in the summer months a ground crew might only collect 10–20 soil samples per day at 250 m intervals in heavily forested and/or rugged terrain, whereas a helicopter crew can comfortably expect to get 300 samples a day at the same sampling interval – with the added bonus of not having to fight off the flies and mosquitoes! Sometimes the vegetation can be the more informative medium, and sometimes the soils. If, from test samples, the soils are clearly superior, then the extra effort should go into launching a soil-sampling programme. However, if the vegetation is the more informative medium, or it is as equally informative as the soils, then the expediency of the treetop sampling procedure is well worth considering.

Cones

The previous section demonstrated that there is a high cone yield from the tops of black spruce. The typical bulbous top to this species is largely a function of an exceptionally high abundance of cones, and this phenomenon is not characteristic of all conifer species: locally, white spruce, pines and fir have high cone concentrations in their uppermost parts, but not as consistently or as densely as black spruce. As a result, for many species any plans to conduct a survey using only the cones might be thwarted by a lack of cone abundance.

Cones yield even less ash (0.2–0.5%) than twigs (~2%), therefore when reduced to ash, intuitively it would be expected that the metal content would be relatively high, in which case a strong anomaly to background ratio would make the cones a desirable medium. However, not all elements make their way into cones in the same proportions that they do into twigs. The twigs act as a reservoir from which cones select the desirable and essential elements for further propagation via their enclosed seeds.

There are now a number of tree canopy tourist attractions around the world at which a walkway built at treetop level permits a view of the strange and diverse ecosystems that evolve at the top of the forest. There is an abundance of new observations steadily being released to the scientific community and the populace at large. By the same token, the world at the top of a black spruce tree is very different from the lateral branches lower down the tree. As described in the previous section, the dense ball of cones contains a stem, many small lateral twigs laden with needles of several ages, newly forming small cones, old closed cones, older open cones that have dispersed their seeds and partially degraded cones that may have simply rotted or been partially eaten by animals or insects. The occasional insect crawls out and (rarely) bird feathers are part of the local ecosystem (Fig. 4-9).

An alternative to gathering cones from the air is to collect cones that have fallen to the ground, but this requires careful sorting by species unless there is a monoculture stand. In addition, it would be desirable to use only cones of similar condition, because further complications arise.

- Old cones yield higher concentrations of metals than new green cones and therefore the two should not be mixed when preparing samples for analysis.
- Cones that have opened up to disperse their seeds have a different composition, because some elements are concentrated in the now-scattered seeds. Therefore, open cones should not be mixed with closed cones – even if they are both old and suberized (suberin is hardened woody tissue that develops as the tissues age).

As part of an airborne survey to collect tops from lodgepole pine (*Pinus contorta*) in central British Columbia, the suberized cones were separated from the twigs and needles and sorted according to whether they were (1) Closed (with seeds contained), or (2) Open (seeds lost). A few trees also had some green immature cones. Table 4-XIX summarizes the data received from INAA of open and closed cones

Fig. 4-9. Composite sample from the top of a black spruce – typical needles, twigs and cones, with an unusual presence of matted feathers (a pellet regurgitated by an owl).

collected from nine trees. This demonstrates that for some elements (e.g., Cr, Cs, Mo, Rb and Zn) it would not compromise analytical data to mix open and closed cones as the concentrations proved to be quite similar. However, closed cones tended to have higher concentrations of K and Ni, and open cones had higher concentrations of Au, As, Br, Ca, Co, Fe, La, Na and Sb. From these data it appears that much of the K and Ni were lost with the seeds when the cones opened, whereas other elements were retained in the woody, dead tissues. Data from the few samples of immature (green) cones (not included in this summary) showed that they were relatively depleted in As, Cr, Fe, La, Na, Ni, Sb and Zn, but enriched in K (significantly), Br, Mo and Rb.

Given the complications that surround the collection of a consistent set of cone samples, it would probably be better to avoid their use as a sample medium for biogeochemical exploration. Analysis of cones carefully selected from a treetop-sampling programme could be used as complementary information to substantiate data obtained from twig or needle tissues.

Stunted trees of the Tundra

Toward the northern margins of the boreal forests, plant life endures extreme cold and long winters with short days. In this zone (e.g., north of about 60°–65°) trees are stunted and add very small increments of growth each year. A 20 cm spruce or birch might be 50 years old or more. If the black spruce are less than 20 cm high a vegetation sample should comprise whatever is practical (e.g., all of the above-ground

TABLE 4-XIX

Comparison of the relative concentrations of elements in open and closed cones from the same nine trees

	Relatively enriched in:		Similar in both (out of 9 trees)
	Closed cones (No. of trees)	Open cones (No. of trees)	Open and closed (No. of trees)
Au	1	5	3
As	0	9	0
Br	3	5	1
Ca	0	8	1
Co	0	6	3
Cr	0	4	5
Cs	1	1	7
Fe	2	7	0
K	5	0	4
La	0	6	3
Mo	1	1	7
Na	0	7	2
Ni	4	3	2
Rb	1	0	8
Sb	0	9	0
Zn	0	1	8

parts), but it remains important to be consistent and maintain a constant maximum diameter of twig/stem. Back in the laboratory, after the sample is dried and the needles have been removed, it is then easy to be consistent in sub-sampling the twigs of similar diameter and age.

In this environment, dwarf birch (*Betula nana* or *Betula glandulosa*) or various species of willow are commonly only a few centimetres high, so a collection of the spindly twigs with leaves is appropriate. From a combined sample of 50 g, about 40 g is leaf tissue, and for most practical purposes the leaves are the preferred medium, because of their ease of collection, preparation for analysis and informative value.

For the small evergreen shrubs (e.g., Labrador tea (*Ledum groenlandicum* and *L. palustre*) and crowberry (*Empetrum nigrum*)) the simplest procedure is to collect all of the aerial portions. These two Labrador tea species are very similar in composition, so as far as is known it is unnecessary to distinguish between the two. Crowberry occurs mainly in North America, but also in the southern Andes. About 50 g of these species will suffice for most survey objectives, and either the leaves or stems can be used in biogeochemical exploration.

LFH/forest litter/humus

These are not biogeochemical samples *sensu stricto*. However, since they are composed primarily of organic material they can be considered to fall under the broad umbrella of biogeochemical exploration. When dead vegetation decomposes it can act as a chemical sink for those metals that are readily immobilized by the generally acidic and reducing conditions associated with the organic matter. This material can, therefore, serve as a viable sampling medium in some environments.

The terms LFH and Forest Litter are synonymous, but the term humus is restricted to just the decayed material. LFH horizon of soil nomenclature comprises the uppermost layer of the forest floor, in varying stages of decomposition, and so it is an environment with a high level of microbial activity. The 'L' stands for 'leaf', 'F' for 'fibric' and 'H' for 'humic'. The leaf component is all of the fallen plant debris that is virtually undecomposed, and comprises leaves, twigs, fragments of bark, cones, seeds, roots as well as any other organic debris from either the Plant or Animal Kingdoms. The fibric material is this same material, but partially decomposed. The humic component is the well-decomposed material, and it commonly has an admixture of mineral grains. LFH, several centimetres in thickness, is common in most forested environments, but it is ephemeral in dry conditions where it is not always present in sufficient quantities to be used for a geochemical survey. It is impractical for a geochemical survey to try and separate the L from the F from the H, so when used it is treated as a single combined sample of the three components. Typically, a handful of this organic material (commonly 5 cm in thickness) is collected from immediately beneath any moss layer that might be present.

A detailed account of the nature and chemistry of humus and its contained compounds is given in Stevenson (1994). Given that the humic layer on the forest floor is made up of a variety of plant species and tissue types, it is to be expected that some inter-site variations in humus chemistry can be attributed to the various components of the forest from which the humus sample is derived. In order to minimise this variation, an approach is to collect all humus samples, wherever possible, from beneath a species of tree or shrub that is found throughout a survey area. A survey conducted in northern Saskatchewan focused on dead tissues of shrub alder (Dunn, 1998c). Field crews were instructed to collect from beneath mature alders. However, the humus is an inconsistent sample medium and the human factor entered the equation, generating some inevitable variation in sampling procedures amongst field crews. On later inspection, many samples were true humus, but others ranged from handfuls of rotted logs at one end of the spectrum to pebbly material with a high inorganic content at the other. Admittedly, at the end of a long hot bug-infested day it is easy to become a little lax in procedures; and in heavy rain and darkened dense forest it can become difficult to be consistent because the wet humus may be confusing to discern.

In this survey, out of the original collection of samples approximately 20% were discarded because they were considered inorganic-rich and atypical of the main

set of humus samples. In preparing the remaining samples selected for analysis, a simple procedure was devised to place each sample in a plastic colander with 2 mm openings through which the material was pressed by gently swirling it by hand. Sieving eliminated pieces of rotted wood and intact twigs and cones. The <2 mm portion of each sieved sample was collected in a large aluminium oven tray, and shaken by hand to allow gravitational separation of any inorganic particles. This proved to be a simple, but effective, method for eliminating the majority of the inorganic material, but certainly not all of the fine particulates. As so often happens in the real world of conducting a geochemical survey, a best compromise has to be taken.

Figure 4-10 is an example of the results for Au from that survey. Sites of known Au mineralization were evident, along with other sites yielding relatively anomalous concentrations worthy of further investigation. A lesson learnt from this study was that some samples were collected too close to the dusty roads, because plots of Na, K and Fe (and Fe-related elements – Hf, Sc, Th, REE) clearly follow the trend of the main unpaved highway along which large trucks frequently travel north-eastward to service the uranium mines of northern Saskatchewan. To avoid the main effects of this dust, samples of surface soils or vegetation should be collected at least 100 m from the road.

In western Australia the litter has been found to be an effective medium to use for exploration (Matthias Cornelius, personal communication), especially in areas dominated by 'mulga' (*Acacia spp.*).

Similarly in Brazil the litter, or mull, accumulates metals and reflects underlying mineralization (Cornelius et al., 2007). As part of that study litter samples were collected from the surface by hand following clearing of a small area from living vegetation. The litter layer was mostly 10–50 mm thick and contained both decaying and freshly fallen plant matter. Whereas care was taken to minimize the amount of soil particles trapped in the fine mesh of rootlets that occurred at the base of the litter, there were some sites where the litter layer was very thin and contained a considerable amount of soil, possibly washed from upslope. In a situation like this, it is difficult and time-consuming to completely separate out the inorganic material, and so allowance for some inorganic contamination must be made when interpreting the analytical data. Samples were taken over a gossanous base metal deposit. The element suite that best identified mineralization was Cu, Pb, Zn, As, Mo, Sb and In. In a few bark samples from Imbauba (*Cecropia spp.*) the best indicators were Pb, Mo and Sb.

Flowers, seeds, spores and pollen

Although each of these media can locally accumulate certain metals, they are only mentioned briefly, because they are rarely in sufficient abundance or are impractical to collect for a geochemical survey.

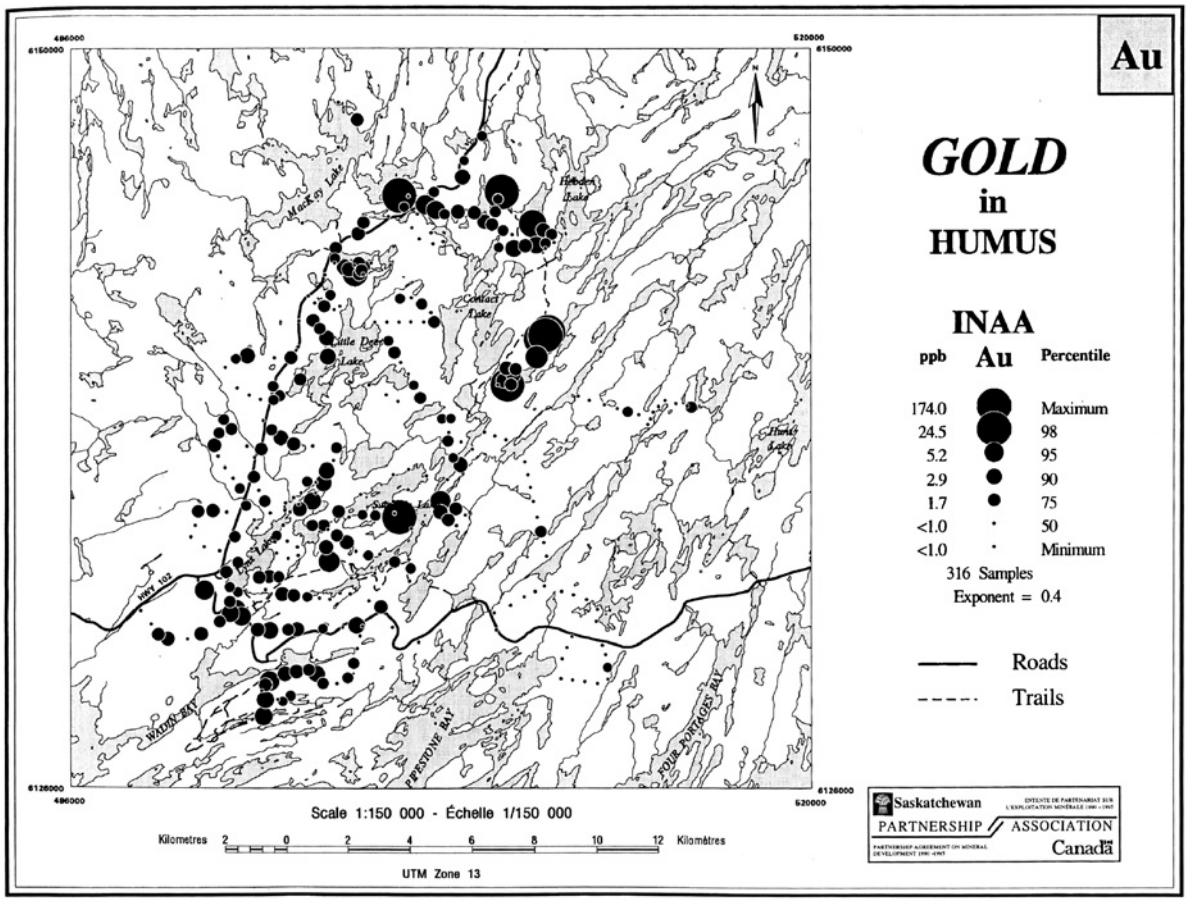

Fig. 4-10. Gold in dry humus – northern Saskatchewan (Dunn, 1998c).

Seeds generally do not represent a suitable sample medium, because of either difficulty of collection or irregular distribution of a species from which seeds can be collected consistently over a survey area. Acacia seeds from western Australia have been analyzed and found to contain Ni concentrations higher than those in associated litter, twigs and foliage (M. Cornelius, personal communication).

The spores from ferns collected in the western Amazon have been analyzed and the data compared to the fern leaf tissue (Table 4-XX). With few exceptions elements were at similar concentrations in both tissues, or higher in the leaf tissue. Since most fern samples do not have an overabundance of spores, if some prove to be particularly laden (abundant spore sacs on the underneath of the fronds, and obvious from the yellow powder that they generate when dried) it can safely be assumed that the integrity of the survey will not be compromised, although slightly better comparisons between samples can be made if surplus spores are not included in the sample that is to be analyzed.

Flowers are sufficiently ephemeral to be disregarded as a suitable sampling medium for a biogeochemical exploration programme. Relevant information on flower chemistry is scant. However, the pollen from the flowers has been used with some success using bees as the collecting agent. Bee pollen analyses can help in identifying metalliferous areas, since metals may accumulate in the pollen and bees rarely forage more than a few kilometres from their hives. By setting up a network of hives some regional biogeochemical prospecting can be undertaken by simply collecting and analyzing pollen from each hive. A number of researchers experimented with this procedure in the 1970s and 1980s, but there appears to be little or no published follow up since that time. Warren (1980), and Warren and Horsky (1984) provided some baseline data from studies in southern British Columbia and collected samples from the platiniferous Stillwater Complex of Montana; Free et al., (1983) published data from England; and in Saskatchewan, Wallace and Jahren (1986) published results of some of their work. The main findings of these studies are summarized in Table 4-XXI.

Summary

Unless there is sufficient prior knowledge of the biogeochemical characteristics of common species in a proposed survey area, an orientation survey is advisable, wherever feasible and practical. This involves collecting representative species and tissues from selected sites that preferably include a background area and an area of known mineralization. Analysis of these samples will determine the following.

- firstly, if the biogeochemical method appears to provide information of value to an exploration programme and
- secondly, the sample medium that provides the best geochemical signature.

TABLE 4-XX

Ferns from the western Amazon. Comparison of fern fronds with the spores retrieved from each sample. Concentrations in dry tissue by ICP-MS on nitric/aqua regia digestion

	Site 1		Site 2		Site 3	
	Frond	Spores	Frond	Spores	Frond	Spores
Ag (ppb)	146	204	7	13	122	80
Al (%)	0.14	0.12	0.41	0.08	0.01	−0.01
As (ppm)	0.3	5	0.3	0.9	1	1.3
Au (ppb)	16.8	9.7	0.6	−0.2	14.4	9.7
B (ppm)	14	9	11	5	19	6
Ba (ppm)	15.7	11.9	25.7	20.9	1.4	0.7
Bi (ppm)	−0.02	0.07	−0.02	−0.02	−0.02	−0.02
Ca (%)	0.24	0.2	0.06	0.09	0.21	0.09
Cd (ppm)	0.06	0.08	0.07	0.04	0.93	0.34
Co (ppm)	0.06	0.18	0.51	0.49	0.08	0.05
Cr (ppm)	2.13	2.61	2.02	2.02	1.84	1.9
Cs (ppm)	0.329	0.242	1.992	1.126	15.826	8.783
Cu (ppm)	15.65	13.19	18.32	10.59	16.89	10.25
Fe (%)	0.013	0.076	0.004	0.012	0.007	0.009
Ga (ppm)	0.1	0.2	0.1	−0.1	0.1	−0.1
Hg (ppb)	3943	1066	41	11	786	212
K (%)	1.4	0.93	1.01	0.67	1.56	1.26
La (ppm)	1.31	0.71	6.03	1	1.12	0.21
Mg (%)	0.187	0.211	0.167	0.193	0.165	0.139
Mn (ppm)	659	643	287	453	660	316
Mo (ppm)	0.22	0.39	0.05	0.04	0.12	0.07
Na (%)	0.002	0.002	0.004	0.002	0.002	0.001
Ni (ppm)	0.6	1.4	16.3	12.4	0.2	0.2
P (%)	0.146	0.157	0.114	0.155	0.185	0.189
Pb (ppm)	3.96	10.9	0.34	0.87	2.75	1.72
Rb (ppm)	67.8	44.6	69.5	44.2	181.4	129.6
S (%)	0.15	0.05	0.14	0.05	0.2	0.11
Sb (ppm)	−0.02	0.16	−0.02	0.04	−0.02	0.02
Sc (ppm)	0.2	0.3	0.1	0.3	0.2	0.4
Se (ppm)	−0.1	0.1	−0.1	−0.1	−0.1	−0.1
Sn (ppm)	0.05	0.11	0.02	0.05	−0.02	0.02
Sr (ppm)	9.9	6.1	17.1	11.1	0.6	−0.5
Te (ppm)	−0.02	−0.02	−0.02	−0.02	−0.02	−0.02
Th (ppm)	0.01	0.03	−0.01	−0.01	−0.01	−0.01
Ti (ppm)	7	18	5	6	7	7
Tl (ppm)	0.02	0.02	0.04	−0.02	−0.02	−0.02
U (ppm)	0.01	0.02	−0.01	−0.01	0.02	−0.01

Continued

TABLE 4-XX *Continued*

	Site 1		Site 2		Site 3	
	Frond	Spores	Frond	Spores	Frond	Spores
V (ppm)	−2	−2	−2	−2	−2	−2
W (ppm)	−0.1	0.2	−0.1	−0.1	−0.1	−0.1
Zn (ppm)	29.5	34	22.6	10.8	61.3	21.1

For typical boreal forest regions there are sufficient published studies that can be referred to for selecting an appropriate sample medium such that this orientation phase is no longer necessary. However, it may be prudent for an exploration manager to conduct a brief survey to verify that the biogeochemical method might be an effective approach for a particular survey area and style of mineralization. In other environments where there is limited published information, such as central Africa or the Amazon, there may be sufficiently diverse flora that an orientation survey could save considerable expense.

The various examples presented in the tables and figures of this and the previous chapter show how diverse the chemistry can be of different plants and their component parts. The example of Mo in the Amazon (Fig. 4-3) shows that a small survey can provide valuable focus for conducting a large survey. Furthermore, once a database of that sort is available, quick judgement calls can be made in the field as to what might be suitable to collect should an area of interest not contain any of the preferred sample media. The western Amazon example shows that, regardless of the absolute values, the areas of Mo enrichment were clearly discernible from analysis of each of the four samples that were investigated. Given this similarity of patterns, the following considerations then came into play for follow-up surveys involving a larger area and/or a larger number of samples.

- Which is the most common plant species in the survey area?
- Which is the easiest and least time-consuming to collect? The 'time' aspect is not usually a major consideration, because a great advantage of the biogeochemical method is that it can be undertaken in considerably less time than for most other geochemical sample media – it only takes a few seconds to collect a handful of leaves, and marginally longer to select and snip twigs, or scrape bark scales.
- Does each of the sample media provide values substantially above the limit of detection of the analytical method to be employed, or does one of the sample media have many values below detection? This can be important, because the geochemical relief of data at low concentrations can sometimes be of value to an exploration programme. Wherever possible, select a sample medium that is likely to provide the highest concentrations of the elements of interest.
- Which sample medium is the easiest to process? Additional costs can occur for the preparation of difficult samples; large samples of wood or chunks of bark involve additional laboratory costs because of the time it takes to reduce them to a particle

TABLE 4-XXI

Analyses of pollen from beehives. Average concentrations in dry pollen

Location		Reference	Cd (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Mo (ppm)	Pb (ppm)	Zn (ppm)	Ag (ppm)	Au (ppb)	Pd (ppb)	Pt (ppb)
Background areas													
Okanagan	British Columbia	Warren (1980)	0.3	12	77	20		2	32				
Omineca	British Columbia	Warren (1980)	0.6	4	57	39		<3	54				
Fraser Lake	British Columbia	Warren and Horsky (1984) Wallace and Jahren (1986)					0.5						
Gordon Lake	Saskatchewan	Dunn (unpublished)					<0.1			<0.2	0.02		
Daffy Green (Harpenden)	England	Free et al. (1983)		11	197	82		1.1	68				
Mineralized areas													
Trail (near smelter)	British Columbia	Warren (1980)	4	13				151	353				
Kamloops	British Columbia	Warren (1980)	1	54				3	33				
Endako (Mo mine)	British Columbia	Warren and Horsky (1984)					48						
Ashloo mine	British Columbia	Warren and Horsky (1984) Wallace and Jahren (1986)								0.1	0.4		
Anglo-Rouyn (Au), Sask.	Saskatchewan	Dunn (unpublished)			185		0.4		42	<0.2	1		
Cheddar Gorge (Cu/Pb)	England	Free et al. (1983)		17	215	138		3.8	118				
Kit Hill (Cu/Pb, Cornwall)	England	Free et al. (1983)		18	116	175		2.5	81				
Stillwater PGEs (samples provided by H. Warren)	Montana, USA	Dunn (unpublished)									1.7	2.9	<2

size suitable for analysis. In general, the easiest samples to prepare are conifer needles, deciduous leaves (although some may have tough central veins), followed by soft bark (conifer scrapings), soft twigs (conifers), hard bark (chunks from deciduous trees), hard thorny twigs (e.g., acacias) and cones and chunks of wood being the most time-consuming.
- Consider if the survey is to be a one-time event. If it is possible that a second phase of vegetation sampling might be required, then it might be advisable to collect also a medium such as outer bark that is already dead tissue and so seasonal variations in chemistry are not a consideration.

Finally, all things being equal collect whatever is easiest to obtain. If there is about as much information from leaves as there is from stems, then while at the sample station, the leaves can be readily removed from the stems thereby cutting down on sample bulk, freight costs and sample processing costs. However, if in doubt, collect stems and leaves, and let the analytical laboratory undertake the processing. One cautionary word, though, is that leaves from deciduous plants should be in a similar state of maturity because of seasonal changes in chemistry. This occurs, too, in stems and leaves of evergreens but is usually less pronounced. A final note of caution is that because of these seasonal changes, resampling of live tissue at a different time of the year is likely to generate different concentrations of many elements, so do not expect to get exactly the same analytical results. If returning to a survey area for further collection, obtain a few samples from trees already sampled during the first pass in order to assist in normalizing the data to a common base.

As a general rule, because of the speed of biogeochemical sampling, collect as many samples as possible on a first pass. It is not necessary to send all for analysis immediately; however, they should all be dried as soon as possible. Once dry, samples can be archived for future analysis in the event that interesting anomalies arise from the first-selected samples. Tests have shown that the trace element chemistry of dried samples is stable for many years – e.g., samples of Douglas-fir needles collected in 1988 were re-analysed in 2005 and yielded very similar values (Dunn et al., 2006a,b).

SAMPLE COLLECTION

Precautions

Procedures are mostly simple, but before conducting a survey a number of precautions need to be taken. The basic rule is to 'be consistent' – collect the same type of plant tissue and the same amount of growth, all from the same species, and as much as possible collect from trees of similar appearance and state of health.

A cardinal rule is to avoid contact with metals during sample collection. Jewellery represents an extremely concentrated point source of the metal of which it is composed, consequently rings (especially precious metals) and other metal jewellery should not be worn while collecting or handling biogeochemical samples. Even a

bracelet or necklace can be a source of contamination, because by adjusting their fit or inadvertently touching these items a rich source of metal can be transferred to the field sample. In order to demonstrate the magnitude of the problem to some student assistants a test was conducted. While wearing no gold jewellery, a sample was first divided into two portions. One half was prepared for analysis (separation of leaves from twigs) and an assistant wearing a gold ring prepared the other half. Remaining preparation procedures were identical, and the two samples were submitted for analysis. The 'no ring' sample yielded an analysis of ~20 ppb Au in twig ash, whereas the sample prepared while wearing the ring returned an analysis of 150 ppb Au in ash. Such a concentration is sufficiently anomalous that, if it were a natural level, it would warrant some field follow-up investigations.

Similarly, lotions, creams and plasters should not come into contact with field samples, because they may contaminate the samples (especially with Zn and B) and generate false anomalies. The use of anti-dandruff shampoos should be avoided, because they contain Se that can be a useful pathfinder element for some precious metal deposits. In short, all reasonable precautions should be maintained and field crews should remain alert to all possible sources of contamination.

Leather gloves provide effective barriers to most sources of vegetation contamination during sampling. The chemical variations among plant tissues are generally small, and so there is unlikely to be any measurable contamination from saps or leaf stains that may adhere to gloves. Saps contain much lower element concentrations than plant tissues, and a smear of sap transferred from one sample to the next is quantitatively only a very small amount of contamination. To put this into perspective, the sap smear may weigh only a few milligrams, whereas the field sample would typically weigh about 10,000 times more than this (e.g., 50 g). If, for example, the sap contained 1 ppb Au (an unusually high concentration for fresh sap), in a worst case scenario transfer to the next field sample would be only about 10,000th of 1 ppb, i.e., 0.0001 ppb Au which is a concentration well below the detection of ICP-MS analytical instrumentation, and represents a concentration that is about 1/1000th of a typical background value of Au in fresh plant tissue. As a result this is not a problem.

Dust from roads and trails can contaminate samples, and even rigorous washing does not always fully eliminate this 'anthropogenic' imprint. If samples are collected within 100 m of a busy and dusty trail or a paved road, the resulting element distribution patterns commonly give rise to a string of anomalies that seem to fortuitously follow the road. Figure 4-11 shows a plot of ash yields of black spruce twigs collected at increasing distance into the forest from a very dusty road in northern Saskatchewan heavily used by large haulage trucks. Typically, in this part of Canada in forest far removed from any source of contamination, black spruce twigs (most recent 10 years of growth) yield about 2% ash. The ash yield can be used as an estimate of the degree of particulate contamination. Figure 4-11 shows that at 25 m from the dusty road the ash yield was 3.7%, and with increasing depth into the forest this trailed off to 2% at a distance of 300 m. The area from which these samples were collected is extremely dusty in the height of summer, with extensive plumes of dust that

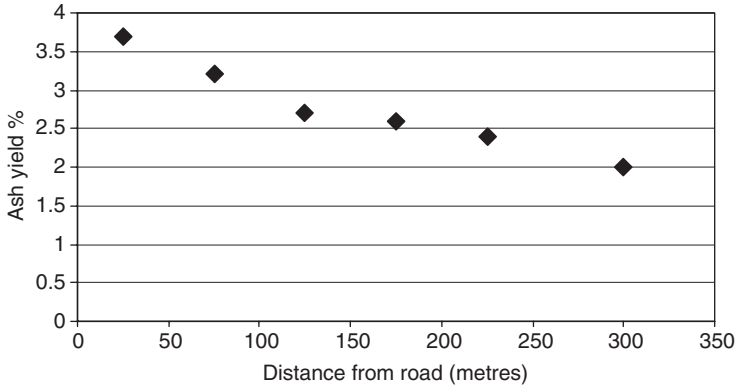

Fig. 4-11. Northern Saskatchewan – changes in ash yield of black spruce twigs with increasing distance from dusty road.

billow out from behind the large trucks, and so it is an extreme environment of dust contamination rendering trees close to the road almost white with dust. From other locations in the northern forests, where the dust levels are lower, it can be expected that there is little dust contamination at a distance of 100 m from the road. In the present example (Fig. 4-11), at 125 m from the road the ash yield was down to 2.7%.

Even along little-used forest trails it is advisable to collect samples from 50 m into the woods. Consequently, as a general rule it is best to sample at least 100 m from a road and 50 m from a forest trail. Elements that might indicate dust contamination are Fe and the high field strength elements – notably Ti, Hf, Nb, Th and Zr. If the road surface is paved with aggregate from a nearby quarry, borrow pit or (even worse) tailings pile, it can be expected that samples from close to roads will have elevated levels of the metals contained within the local excavations. It is important to be constantly aware of this potential problem. Due consideration should be given to the local geology, and effort should be made to determine what is the most likely source of road aggregate. Conversations with local people and anecdotal information can often help to find out where the aggregate may have come from.

In one situation a consistent Co anomaly was found to follow the trend of a dusty road in central British Columbia (Fig. 4-12 – eastern trail). From questioning local people who knew the long-term history of the area it was established that the road aggregate probably came from two borrow pits dug into mafic/ultramafic bodies. Analysis of these rocks confirmed that they contained elevated Co levels, and so this anomalous trend could subsequently be attributed to contamination from road dust.

Another example to demonstrate why samples should not be collected from close to major highways comes from a plot of Pb in pine bark (Fig. 4-13). In this case there is a clear relationship between Pb in lodgepole pine bark and the location of a major paved highway. Although Pb in gasoline has been banned in Canada for many years, it appears that a residual signature remains in the pine bark from samples along the roadsides. However, it seems, also, that there is a mixed signature in the centre of the

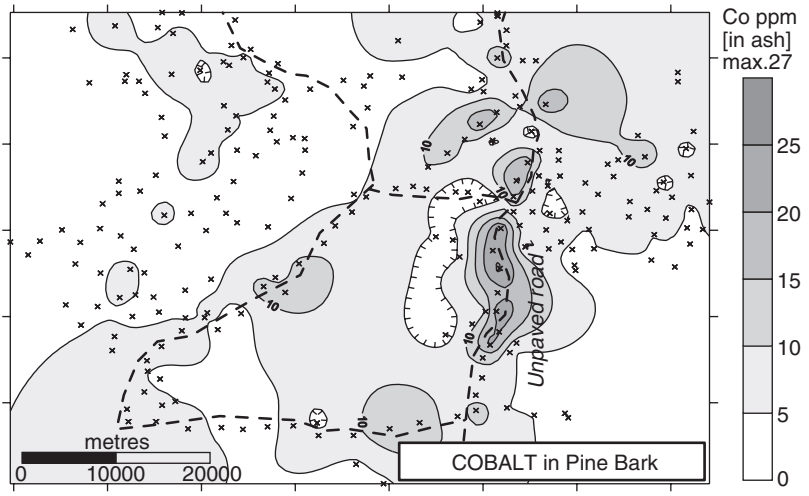

Fig. 4-12. Cobalt in the ash of lodgepole pine bark, Nechako area, central British Columbia. Crosses indicate sample locations.

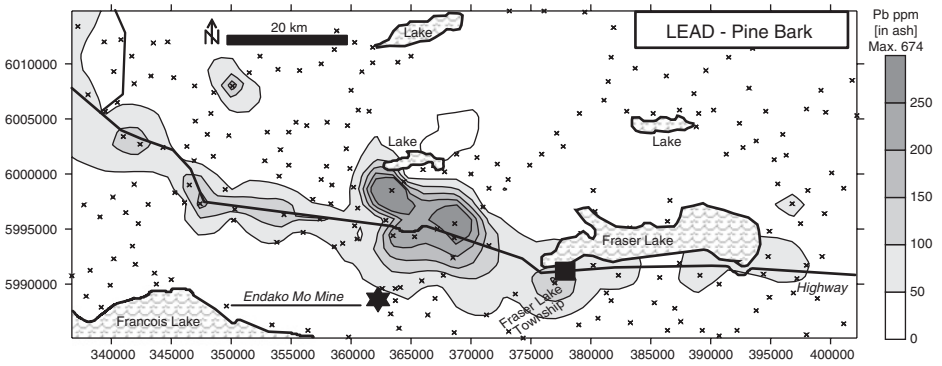

Fig. 4-13. Lead in ash of lodgepole pine bark, Endako, central British Columbia. Crosses indicate sample locations.

map, in that high concentrations of Pb occur to the north of the Endako Mo mine. This area has several sulphide-rich gossans, and the Pb anomalies probably relate to these occurrences.

Field accessories

Frequently asked questions include what special collecting tools are required? This includes the type and size of sample bags and the appropriate tools for scraping and snipping. Figure 4-14 illustrates accessories for vegetation sampling that are required in addition to the usual field items that a geologist would carry. Shown are

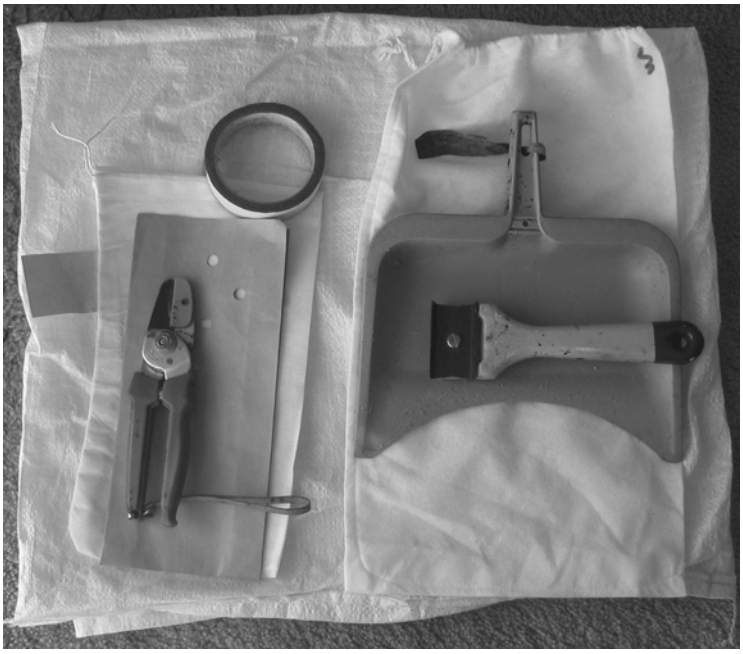

Fig. 4-14. Typical field accessories for vegetation sampling.

pruning snips resting on a 'kraft' paper soil bag and a small polypropylene bag with a drawstring; a paint scraper (for outer bark scales) and modified dust-pan resting on a larger polypropylene bag with drawstring (for larger twig + foliage samples). The roll of masking tape is used for closing the kraft bags. All items are resting on a flour/rice sack, folded in half, that can be used for easy shipment of the sample collections.

In more detail, these items and others that might be of use are:

- A pair of hardened steel pruning snips, preferably Teflon-coated anvil type, from any hardware or gardening store. Some field personnel prefer snips with a scissor-action, and it is really just a matter of preference. Usually the anvil type is more efficient, especially for cutting tough twigs or removing thick branches to gain access to the required tissue (e.g., bark of a tree).
- A hardened steel paint scraper for scraping bark, and either a plastic dustpan or large paper bag for collecting the flakes of bark. The dustpan is particularly suitable because the bark particles tend to fly off in all directions when scraped, and the dustpan readily catches most of them. Standing in an awkward position on a windy day it is frustrating if half of the scraped particles fall to the ground. The dustpan increases the percentage of particles caught, and it is easy to pour the scrapings into the sample bag. In order to streamline operations, a semi-circle can be cut from the lip of the dustpan so that it conforms better to the contour of the tree.

- On occasion a hatchet may be useful in the [unusual] event that collection of thick bark is required.
- A hunting knife can be substituted for the paint scraper. The blade should be angled upward when scraping down so that it does not dig into the inner bark. Inner bark should not be mixed with outer bark because of its substantially different composition. Although effective, the knife is less efficient than the paint scraper, and if gloves are not worn it is inevitable that knuckles will be skinned before the operator learns the optimal procedure.
- A roll of masking tape for closure of paper sample bags. Staples are convenient in the field, but tedious for the laboratory to deal with and on occasion a staple has been known drop into a sample that is submitted for analysis, generating unusual metal levels if it goes un-noticed.
- Leather gloves prevent any contamination from jewellery or lotions. When working in arid environments they become virtually essential where thorny species are present. Also in jungle environments they are useful for brushing off the omnipresent stinging ants.
- A large backpack; because if twigs or foliage is the chosen sample medium the volume of material collected soon becomes quite large, but not heavy. Usually, it is impractical to separate foliage from twigs in the field since separation is most easily performed back in the laboratory after drying. The less sample handling in the field the better, especially since weather conditions are not always conducive to spending extra minutes at each sample station and, in extreme weather, it becomes very tempting for the samplers to take short cuts that could compromise the sample integrity.
- For large surveys, heavy-duty plastic garbage bags or large flour/rice sacks are useful, and they can be left full of samples at the ends of traverses or cut lines to be picked up at the end of the day.
- A $\times 10$ magnification hand lens to help in species identification, and in counting growth rings on twigs to determine the age of the samples that are collected.

Sample bags

Intuitively, many people embarking on a biogeochemical survey tend to select plastic bags for containing their samples. However, this is not an appropriate choice because once samples have been collected moisture is readily released from plant tissues in the warmth of a backpack, such that by the end of the day the contained samples become a soggy mess. Moulds and slimes soon develop and sample integrity is lost as the closed chemical system of organic material contained within the plastic bag becomes a melting pot of degraded tissue.

- For bark scales that are scraped from a tree trunk, 'Kraft' (heavy brown paper) 12×28 cm gusset soil sample bags are suitable. The paper has a pH of 5.5 and no heavy metals are used in the glue. A short strip of masking tape is ideal for closure,

although flagging tape can be used to thread through the punched holes in the sample bag. These bags are tough and wet-resistant so rarely end up soggy or torn at the end of the day.

- For twigs and foliage [also mull, humus or peat] a fabric bag is preferred but not essential. An example of a suitable bag is the "Sentry Sample Bag" (or similar), that can be seen on webpage www.csinet.ca/files/catflyer/CFE-Catalog.pdf. These bags are made from spun-bonded polypropylene that is a strong and very lightweight fabric with excellent filtration qualities, such that moisture is readily released from the enclosed materials. They have a white polished drawstring making them easy to close under all weather conditions. They can be easily marked with indelible felt markers. The bags are rot and mildew resistant, and are supplied in five sizes of which the most suitable for biogeochemical samples are $\sim 14 \times 22$ cm and $\sim 18 \times 33$ cm. Their current cost is approximately $1 each. Chemical analysis of these bags shows that they have trace metal concentrations below the detection limit of ICP-MS, except for barely detectable (sub-ppm) concentrations of Cu, Pb and Zn. Consequently, the bags can be used with the assurance that they will not transfer any detectable contamination to the contained samples.
- Heavy-duty coarse brown paper bags (e.g., 7–9 kg hardware bags) can be used if conditions are dry, but they are somewhat more cumbersome. A size of $\sim 20 \times 30$ cm is usually suitable. Even lightweight brown paper lunch bags can be used in dry conditions, but double-bagging is recommended and samples with a high moisture content (such as leaves) degrade the paper to a pulp in quite a short period of time. Consequently, these bags should be used in emergency situations, only, and the vegetation transferred to more robust bags as soon as possible (preferably the same day). Paper bags can vary considerably in composition, and since brown paper is typically recycled from pulp, the metal content can be substantially higher than that contained in fabric bags. This is not a problem provided none of the paper bag itself gets processed with the vegetation samples.
- Plasticized aerated bags with drawstrings are tough, light and convenient, but less desirable, because samples should not be left in these bags for several weeks or they will grow mould. If mould should develop, the samples are unpleasant to handle and some remobilization of elements from the plant tissues to the mould takes place.
- Calico cloth bags should be avoided because they may rot and contaminate the sample from the fungicide with which they are commonly treated. Organic samples from the tropics are highly biologically active and acidic, such that natural materials (e.g., cotton bags) readily decompose. Analysis of these bags has revealed up to 50 ppm As and/or 50 ppm Sb in the material of dry, new calico bags that have been treated with fungicides. Other elements somewhat enriched are Ba, Fe, Mg, Na, Au and Sn. Whereas leaching of As and Sb from the bag to its contents may be of concern, concentrations of the other elements are not likely to be of significance, because either they are already present in relatively high concentrations (e.g., Ba, Fe, Mg) or relative to the bulk of the vegetation sample, the addition of metals from the bag to the vegetation is likely to be insignificant (e.g., Au, Sn).

Sample size

This depends on the objectives of the survey. In general, if a survey involves bark scales, a 'kraft' soil bag should be at least half-filled (30–50 g of material). If twigs and foliage are to be sampled, a combined (fresh) weight of ~200 g is preferred. As a broad rule of thumb, a sample of this size can be expected to contain about 50% moisture, leaving 100 g of dry tissue. Of this 100 g, for many species 70–80% is foliage, leaving 20–30 g of twig and 70–80 g of foliage. If leaf tissues are the required sample medium, 100 g samples provide an over-abundance of material and sample size can be reduced by half. However, if the twigs are required, then the original 200 g sample of fresh material generates the appropriate amount of dry twig (20–30 g). Smaller samples can be collected without compromising a survey, and now that analysis of 1 g samples of dry tissue by ICP-MS provides highly precise and accurate data for most elements, an initial sample weight of ~50 g of fresh tissue is adequate. Tests have shown that for most species 50 g provides a sufficiently representative sample of vegetation, and sometimes even just a few grams of tissue (e.g., small leaves, such as occur on some species of *Acacia*, *Artemisia* [sagebrush] and hardy desert plants) can provide a representative sample of foliage from the plant as a whole and generate precise and meaningful results.

It is a rare situation where trunk wood is the medium of choice, but a considerably larger sample needs to be collected, especially if samples are to be reduced to ash prior to analysis. In order to generate 1 g of ash from most conifers about 400 g of trunk wood is required, because the ash yield is sometimes as low as 0.25%. If dry trunk wood is to be analysed the laboratory is likely to charge extra for milling, because it is a time-consuming task to reduce chunks of wood to the powder required for analysis.

A consideration when deciding upon the size of sample to collect is the range of elements for which data are required. Some elements are present in such low levels in plants that tissues need to be reduced to ash in order to preconcentrate the elements prior to analysis. This is particularly true of Be, Bi, Ga, Ge, In, some high field strength elements (e.g., Nb, Hf, Ta, Zr), platinum group elements, some REE, Re, Se, Te, Tl, U, V, W and sometimes Au and Sb. Many of these elements are commonly useful 'pathfinder' elements for kimberlites and precious metal deposits; consequently, unless a high resolution ICP-MS is available for determining their concentrations in dry tissue, in addition to analysis of dry tissues it is worthwhile requesting the analytical laboratory to reduce 15–30 g of dry tissue to ash for supplementary data on the pathfinder elements. This topic is dealt with in more detail in the sections on 'ashing' and analysis (Chapter 6).

Samples required for quality control

In addition to the survey samples, sufficient additional samples should be collected to determine the reproducibility of the biogeochemical data. A 20-sample block

Sample		Sample	
1	*Field Duplicate 1*	11	*Field Duplicate 1*
2	*Field Duplicate 2*	12	*Field Duplicate 2*
3		13	
4		14	
5		15	
6		16	
7		17	
8		18	
9		19	
10	Standard	20	

Fig. 4-15. Typical 20-sample block QA/QC scheme used for sampling and analysis of geochemical field samples.

'Quality Assurance/Quality Control (QA/QC) scheme' similar to protocols used for Geological Survey of Canada regional geochemical surveys is outlined in Fig. 4-15.

A minimum requirement should be one standard of known composition (discussed in Chapter 6) and one field duplicate in each block of 20 samples, although some geochemists prefer to include an extra set of field duplicates at sites 1 and 2 of each block. Using the scheme outlined (Fig. 4-15) bags can be pre-numbered in order to ensure that sample numbers are not missed or duplicated and that the standards and field duplicates are not forgotten. The bags for the controls (standards and duplicates) should remain empty. Marking the bags for controls in different colours helps the field samplers to remember to collect a duplicate field sample. Once samples are collected, some workers prefer to randomize the samples submitted for analysis. Laboratories will usually undertake this procedure for a small extra cost.

Checklist for vegetation sample collection and site observations

It is advisable to prepare a field sheet for filling in sample numbers, species, tissues and locations. Before going into the field the numbers allocated for QA/QC should be clearly marked on the field sheets. Columns for a few simple observations to be entered on the field sheets may help in data interpretation. Suggested protocols include the following:

- Ensure that the correct species is collected (essential).
- Since there is not always the desired species growing at exactly the required sample station, samples should be collected within a radius of 5 m from the preferred station, although the density of the sampling grid and species distribution may dictate a larger radius. If there is nothing within this radius, then, after considering the scale of the survey, a judgement must be made as to whether it would be worthwhile to collect from farther away (noting coordinates of the sample site) or abandon that site and move on to the next. If a chosen sample interval is 25 m, then

about 5 m is likely to be the farthest for digression from the desired site. However, for a more regional survey, such as 200 m spacing, 50 m would be acceptable. This requires a judgement call on the part of the survey party leader, taking into account the nature of the target mineralization. If the objective is to pinpoint a narrow gold-bearing quartz vein, then a close sample interval with only minor digressions from the desired sample location should be accepted. However, for a massive sulphide body a much greater divergence from the preferred sample interval might be acceptable. If no sample is available after these considerations, the pre-numbered sample bag should be retained as an empty bag and field notes marked accordingly.

- Where possible, trees with similar amount of growth/appearance and state of health should be collected, and notes made of any samples that do not conform to the norm.
- It is usually practical and advisable to collect samples at about chest height from around the circumference of the tree or shrub. This is not imperative, but it is good practice for maintaining consistency in the approach to sampling.
- Inter-sample contamination is rarely measurable, so it is not necessary to clean utensils between sample sites, but particulates should be shaken off the sampling tools.
- Although it is desirable to collect from more than one tree or shrub around a sample station this is not always practical. In fact it may often be the case that for part of a survey area it is possible to collect from more than one plant at many sample stations, but at other sample stations there is only a single specimen. Consequently, if it has been mandated from the start to collect from 3 or 5 trees, then the solitary tree found at a sample station will introduce a bias to the sample programme. It is usually sufficient to collect from a single tree – the roots have done the chemical integration of the substrate.
- Site selection and representative samples are critical to the definition of regional, rateable anomalies.
- The degree of normal ground moisture (e.g., dry, moist, wet and boggy – excluding temporary affects of rain) can be a critical observation, so it is advisable to keep adequate notes.
- Note the general cover of other common species (e.g., abundant, few, etc.).
- Note the relative slope and aspect (e.g., gentle dip to N; steep dip to SW, etc.). This might prove to be a controlling factor for some elements. It is easy to provide a qualitative assessment and subsequently analyse the data to seek statistically significant associations.
- For transport out of the field the bags containing the samples can be placed in flour or rice sacks, or into cardboard boxes. Samples should not be sealed in plastic containers because moulds may develop and samples will rot. If there is only a short period between the collection of samples and their arrival at the laboratory (two or three days) they could be shipped in hard plastic containers or metal rock barrels. Containers of these types are less desirable, and should only be used when samples have been partially air-dried. They should not be used if the samples are wet.
- Although not essential, another parameter that might help in data interpretation is the pH of the soil. A simple ($20) garden soil pH meter is adequate.

Field drying and shipping

Whenever possible, at the end of the day sample bags should be spread out in a warm and dry place. If it is sunny they can be put out in the sun to dry off as quickly as possible. An added bonus is that the loss of moisture helps reduce shipping costs.

In the event that a drying oven is available, the samples, still in their closed bags, can be stuffed in a drying oven (they do not need air space around) until they are crisp and all moisture has been driven off. If the oven has a fan, 24 h at 70 °C is usually adequate. If it does not have a fan, then at least double that time is usually necessary.

If possible, samples should be sent to the analytical laboratory within a week or two of collection. If samples are to be sent to a laboratory in another country it is advisable to inform the selected analytical facility well in advance so that they can ensure that the appropriate import permit is in place. Boxes should be clearly marked 'DRY PLANT TISSUES FOR DESTRUCTIVE CHEMICAL ANALYSIS – NOT FOR PROPAGATION'. However, with constantly evolving security procedures, some countries may require alternative procedures: check with local authorities.

ALTERNATIVE SAMPLES

Over the past 50 years almost every imaginable type of biological medium has been examined and tested to ascertain its potential value to help in locating mineral deposits. Robert Brooks outlined a number of these media in two of his books (Brooks, 1983; Brooks et al., 1995). The latter publication, which is out of print, devoted 42 pages to less conventional sample media. These are summarized in the following sections with additional material from more recent studies.

Saps

The most dramatic example of metal hyperaccumulation in sap is that of the Sève Bleue tree (*Sebertia acuminata*) from New Caledonia. Its name comes from the bright blue sap that derives is colour from the extraordinarily high concentrations of Ni that it contains (Jaffré et al., 1976). This tree is endemic to serpentine soils of New Caledonia, and its latex (sap) contains a phenomenal 25.74% Ni on a dry weight basis or 11.2% Ni fresh weight, representing a far higher Ni content than any other living material. The nickel is present largely as a citrate and as $Ni(H_2O)_6^{2+}$ (Lee et al., 1977). Experiments show that $Ni(NH_3)_6^{2+}$ reacts virtually instantaneously with H_2O to form $Ni(H_2O)_6^{2+}$, and so it may be that the former compound develops through reaction with Ni and N at the roots and is dissolved by rainwater to be taken up in the sap as $Ni(H_2O)_6^{2+}$.

A few studies have investigated the trace element content of saps from trees in the boreal forests. Although not a particularly practical method of prospecting, given

that trees need to be tapped and there is only a limited period in the spring when there is sufficient sap-flow for collection, a summary of the literature is provided since there are claims that saps can be useful in locating mineralization. Furthermore, with the advent of ICP-MS saps might be worth further consideration, since essentially they represent metal-rich water.

Kyuregyan and Burnutyan (1972) demonstrated that plant saps could be used in exploration for Au, because the Au content was significantly higher than that of other aqueous extracts of the plant material or of the underlying soil.

Saps from birch are the most studied, both because of the ubiquity of birch in the boreal forests (especially Siberia and Finland) and their strong sap flow in the spring. Krendelev and Pogrebnyak (1979) conducted a sampling programme in an area of permafrost over an intensively fractured and hydrothermally altered gold stockwork in Transbaikal (the Ozernoye complex), where most of the Au is associated with pyrite and there is a cover of 0.5–4 m of unconsolidated sediments. Trees were drilled and polyethylene tubes inserted into the heartwood. The mean of 73 samples was reported as 0.011–0.33 ppb Au. Over a zone of Zn-rich ore concentrations reached 17.2 ppm Zn. They concluded, too, that in the vascular system of the birch species studied (*Betula platyphylla*) there is no biological barrier against the absorption of zinc. They found that the best anomaly to background contrast was obtained by calculating the Zn/(K + Ca + Mg) ratio.

Using the same birch species and the same methodology, Zaguzin et al. (1981) determined the Mo and W content of sap over a Mo stockwork (maximum in sap of 14 ppb Mo) and a W quartz vein system (maximum of 3.4 ppb W), with pronounced responses over mineralization.

Another Russian study examined the fluorine content of birch sap and found a strong response with up to 1.57% F directly over fluorite mineralization (Zamana and Lesnikov, 1989).

Harju and Huldén (1990) conducted an exhaustive survey in southern Finland where they collected sap samples from 40 different species of birch (mostly *Betula verrucosa*) over a 10-year period. Sampling covered the 30 days in April to early May during which it was feasible to collect the sap. They found considerable variation in base metal contents of the sap, with a window of about 12 days when the chemistry was moderately stable. On Attu Island, transects were made over a zone of base metal mineralization. Silver, Cd, Zn and Pb showed clear anomalies above the ore body (Fig. 4-16). Highest Pb values were very close to the ore body, and because of the low Cu content of the ore there was a less distinct Cu anomaly.

Studies of saps from other species include the European filbert (*Corylus avellana*) and sugar maple (*Acer saccharum*). Denaeyer-De Smet (1967) found 128 ppm Ca and 173 ppm K in the sap of filbert trees from Belgium.

A study of sugar maple sap from western Quebec involved the determination of elements by ICP-ES and INAA (Geological Survey of Canada, unpublished data by G. Lund). Table 4-XXII shows the average concentrations in nine trees from three

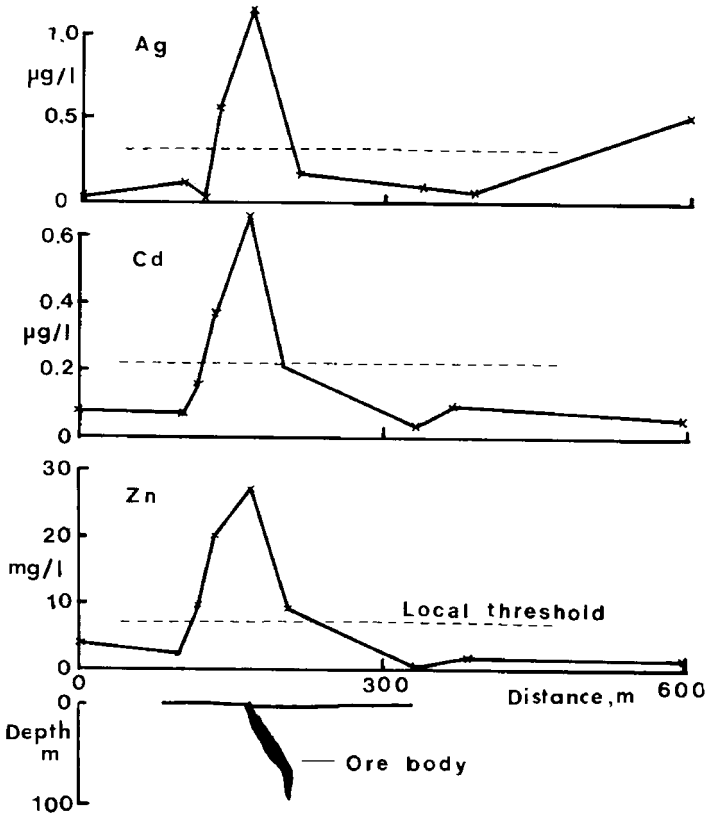

Fig. 4-16. Transect across the skarn-type polymetallic sulphide deposit on Attu Island. Silver, Cd and Zn in birch sap (*Betula verrucosa*). Source: Harju and Huldén (1990).

TABLE 4-XXII

Average concentrations in maple sap (*Acer saccharum*) from the western townships of Quebec. Average of 13 determinations

ICP-ES (ppm)				INAA (ppm)			
Ba	0.2	Pb	0.4	As	0.009	La	0.002
Ca	85	S	3.2	Br	0.1	Na	1.3
Cu	0.02	Si	14	Co	0.05	Ni	0.12
Mg	9	Sr	0.2	Cr	0.02	Rb	0.22
Mn	1.8	Zn	0.3	Fe	2.77	Sb	<0.001
P	1.8			K	106	Sc	0.001

locations, and the pooled reservoir of sap from four locations. For each element values fell within a narrow range.

In the La Ronge Gold Belt of northern Saskatchewan, remote from any mining activity, two samples of solid sap from black spruce were collected. The sap had congealed on the bark from wounds on the trees, and based on some comparisons with maple sap they were estimated to be concentrated about 50-fold from the fresh sap. Without any processing they were placed directly into polyethylene vials and irradiated for 35-element analysis by INA. All elements were below detection except for surprisingly high values for Mo (8 ppm), Zn (1100 ppm) and Au (19 ppb).

In summary, whereas saps are reported to give strong and well-defined anomalies over various types of mineralization, the method has the distinct drawback that sampling can only be undertaken during a very short period each year, and within that period it seems that for some elements there is substantial variation in sap chemistry. Any attempt to undertake a survey of this sort must be well planned and acted upon within a period of preferably no more than 10 warm spring days during sap-flow, that lasts, typically, for 25–30 days. Factors that would favour the use of sap as a sample medium are (1) sap functions as a transport medium of elements from the roots to all growing parts; (2) it is homogeneous in composition throughout a tree (Harju and Huldén, 1990); (3) it is a relatively simple matrix that lends itself to direct analysis by ICP-MS.

Fungi

Macrofungi include all mushrooms, morels, puffballs, bracket fungi and cup fungi. More than 3000 species of mushrooms and their relatives are reported from Western Europe (Moser, 1983) and it is estimated that perhaps 5000 to 10,000 species occur in North America. Some of these species have been found to accumulate extraordinarily high amounts of several elements, including several that are of particular relevance to mineral exploration – notably, with maxima expressed in dry weight, Ag (1253 ppm, Borovička et al., in prep.), Au (7739 ppb, Borovička et al., 2006), Sb (1423 ppm, Borovička et al., 2006) and As (7090 ppm, Stijve et al., 1990). High levels of other elements, including Hg and Se, have also been reported (Allen and Steinnes, 1978; Řanda and Kučera, 2004), and further details are given in the first chapter (Table 1-I). Fungi play a vital role in the concentration and redistribution of elements, and knowledge of their capabilities to concentrate elements is fundamental to detailed understanding of biogeochemical processes.

For the practical purposes of the exploration biogeochemist studies of element concentrations in mushrooms are largely of academic interest, because mushrooms are short-lived and they are rarely found distributed throughout a survey area at sufficient density and abundance to conduct a systematic survey. Furthermore, there are substantial differences in the abilities of different mushroom species to

accumulate metals, and so detailed knowledge is a requirement and the field or laboratory assistance of a mycologist would be highly advantageous. If mushrooms are found growing in areas of specific interest, the collection and analysis of samples could throw light on the chemistry of the substrate. They do require special care in collection and handling. The conventional paper or cloth bag used for collecting bark, twig or foliage samples is inappropriate for mushrooms. They have high water content (80–90%), and if packed among other samples during a field traverse they tend to be smeared into the sample bag by the end of the day.

Moss

The bryophytes consist of three groups: the Marchantiophyta (liverworts), Anthocerotophyta (hornworts) and Bryophyta (mosses). Mosses are non-vascular plants (i.e., they do not circulate fluids) and therefore are generally considered to be of limited use in biogeochemical exploration, although some interesting case-history studies have produced useful results pertinent to exploration. They do not have flowers or seeds, and their simple leaves cover thin stems.

Brooks (1982, 1995) considered that the best potential for mosses in biogeochemical prospecting lies with aquatic species, primarily because of their ion-exchange capabilities. They occur anchored to rocks in swiftly flowing streams, but their small rhizoids act merely as 'holdfasts', much like seaweeds, and supply minimal nourishment to the plants.

Several studies using aquatic mosses have focused on U mineralization. In New Zealand, Whitehead and Brooks (1969) published a study of aquatic mosses from the Lower Buller Gorge region and demonstrated a strong relationship of U in moss to known mineralization. Wenrich-Verbeek (1980) reported that mosses growing in stream water with 5 ppb U contained 1500 ppm U (a concentration factor of 300,000) and that there was a positive correlation with the stream sediments. Shacklette and Erdman (1982) reported up to 1800 ppm U in ash of *Brachythecium rivulare* from central Idaho.

Studies in Cu-rich areas have reported an extraordinary 2.4% Cu in *Pohlia nutans* growing near a spring in a cupriferous bog in New Brunswick, Canada (Boyle, 1977). Erdman and Modreski (1984) reported high concentrations of Cu and Co in the ash of bryophytes from the cobalt belt of Idaho. Studies in Alaska by King and his co-workers reported on the uptake of Ag (King et al., 1983a), As (King et al., 1983b), Sn (King et al., 1983c) and Cu, Pb and Zn (King et al., 1983d).

Shacklette (1965, 1967, 1984) has provided comprehensive reviews of trace elements in both aquatic and terrestrial mosses. With respect to aquatic mosses he noted the following positive aspects.

- They have the ability to absorb very high concentrations of elements.
- They integrate the geochemical signature of the flowing water over a long period.

Negative features noted were as follows:

- Aquatic mosses are not present in all streams and they cannot tolerate desiccation during periods of low water flow.
- Different species have different abilities to absorb trace elements, and so specialized expertise in species recognition is a requirement (much the same as specialized expertise is required in the collection of mushrooms).
- Contamination of moss by inorganic material is a problem that is difficult to overcome, because fine particles are difficult to separate from the plant tissue.

In the collection of aquatic mosses samples should be thoroughly rinsed in the water of the stream from which they are collected before placing them into plastic bags. In the laboratory they should be examined and hand picked to obtain as clean a moss sample as possible. Lenarčič and Pirc (1987) describe a mechanical method for rapidly cleaning aquatic mosses from carbonate terrain.

The most widely studied and most commonly analysed species of terrestrial mosses are the feather mosses *Hylocomium splendens* and *Pleurozium schreberi*. Their use is primarily in monitoring airborne heavy metal pollution, especially in northern European countries (Rühling and Tyler, 1968; Niskavaara and Äyräs, 1991; Steinnes, 1995; Reimann et al., 1997, 2001, 2006; de Caritat et al., 2001). In exploration, terrestrial mosses have been shown to accumulate a wide range of elements and several studies have related concentrations to geological substrates and mineralization. In Finland, Lounamaa (1956) successfully differentiated between three underlying lithological substrates by examining the Ni content of nine species of moss. Also in Finland, Erämetsä and Yliruorakanen (1971a,b) determined REE and 6 additional elements in 22 mosses, and found up to 180 ppm U in dry tissue of *Rhacomitrium lanuginosum* (common in cold and bleak areas of the northern hemisphere).

In New Zealand, Ward et al. (1977) analysed *Hypnum cupressiforme* from a mining area and reported maximum concentrations in dry tissue of 5 ppm Cd, 34 ppm Cu, 202 ppm Pb and 156 ppm Zn. In New Caledonia, Lee et al. (1977) found 300 ppm Ni in *Aerobryopsis longissima* that was growing on a vascular plant (*Homalium guillainii*) known to hyperaccumulate Ni. Plouffe et al. (2004) determined the Hg and Sb concentrations of several mosses and lichens collected from the vicinities of two past Hg-producing mines and areas remote from known mineralization in British Columbia. Their observations supported a large body of evidence that the mosses and lichens are sensitive bioindicators of environmental concentrations.

With respect to terrestrial mosses, Reimann et al. (2006) note the following:

> moss plant surfaces and rhizoids to not perform any active metal ion discrimination … It is assumed that they receive their nutrients directly from the atmosphere. Element concentrations as measured in moss samples are supposed to represent the accumulated load of the last 2–3 years (resulting in concentrations) much higher than in … other plants

At this time it appears that both aquatic and terrestrial mosses can on occasion assist in the biogeochemical prospecting and in the differentiation of geological

substrates, but there are limitations to the use of both types of mosses. Aquatic mosses may be useful integrators of stream water chemistry and that ability in itself is a useful application. Terrestrial mosses can reflect the composition of the local microenvironment and therefore provide a snapshot of the chemistry of the substrate to which they adhere, but there remains the significant complication of airborne contamination that limits the usefulness of this medium for biogeochemical prospecting.

Seaweed

Seaweeds are marine macroalgae of which more than 40,000 species are known (Vinogradov, 1953). They can be classified into three main groups according to their habitat and colour. The green seaweeds (Class Chlorophyceae) are mostly from the upper tidal zone; brown seaweeds (Class Phaeophyceae) are mostly in the mid-tidal zone; and red seaweeds (Class Rhodophyceae) are mostly from the low tidal zone. In temperate to warm seas the more rigid coralline algae (Class Corallinaceae) are common. Seaweeds are primitive non-vascular plants and so they accumulate nutrients and trace elements by direct absorption from surrounding seawater. Unlike land-based vascular plants that, through their root systems, absorb much of their nutrient requirement from soil, seaweed has no true root system to perform a similar task. The equivalent structure is a 'holdfast' that secures seaweed to the underlying rock or sediment. Element uptake occurs by simple ion exchange in which cations in the water are exchanged after membrane transport on to intercellular negatively charged polysaccharides (Skipnes et al., 1975). Some elements are transferred to specific cellular sites where they are probably bound to polyphenols (Jensen, 1984).

Haug (1972), following on from several pioneering studies of seaweeds (Vinogradov, 1953; Black and Mitchell, 1952; Bryan, 1969), suggested that seaweed might be a useful sampling medium for detecting influx of metals into the marine environment. Subsequently, several mineral exploration surveys have involved the analysis of seaweed (e.g., Fuge and James, 1973, 1974; Haug et al., 1974; Lysholm, 1972; Bollingberg, 1975; Morris and Bale, 1975; Sharp and Bölviken, 1979; Bollingberg and Cooke, 1985; Dunn, 1990, 1998a; Dunn et al., 1993b). Bollingberg (1975) collected two species of rockweed (*Fucus distichus* and *F. vesiculosus*) near a base metal deposit in western Greenland. Lead concentrations increased from a background level of 1 ppm Pb to 200 ppm near the mineral deposit, while Zn increased from 1.6 to 488 ppm, Cu from 1.9 to 8.9 ppm, and Cd from 0.57 to 8.0 ppm. In subsequent studies of that area, carried out after the Black Angel mine had gone into production, increased levels of metals were recorded in the same species of seaweed, reaching maxima in dry weight of 611 ppm Pb and 2060 ppm Zn (Bollingberg and Cooke, 1985).

Sharp and Bölviken (1979) found increased concentrations of Ni and Mn in brown seaweed that were associated with the substrate, and attributed increases in Zn, Cd, Co, Fe and V to influx of fresh water.

Down-slope from the carbonate-hosted copper–gold deposit of the former Little Billie mine on Texada Island in south-western British Columbia, nine species of seaweed were reduced to ash and analysed by INAA (Dunn, 1990). No species showed a clear tendency to accumulate Au or Cu, but the brown seaweeds had moderate levels of As, Sr and U, and the green seaweeds were relatively enriched in Al, Fe, Mo and REE.

Elsewhere in southwestern British Columbia acid rock drainage seeps down a mountainside from the former Britannia Cu mine into Howe Sound. For a distance of more than 1 km on either side of the drainage from Britannia into Howe Sound there is no seaweed growth. The only seaweed in this fjorded Sound is the rockweed *Fucus gardneri*, and where it first appears 1 km down the coastline from the mine site it is stunted and contains 3200 ppm Cu in ash (1000 ppm Cu dry weight, or 200 ppm wet weight – Dunn et al., 1993b), attesting to the high degree of metal accumulation that the seaweed can withstand before concentrations become detrimental to its growth. Background Cu concentrations in ash of this species are 60–70 ppm Cu (20 ppm dry weight). Zinc in ash reaches a maximum of 1540 ppm Zn compared to background levels of 40–160 ppm Zn (12–50 ppm dry weight)

Brown seaweeds can accumulate more As than the green or red varieties and *Sargassum* appears capable of scavenging As to a higher degree than other genera. Near a zone of undisturbed As-rich mineralization on the east shore of Bowen Island, near Vancouver, dry tissue of *Fucus gardneri* yielded 7–12 ppm As and a single sample of *Sargassum* contained 36 ppm As (Dunn, 1990, 1998a).

In the absence of mineralization many seaweed species, especially the brown seaweeds, concentrate in excess of 1 ppm U (dry weight) (Dunn, 1998a). This is an enrichment that is two orders of magnitude higher than the background level of 10 ppb U that is typical of land plants (Table 1-III).

Relative to land plants, there are several species of seaweed that have been shown to concentrate the following elements (Dunn, 1998a).

- Iodine, especially in brown seaweeds (e.g. *Laminaria*).
- Bromine in brown seaweeds (e.g., *Laminaria* and *Fucus*), and in the red seaweed *Prionitis*.
- Arsenic in brown seaweeds, notably *Sargassum*.
- Uranium in brown seaweeds, notably *Fucus*.
- Strontium in some brown seaweeds (*Fucus* and *Sargassum*).
- Vanadium in the green sea lettuce *Ulva*.

In areas of elevated levels of metal flux into the marine environment Pb, Cu, Cd and Zn can be accumulated by seaweeds to high levels before toxicity inhibits their growth. Consequently, there is considerable potential for applying seaweed chemistry to the exploration for minerals along the enormous extent of the world's coastlines. A comprehensive reference to the botany and identification of seaweeds is Scagel (1967).

GEOZOOLOGY

This term was coined by Brooks (1983, p. 87) to 'describe all methods of prospecting involving the direct or indirect use of animals, whether these methods be visual or involve chemical analysis'. The topic is discussed only briefly in the following pages, because there is little to add to the entertaining accounts presented by Brooks (1983, 1995). Much of the following is a summary from Brooks' publications.

It appears that geozoological methods date back even further than geobotanical methods. Herodotus, in 450 B.C., wrote that in the search for gold in India there occur:

> in this desert a kind of ant of great size – bigger than a fox though not as big as a dog. These creatures as they burrow underground throw up the sand in heaps just as our own ants throw up the earth … The sand has rich content of gold, and it is this that the Indians are after when they make their expeditions into the desert.

It was long considered that the 'ant bigger than a fox' must have been an exaggerated description of a termite, since termite mounds are local sources of metal enrichments where the termites have brought up mineralized fragments from some depth. However, recent studies of ancient languages have pointed out that the Persian word for 'marmot' is very similar to that for 'ant'. The conclusion, therefore, is that those ancient observations of the 'ant bigger than a fox' were actually marmots, and it was they, who during excavation of their burrows brought gold-rich particles to the surface.

On this topic of burrowing creatures, one of the best-proven geozoological methods and potentially the most useful is that of letting termites act as 'field assistants' (Brooks, 1995). The use of termitaria in prospecting for minerals has been examined by several workers, principally in central and west Africa (e.g., Tooms and Webb, 1961; West, 1970; Gleeson and Poulin, 1989), but also in India (Prasad et al., 1987), Russia (Glazovskaya, 1984) and Australia (Petts and Hill, 2006). In South America, their application seems to have been neglected. West (1965) explained the rationale for using termitaria as follows.

> Termite colonies … cannot exist without water … and their water-carrying passages extend down to the 6-m level in the (Leopard) mine which is the present water level … In mining their way downwards to water, they remove the necessary spoil and bring it to the surface to be deposited in the form of a heap known as an ant heap … The plotting and sampling of termite heaps, irrespective of the nature of the overburden such as Kalahari sands, is a useful and accurate method of prospecting with this advantage – the onerous part of the work has already been done by the termites at no cost to the prospector.

Indirectly, even goats have assisted the prospector. Around A.D. 850 the Cu-Zn mine at Falun in Sweden is reputed to have been found after a goat returned from the hills with a red stain on its horns. When the owner followed the goat the next day he

found an iron-rich outcrop that subsequently was developed as the iron mine that finally closed down more than a thousand years later in 1992.

Another indirect use of animals is information that has been provided by birds. A rather impractical approach to Au exploration was described by Razin and Rozkhov (1966), since it involved the analysis of dried tissue of Siberian birds. The 'Common bunting' (*Emberiza leucocephalus*) yielded the highest level, with 50 ppb Au.

Other animals that have from time to time been analysed for their metal content in metal-rich areas (although rarely with the specified intention of using the data as a guide to locating mineralization) include mule deer (antlers – Jones, 1970); roebuck (Oslany Au-mining region of Slovakia – Babička et al., 1945); and base metals in domestic livestock from southern India (quoted in Brooks, 1995).

The sensitive noses of dogs have assisted some prospectors. In several countries with glacial overburden (notably in Scandinavia) dogs have been trained to scent out sulphide-rich deposits boulders concealed by glacial drift (Kahma et al., 1975; Mattson, 1989).

Most of the work involving the chemical analysis of fish to assist in prospecting has concentrated on freshwater fish, because marine fish are not sufficiently territorial to be of use. Warren et al. (1971) analysed the livers of 96 trout collected around British Columbia. Of 17 localities where livers contained >60 ppm Cu (wet weight), 4 were from areas with known mineralization and another 7 were from sites where the geology favoured Cu deposits, and therefore merited further investigation.

The Mo content of trout and salmon livers was studied by Ward (1973), and a good correlation was observed with the Mo content of stream and lake waters. Trout livers contained a mean concentration of 2233 ppm Mo (wet weight) compared to 100 ppm Mo in salmon livers.

In New Zealand, Brooks et al. (1976) determined the Cd, Cu, Fe, Hg, Mn and Zn content of livers from 111 trout and found a good correlation between elemental levels and the degree of geothermal activity.

Whereas some significant relationships between metal deposits and various creatures from the Animal Kingdom have been observed, for the most part these are scientific curiosities rather than techniques for adopting as systematic surveys to assist in the exploration for mineral deposits.

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Chapter 5

SURVEY DESIGN AND COMPARISONS WITH OTHER SAMPLE MEDIA

INTRODUCTION

The design of a biogeochemical sampling programme to explore for minerals involves essentially the same considerations and procedures as for any type of exploration geochemical survey. Additional points to bear in mind include:

- Unlike a stream, lake or soil geochemical survey for which a pre-designed distribution of sample locations can be established by referring to a topographic map, the coverage obtained from a proposed biogeochemical survey is only as comprehensive as the distribution of the species selected for collection. Consequently, whenever possible, a quick reconnaissance assessment of plant coverage should be made. In North America, there are plant distribution maps published by many jurisdictions, and they can often be purchased or they may be consulted in local forestry offices. A short visit to a local forestry office can be valuable for up-to-date information on access, logging operations and the locations of any recently cut areas from which samples had been desired. For some areas there are 'Biogeoclimatic' maps available (e.g., British Columbia, Pojar et al., 1987) which are very useful for assessing species coverage.
- The selection of sample spacing should be commensurate with the distribution of the desired species, especially if a moderately regular grid is required. The closer the sample spacing, the greater the divergence that may be required from a preferred sample location in order to find a specimen of the tree or shrub selected for the survey. Given this reality of the sometimes irregular plant distribution patterns of trees in undisturbed forest, for detailed sampling programmes the geochemist should be prepared to end up with an uneven grid, with perhaps some gaps where there is a recent clear-cut, or where a change of elevation is sufficient that a different plant assemblage occurs. However, in practice a survey in northern and temperate forests usually results in good over all coverage of an area, with only minor gaps in the preferred sampling pattern.
- A plant's root system steadily integrates the geochemical signature of several cubic metres of the substrate – all soil horizons, interstitial water, and perhaps penetrates through regolith or glacial deposits into bedrock fractures. The physical power of roots is enormous, such that small fractures can be wedged apart and propagated as an enlarging root advances downward or laterally. As a consequence of the sometimes great extent of root systems, a biogeochemical signature is normally a

selective mixture of any or all of the soil/water/bedrock chemical components, and that signature is unlikely to be identical to the geochemical signature of any of the individual media penetrated by the roots. Given the complex interactions of physical, chemical and biological dynamics in the natural environment of a forest or scrub-land, in most parts of the world it is unrealistic to expect a biogeochemical signature to be the same as that from an underlying soil.

- Lateral roots may extend several times the radius of the drip-line (the extent of the branches) of a tree, thereby absorbing elements from a large area. A large coniferous tree in a wet forest regime commonly has roots that mostly spread laterally and the majority are contained within 0.5–1 m from the surface; but given favourable conditions they can penetrate deeply into the ground (e.g., a depth of 12 m for Douglas-fir in limestone caves of southern Oregon). In general, deciduous trees tend to penetrate more deeply into the ground than conifers. Roots of shrubs from arid regions are mostly within a few metres of the ground surface, but may extend for tens of metres into the substrate. Phillips (1963) reported roots (probably from mesquite, *Prosopis* spp.) excavated by mining operations in Arizona at a depth of 53 m, and Cannon (1960) reported evidence of juniper roots in mine shafts at a depth of 61 m. A concise review of root systems is given by Richards (1986) and of metal transfer from soils to roots by Arienzo (2005).

Roots can extract nutrients and other elements from a large radius and depth, so in designing sample spacing for a detailed survey some consideration should be given to the area of influence extended by root systems. This is not an easy task, because local environments including depth to the water table or the presence of indurated zones such as hard pans and caliches, influence root structure and penetration. In short, with a few exceptions outlined below, there is usually no advantage in reducing sample spacing to less than 25 m, because any closer spacing is unlikely to provide significantly more information than a 'field duplicate'.

An integral part of a survey design is to consider at the outset the basic analytical protocols that will be used. Modern analytical technology is now sufficiently sophisticated and sensitive that a multi-element analytical package is invariably an advisable choice, because of the minimal additional cost of acquiring data for many elements rather than for just a few. Frequently, valuable information can be derived from a comparison of the spatial patterns of elements that might not usually be considered as relevant to the exploration for a particular commodity. Cadmium and Cs, for example, do not always spring to mind as leading pathfinder elements for epithermal Au deposits, yet their spatial relationship to vein systems is sometimes of value in providing focus for exploration efforts. Consequently, whenever economically possible, the decision should be made to adopt a multi-element approach, regardless of the size and nature of a biogeochemical exploration survey. In institutions where only ICP-ES or AA is available, unless there is a specific requirement to determine the content of just one or two elements targeted at a particular style of mineralization, the objective should still be to analyse for as many elements as possible. Chapters 6 and 7 elaborate on the laboratory components of a biogeochemical survey.

SURVEY DESIGN

The philosophy of the 'the more samples the better' for a geochemical survey is good up to the point where diminishing returns set in, and, of course, a very closely spaced sample survey will generate vast numbers of samples that might render a geochemical survey inordinately expensive. Kovalevsky (1987) provides a table of recommended prospecting grids that are practical for the larger-scale surveys, but at the densities suggested for the detailed surveys would generate far too many samples for practical purposes (Table 5-I).

Whereas detailed surveys of these proportions might have been feasible under the economics of the former soviet regime, the data presented in Table 5-I are not recommended for the modern world of exploration given the enormous numbers of samples that would be generated from surveys at these densities. Taking the extreme case shown of a survey at 1:1000 scale whereby the distance between transects is 10 m and spacing between samples is 2 m, given a current estimated all inclusive cost of \$50/sample (to include all field operations, sample preparation and a 50+ element ICP-MS analysis), the cost per square kilometre would be an astronomical \$250,000! Clearly, a more rational approach is necessary and the following suggestions provide some cost-effective recommendations.

In designing a biogeochemical survey the broad objectives should first be considered, just as would be done for any type of geochemical survey, except for the additional consideration of the type of vegetation coverage that is anticipated. If a large area (i.e., hundreds to thousands of square kilometres) has received little exploration attention, or past work was focussed on only one or two commodities, a low-density multi-element reconnaissance-level exploration programme can assist in delineating broad patterns of metal distribution and in defining large geochemical

TABLE 5-I

Sample densities recommended by Kovalevsky (1987)

Scale	Spacing (m)		Samples/square km
	Between transects	Between sample points	
1:200,000	2000	100–500	1–5
1:100,000	1000	50–200	5–120
1:50,000	500	20–100	20–100
1:25,000	250	10–50	80–400
1:10,000	100	10–20	500–1000
1:5000	50	5–20	1000–5000
1:2000	20	2–20	2500–25,000
1:1000	10	2–10	10,000–50,000

'provinces'. A survey of this type is unlikely to pinpoint mineral deposits, but it can establish the regional geochemical patterns and thereby provide a focus for closer investigation.

An assessment should be made of what is already known about an area: the bedrock geology, structure, any indications of mineralization, the nature of the overburden, topography, drainage and any geophysical targets. If there is a similar lithological substrate throughout a proposed survey area, such as a large sandstone body hosting uranium deposits, a simple approach would be to collect samples in a grid pattern. Complex underlying geology with shears, elongate structures, intrusions and volcanic deposits might warrant a programme that includes greater focus on certain lithological units or structures, depending, too, on the type of mineralization that may be present. If targets are large disseminated volcanogenic massive sulphides or copper porphyry deposits, sample spacing can be at much lower density than if targets are vein hosted mineralization that are unlikely to have generated large geochemical haloes.

SCALE OF SURVEY

This section includes some brief case-history examples of surveys conducted at different sample densities and sample designs. Chapter 11, dealing with case histories, elaborates on some of these.

Low density (1 site per 10 km^2 or greater) – Reconnaissance level

The purpose of conducting a reconnaissance level survey is to look for geochemical 'provinces' (i.e., areas of similar geochemical signature that may extend for hundreds or thousands of square kilometres). From such a survey it may be possible to distinguish suites of elements typical of a particular style of mineralization that extend over a large area. For example, relative enrichments of copper, molybdenum and gold could identify areas favourable to prospect for porphyry-related mineralization. Such surveys are unlikely to define the precise location of a mineral deposit, but they may provide a focus for exploration activities.

In Chapter 4, it is recommended that, in the absence of any prior knowledge of an area, a first step in preparing to conduct a biogeochemical survey is to establish which species are the most widespread within the area of interest. If the biogeochemist has no prior experience of the survey area it is advisable to conduct some preliminary investigations by reading available literature and conducting web searches. Visits to botanical gardens and discussions with local botanists at museums and universities can be extremely beneficial.

An example of the type of procedure that can be adopted for expeditiously obtaining botanical information of relevance to a survey is a project funded under the

auspices of the Canadian International Development Agency (CIDA). A situation arose that required a biogeochemical survey in the Tapajós area of the Amazon, Pará State, Brazil (Dunn and Angélica, 2000). Prior to going into the field, the field party had virtually no knowledge of the flora to be expected. The staging city for the project in Brazil was Belém, where there are the botanical gardens of the Museu Paraense Emilio Goeldi which, along with advice from their botanists, proved useful for providing a first assessment of the common flora that might be present in the survey area, close to the Amazonian village of Creporizão. Additional information of use was obtained from botanists at the University of Belém, from whom an assessment could be made as to which species were likely to be dominant.

In environments of diverse flora such as the Amazon, Central Africa or Indonesia, the field knowledge of local guides can be absolutely invaluable. Half a day with local guides walking through the jungle is usually sufficient to establish the species that are widely distributed, and which can be the focus for sample collection. Typically, local guides know the common names of many species and the identification of their botanical names can be established at a later date by reference to local government, museum or university publications. In the case of the Amazon survey, of particular value were two small publications available in Belém, 'Guia Botânico do Museu Goeldi' and 'Frutas Comestíveis da Amazônia' (Cavalcante, 1982, 1996).

In northern Saskatchewan, near the eastern edge of the Precambrian (Helikian) sandstones that comprise the Athabasca Group, there are some of the world's largest and richest uranium deposits. A small-scale orientation survey in 1979 identified some unusually high levels of U in twigs of black spruce (*Picea mariana*), with >100 ppm U in twig ash compared to normal background levels of <1 ppm U. Over the succeeding years, sampling was extended outward from this area (McClean Lake), and it became apparent the orientation survey happened to be close to the core of what later proved to be an extraordinarily large biogeochemical U 'province'. To establish the limits to this 'Wollaston Uranium Biogeochemical Anomaly' (Dunn, 1981), samples were collected at 5 km intervals along the road leading to the core of the anomaly, and, since there were no other roads in the area at the time, a float plane was used as transport between lakes to collect additional samples at intervals of 5–10 km across the eastern side of the Athabasca Group. As a consequence of logistical demands, there was no consistent grid established, but more than 1000 samples were collected over a large area on an opportunistic basis, with the highest sample density in the core of the anomaly. Although not an ideal survey design, for a broad reconnaissance view of an unknown area, this approach can provide valuable information.

Ultimately, the very low-density sampling of the Athabasca area, with irregularly spaced sample intervals, sufficed to sketch an approximate limit to the 10 ppm U contour, and define the limits of the 50 ppm and 100 ppm U contours. Whereas black spruce twigs usually contain less than 1 ppm U in ash, the regional background of black spruce twigs from much of northern Saskatchewan is 2 ppm U, no doubt because of the world-class grade and extent of the many uranium deposits that have subsequently been discovered in that area.

Results of the Athabasca survey showed that within an area of 10,000 km^2 all trees sampled yielded concentrations of at least 10 ppm U. Inside this vast area, the 50 ppm U contour encompassed 3000 km^2, and the 100 ppm contour extended over an area of 1000 km^2, reaching a maximum concentration of 2270 ppm U at Rabbit Lake (Dunn, 1982). In hindsight, this unique occurrence of a massive biogeochemical uranium province near the eastern margin of the Athabasca Sandstone could have been identified by sampling just 1 tree per 1000 km^2. There is further discussion of this U anomaly in Chapter 11.

The extraordinary size of the Wollaston U anomaly appears to be rare, although a regional Mo 'province' approaching this size has been identified in Russia (Kovalevsky, pers. comm., 1983). Subsequently, a large Mo anomaly defined by lodgepole pine bark (*Pinus contorta*) has been established around the Endako molybdenum mine in central British Columbia. The contour of 20 ppm Mo in bark ash (approximately ten times background) extends for over 5000 km^2 (Dunn, 1999). In the Endako area, although dust from the mining operations contributes to the high levels encountered, historical records indicate similar levels in plants collected from the mine site in the 1960s during the early days of mining operations (Warren et al., 1953; Warren and Delavault, 1965; Warren, pers. comm., ca. 1988).

In addition to identifying major geochemical provinces, low-density surveys can be undertaken to outline large geochemical systems and significant mineral 'camps'. As part of a regional survey of about 20,000 km^2 of Nova Scotia, focussing primarily on balsam fir (*Abies balsamea*), at every fifth fir sample site the outer bark from red spruce (*Picea rubens*) was collected at a sample density of just 1 tree per 50 km^2 within a 5000 km^2 area of eastern Nova Scotia (Dunn, 1989). For this survey, the network of roads and forestry trails was used for gaining access, and the trees sampled were each at least 50 m from any roadside or other sign of disturbance. From simply plotting multiples of the median values of the element concentrations, the distribution patterns of Au, As, Sb and Se each outlined the main gold camps (Fig. 5-1) of which the north-easterly trend from Goldenville to Forest Hill was the most notable.

Additional examples of low-density biogeochemical surveys in Canada include publications from Manitoba (Fedikow et al., 1997a,b, 1998, 1999, 2000, 2002), in central British Columbia (Dunn et al., 1994c, 1996b; Dunn and Hastings, 1998, 1999, 2000), in Newfoundland (Dunn et al., 1995b) and in Alberta (Seneshen et al., 2005).

In Australia, Cohen et al. (1998, 1999) conducted two reconnaissance surveys that included the analysis of foliage from several common plant species. The larger of these surveys encompassed 14,000 km^2 of north-eastern New South Wales and, since it included a comparison with stream sediments, sample sites were close to streams (Cohen et al., 1999). Among their conclusions, they found that genera capable of accumulating high concentrations of trace elements in the vicinity of mineralization included Co and REE in *Eucalyptus*, As in *Callistemon* (bottle brush) and Au in *Casuarina* (River She-oak).

This last-mentioned survey brings up another point for consideration – the preferred topographic location of sample stations. In some areas, there is sufficient

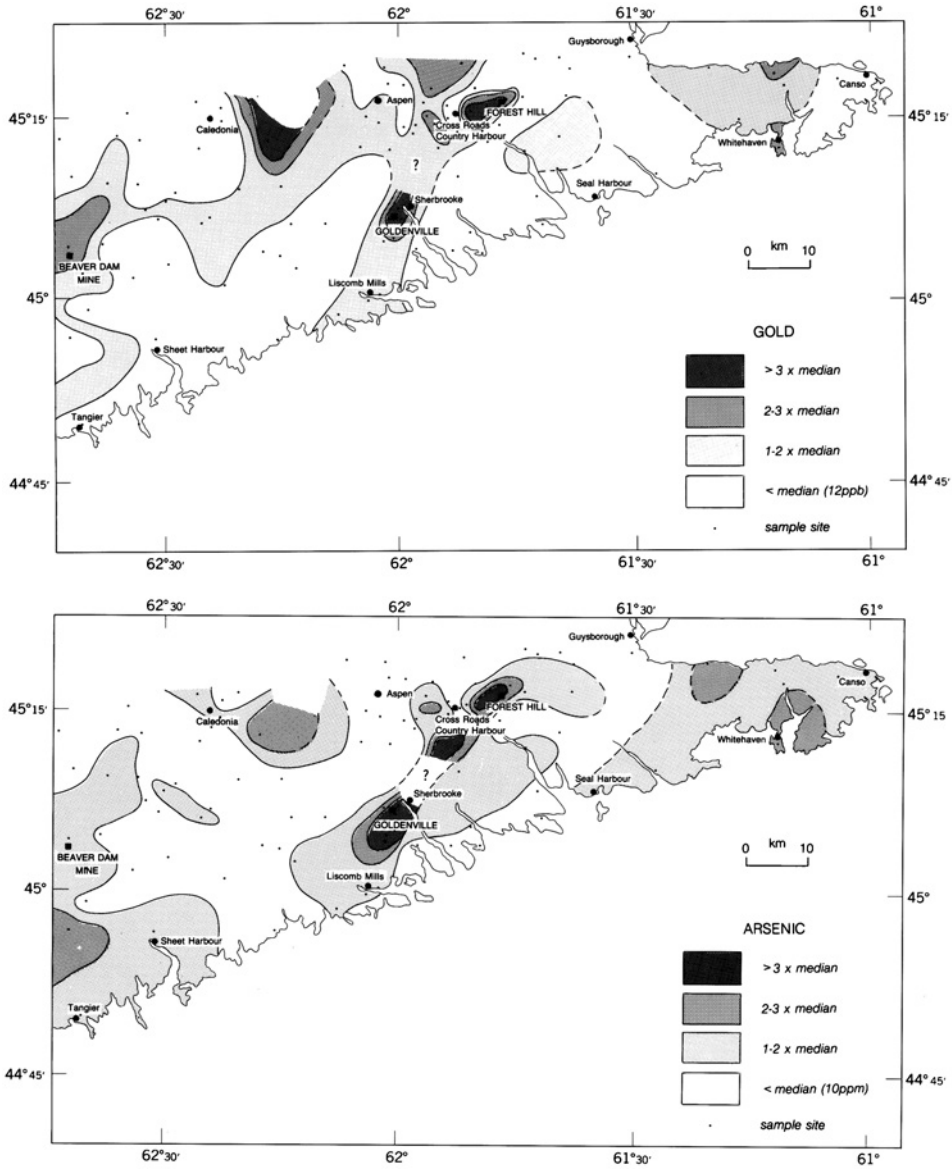

Fig. 5-1. Low-density reconnaissance survey (1 per 50 km^2 over approximately 5000 km^2) of eastern Nova Scotia, Canada, showing concentrations of Au and As in ash of red spruce outer bark (*Picea rubens*). Similar patterns were shown by Sb and Se (Dunn, 1989). Analysis by INAA.

density of streams to permit sample collection from near the base of a slope draining into a stream valley. In areas with moderately thick surficial cover, it is better to collect at this break in slope rather than from valley floors, because in the stream's floodplain plants are likely to be rooted in exotic detritus brought down by streams in times of high water flow. Consequently, the biogeochemical signature of samples from a floodplain may provide a subtle signature that reflects an allochthonous component of material rather than the preferred signature of a nearby source that is more likely to be obtained from a break-in-slope sample location.

Moderate density (1 site per 1–10 km^2)

In the previous section, reference was made to 20,000 km^2 covered by regional biogeochemical surveys in Nova Scotia. Surveys were conducted in early summer on five occasions between 1987 and 1995 at a sample density of approximately 1 site per 8 km^2 using balsam fir twigs (*Abies balsamea*), because this proved to be the most ubiquitous tree (e.g., Dunn et al., 1989, 1992a,b, 1994a,b, 1996a,b; Dunn and Balma, 1997). At most of the fir sample sites, there were also spruce present (red spruce and/or black spruce) and so outer bark of spruce was collected at the same time and additional datasets were published for comparison (Dunn et al., 1992a,b). At that time, the optimal analytical procedures involved reduction of tissues to ash by controlled ignition to 475°C with analysis by INAA, and by ICP-ES (aqua regia digestion) for those elements not readily determined by INAA.

The Nova Scotia regional biogeochemical surveys generated exploration data that defined known and previously unknown areas of metal enrichments. As an example of the signatures obtained, Fig. 5-2 shows U and W in balsam fir twigs (concentrations in ash) from a survey of the south-western part of the Province conducted in the spring of 1991 (Dunn et al., 1994a). The area of anomalous concentrations encompasses the East Kemptville tin/tungsten mine that was in production from 1985 to 1992, and several satellite zones of mineralization that were not exploited. Other elements in the fir twigs that exhibited similar patterns of enrichments included As, Be, Cs, Li, Rb, Sn and Ta. Although some of this signature was probably related to airborne dust it was noteworthy that other sites, remote from known mineralization, yielded some anomalous values of similar magnitude.

The fastest way to establish biogeochemical patterns over large tracts of land is by helicopter. Although the cost of helicopter rental is high, the speed of collecting samples is sufficiently rapid that the over all economics are favourable. At the time of writing, the entire cost per sample to run a survey in remote areas of Canada is around US$120. This is about two-thirds to three-quarters the cost of a regional helicopter-supported lake sediment or stream sediment survey, and includes all components of a survey from planning, collection, analysis to a final report. Sample densities are typically in the range of 1 site per square kilometre to 1 per 4 km^2. Examples of results from helicopter-borne surveys are given in Chapter 11.

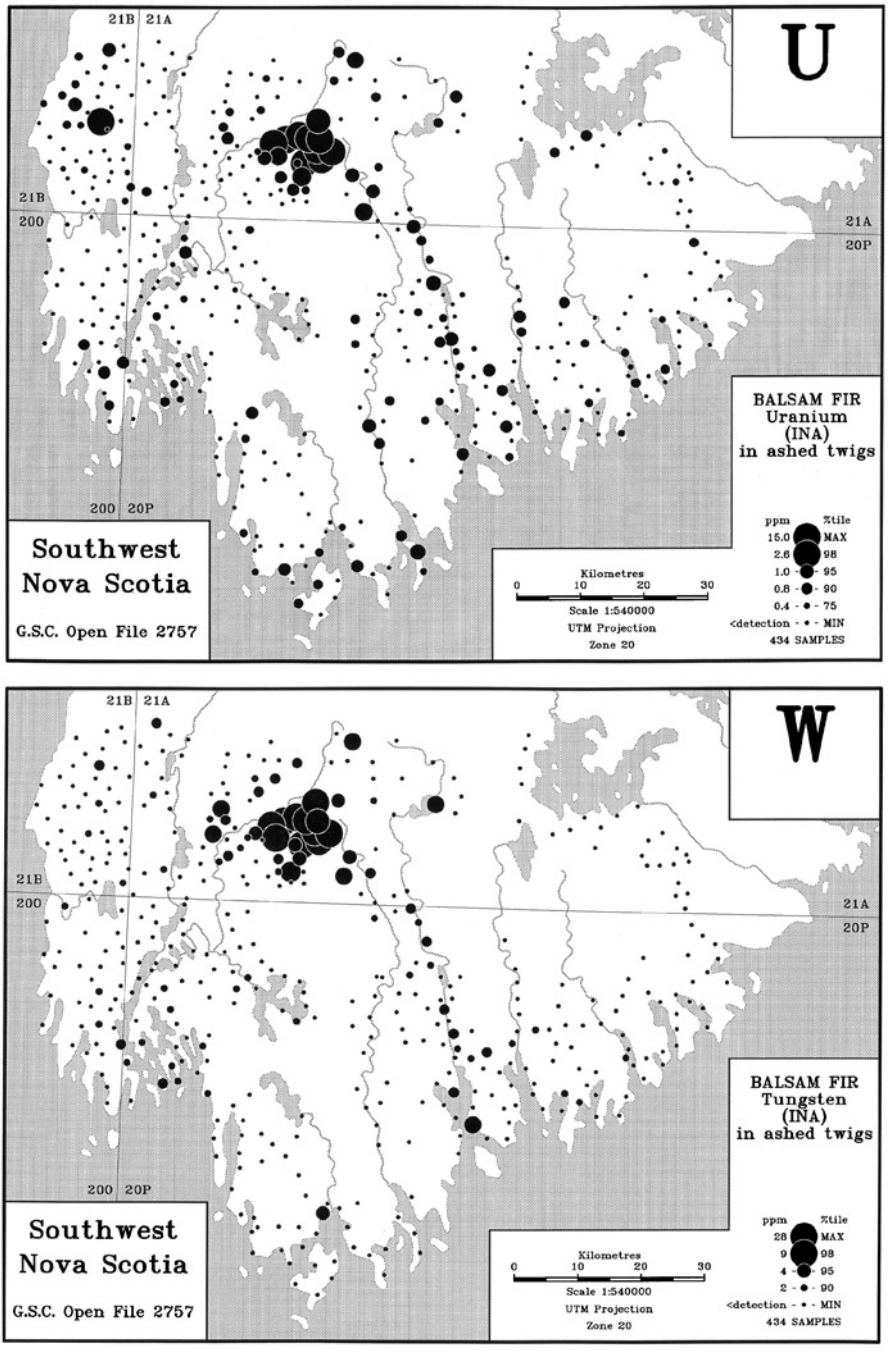

Fig. 5-2. Regional survey of south-western Nova Scotia, Canada, using twigs of balsam fir (*Abies balsamea*). Uranium and tungsten shown as ppm in ash, determined by INAA. Location of disused East Kemptville Sn/W mine centred in area of anomalous metal enrichments (Dunn et al., 1994a).

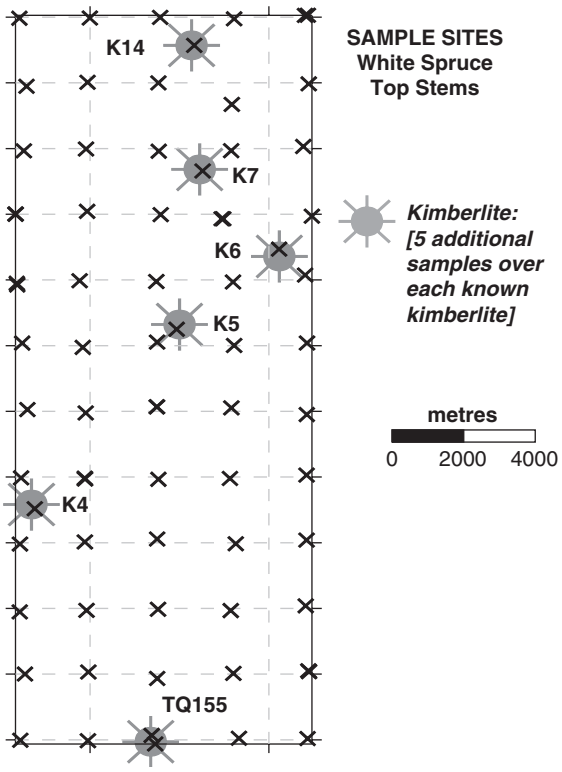

Fig. 5-3. Sample pattern established for collection of tree tops (2 km grid) to assist in locating concealed kimberlites in central Alberta (Seneshen et al., 2005).

A survey designed to locate kimberlites beneath a Quaternary cover in central Alberta involved the collection of white spruce (*Picea glauca*) tops at a sample spacing of 2 km (i.e., 1 site per 4 km^2). An added component to this study was the collection of an additional five samples over known kimberlites, in order to establish their biogeochemical signatures (Seneshen et al., 2005). The resulting sample grid pattern and the locations of the principal kimberlites known at the time of the survey are shown in Fig. 5-3.

In retrospect, the basic sample spacing of 2 km proved too coarse for clearly defining the locations of kimberlites, and 1-km spacing would have been preferable. Some results of this survey are discussed in Chapter 11.

Semi-detailed surveys (1 site per 0.25–1 km^2)

Surveys at this scale can be undertaken by helicopter in difficult terrain, or ground traverses – either on foot, by truck or four-wheel drive vehicles in open arid terrain,

by all-terrain vehicles ('quads') on rough forest trails, or by snow-mobiles along trails during the long cold winter months of the northern continental masses. Biogeochemical sampling may be the only feasible geochemical sampling method in northern climates during the half of the year that experiences snow-covered terrain and frozen ground.

In the rugged Cordillera del Cóndor region of the western Amazon, close to the border between Peru and Ecuador, a survey programme included the collection of foliage from 366 tree ferns (Chonta con Espinas – *Cyathea spp.*) on an even grid of sample stations spaced at intervals of 500 m (i.e., 1 site per 0.25 km^2). Figure 5-4 shows plots of Au and As with the 98th percentile of the dataset selected as the maximum for plotting, such that values above this value are all shown as the same intensity of shading. The former artisanal Au workings at Chinapintza (epithermal Au/Ag) are clearly evident, and a second area (Anomaly A) farther south also shows a Au/As relationship. A strong mercury anomaly was associated with the

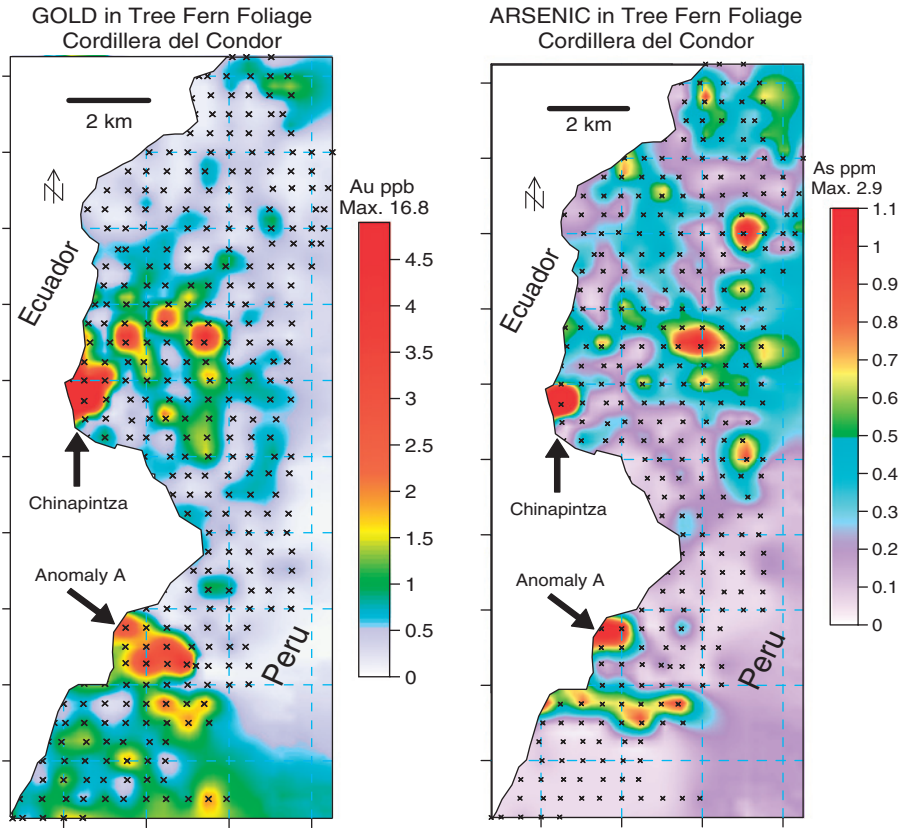

Fig. 5-4. Western Amazon – Au and As in dry foliage of tree fern. Data courtesy of AngloGold-Ashanti. Determinations by ICP-MS on aqua regia digestion of dry tissues.

Chinapintza Au, and Ag anomalies were peripheral to Anomaly A, indicating zoning of the mineralization. Details of this survey are provided in Chapter 11.

In northern Saskatchewan the small but high-grade Rottenstone Ni-PGM deposit was mined out between 1965 and 1968, but there are indications of additional deposits in the area. There is no all-weather road access and the heavily forested terrain has a veneer of glacial deposits. It is cut by streams and rivers and is dotted with many lakes such that geochemical surveys are difficult and very time-consuming to undertake.

To provide a rapid and broad reconnaissance geochemical survey of a 130 km^2 area that had been staked by Uravan Minerals Inc., about 800 black spruce (*Picea mariana*) tops were collected from a helicopter during a five-day period in September 1999. Samples were collected at 500-m spacing, with alternating offset lines to provide minimum distance between sample points in accord with an idealized grid shown in Fig. 5-5.

In practice, this sampling pattern was easy to achieve, and digressions from the designated sample locations were mostly only a few metres in order to locate an appropriate tree or to avoid a lake. Many of the larger lakes had islands on which

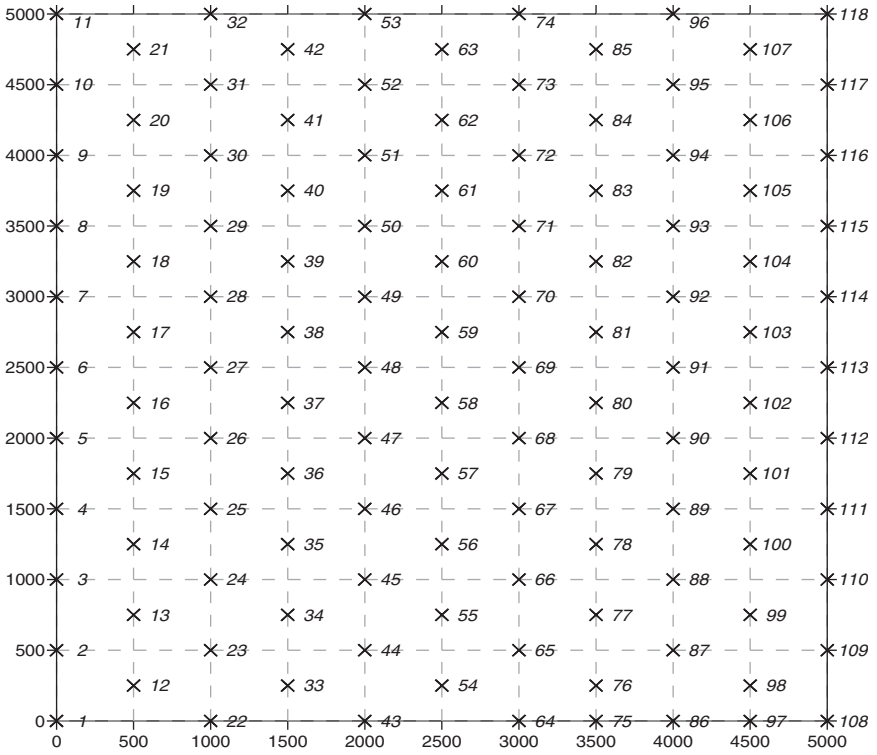

Fig. 5-5. Idealized offset grid sampling pattern to provide minimum distances between sample points.

trees were available for sampling, and the extensive drainage network required that sample sites only be shifted a short distance from the desired grid. The sample interval was reduced to 250 m over a 48 km^2 area of primary interest around Rottenstone Lake that is outlined in the centre of Fig. 5-6. The results of this survey are discussed in Chapter 11.

Detailed surveys (25–200 m spacing)

In desert areas, such as the south-western United States, detailed surveys using widespread species (e.g., sagebrush [*Artemisia tridentata*]) can be undertaken by sampling on a predetermined grid pattern. However, surveys in the dense temperate and boreal forests of North America and the densely vegetated tropical areas of the world are often controlled by logistics – access by road, foot, boat, float-plane or helicopter. In these areas, a sample spacing of about 200 m has proven effective and feasible, even though sampling on a regular grid pattern may be precluded by the presence of streams, bogs, or perhaps the absence of the desired species. In Precambrian Shield terrain of many parts of the world there is commonly a preferred

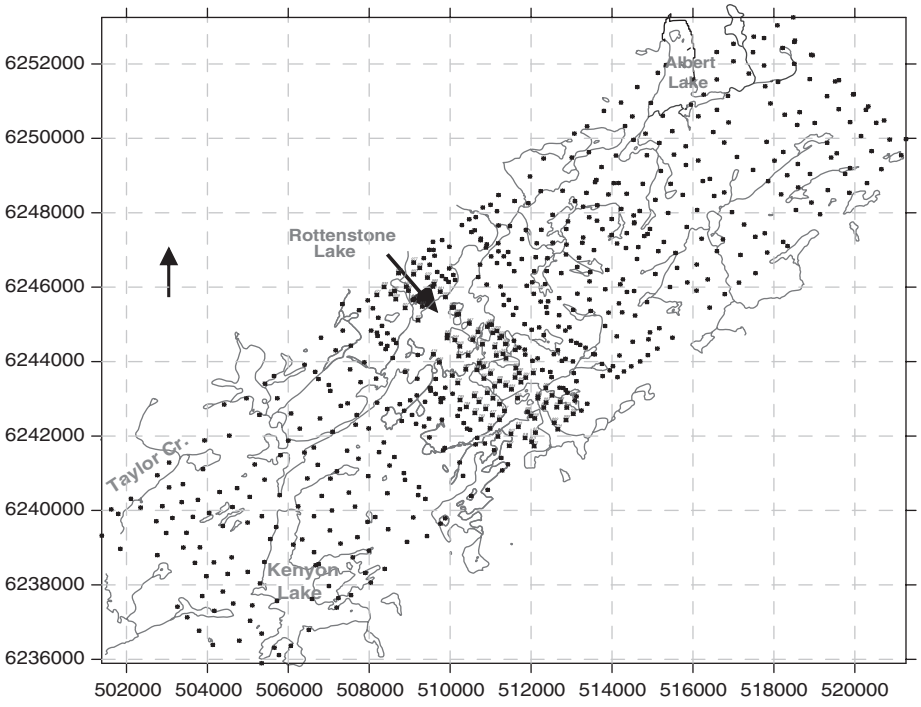

Fig. 5-6. Sample pattern achieved for the collection of black spruce tops around Rottenstone Lake, Saskatchewan.

trend to metamorphic grade and to structure, such that an effective compromise is to sample across strike at 200 m intervals along lines spaced 1 km apart. Extensive surveys at this sample spacing in northern Saskatchewan have used bark of black spruce (*Picea mariana*) and twigs of mountain alder (*Alnus crispa*). Among several zones of anomalous Au concentrations that were identified, one was in an area dominated by alder, and follow up work using alder at closer sample spacing (mostly a 100 m × 100 m grid) identified the spatial relationship among Au, Mo and Cs (Fig. 5-7) in alder twig ash (most recent three years of growth). These three elements

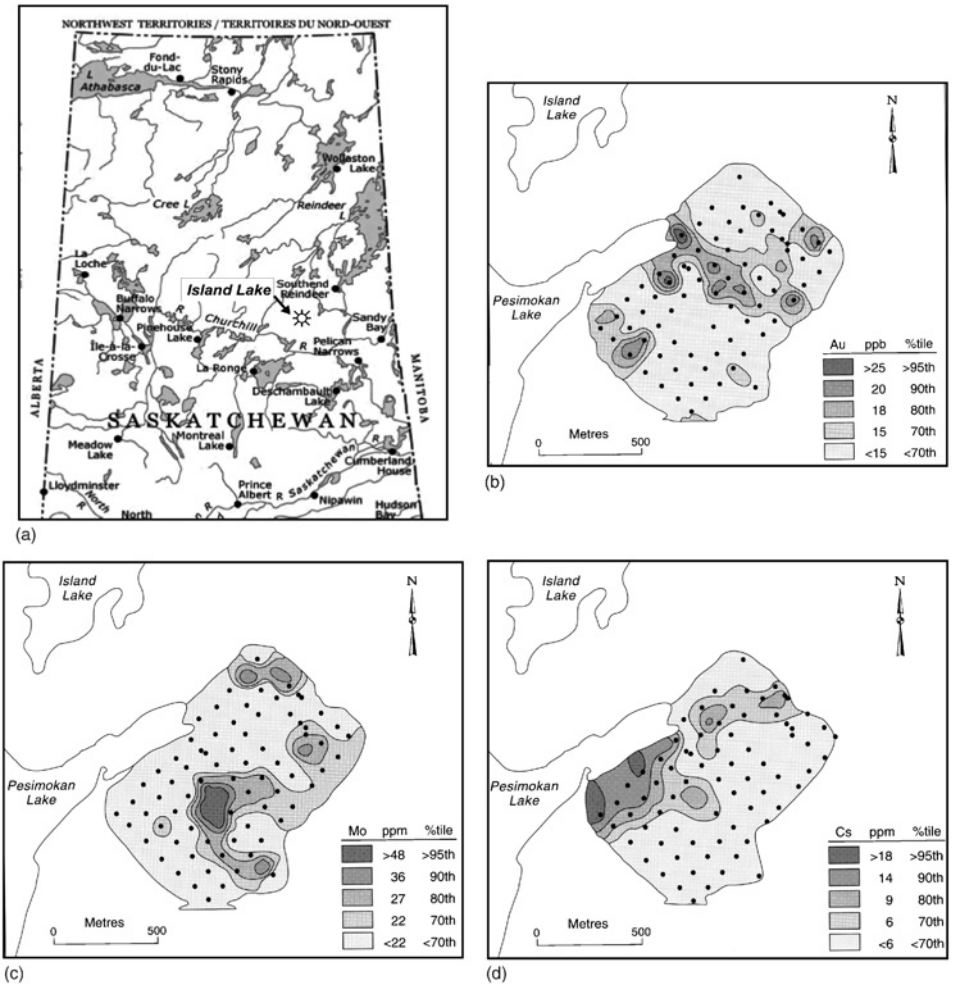

Fig. 5-7. Metals in ashed twigs of alder (*Alnus crispa*) – ash yield approximately 2%: (a) Location of survey area, (b) Gold, (c) Molybdenum and (d) Cesium. La Ronge gold belt of northern Saskatchewan, Canada (Dunn et al., 1990). Analysis by INAA.

in plant tissues quite commonly exhibit a spatial relationship to gold mineralization. Subsequent drilling intersected gold mineralization, but to date only sub-economic grades have been found.

In an area with known or suspected mineralization, a simple orientation survey can be conducted to determine if a biogeochemical response is obtained in common plants. A single line is inadvisable, since the validity of any anomaly that arises may be doubtful. By sampling along two lines oriented normal to the presumed strike of mineralization, a positive signature on both lines would provide an indication of the strike of the mineralized body. It is an expeditious procedure to collect along one line going away from a base camp and a second returning to it. If time permits, of course, greater substantiation of anomalous trends can be obtained from a four-line sampling program. Figure 5-8 shows examples of iodine anomalies in cedar foliage (western redcedar) along two lines that traversed a suspected zone of breccia-hosted Cu porphyry at the Boundary Zone, Mount Polley, in central British Columbia. The trend of the mineralization can be determined from the locations of the anomalies.

From these profiles, a sketch map could be plotted outlining the predicted location and trend of mineralization (Fig. 5-9).

The plot shown in Fig. 5-9 shows sample spacing at 100 m, closing down to 50 m then 25 m over the zone of interest. Similarly, quartz-hosted vein-type gold may require sample spacing at 50 m or as close as 25 m. To sample more closely than this may be impractical or not possible because of the generally lower density of plant distributions, although on occasion, according to local geological conditions, spacing at 10 m may be warranted. In a situation where a narrow quartz vein is known to have irregularly spaced zones of mineralization along strike, sampling may be spaced at 100 m or 200 m along a vein. However, sampling normal to the strike of the vein may be reduced locally to 10 m in order to delineate mineralization. Figure 5-10 shows an idealized situation of an effective sampling pattern over a gold-bearing quartz vein. In this situation sampling over the vein at 10 m intervals has provided a four-point

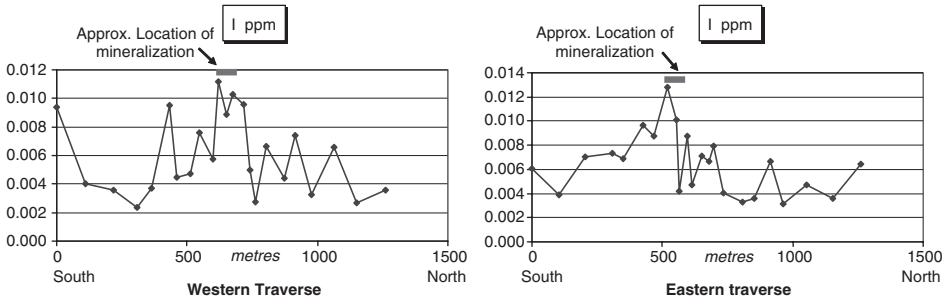

Fig. 5-8. Iodine in dry tissue of western redcedar (*Thuja plicata*) at the Boundary Zone, Mount Polley, British Columbia. Analysis by HR-ICP-MS after a warm water leach (Dunn et al., 2006a,b).

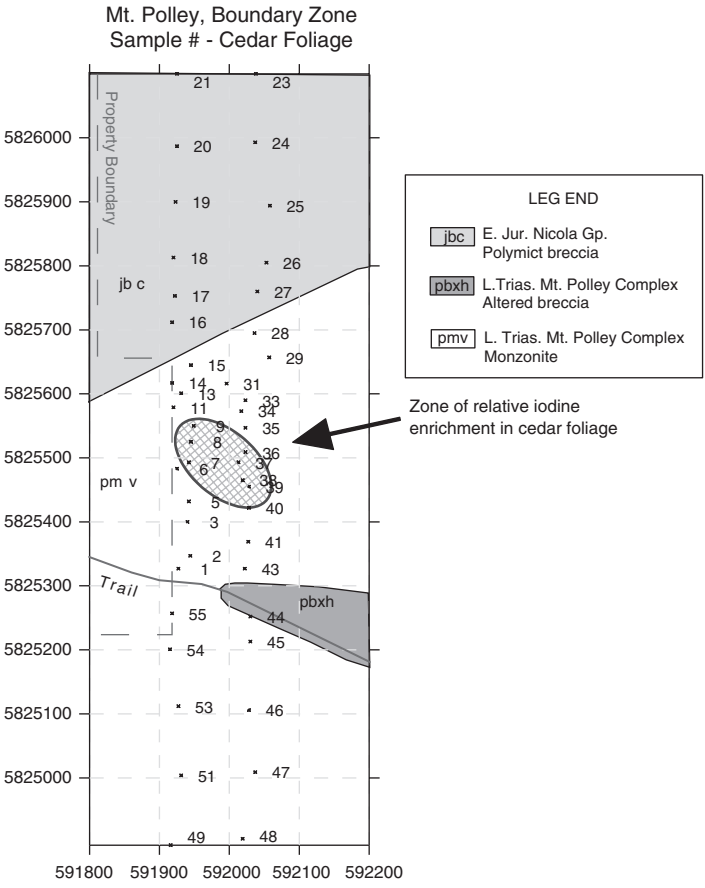

Fig. 5-9. Trend of iodine anomalies indicated in the profiles shown in Fig. 5-8.

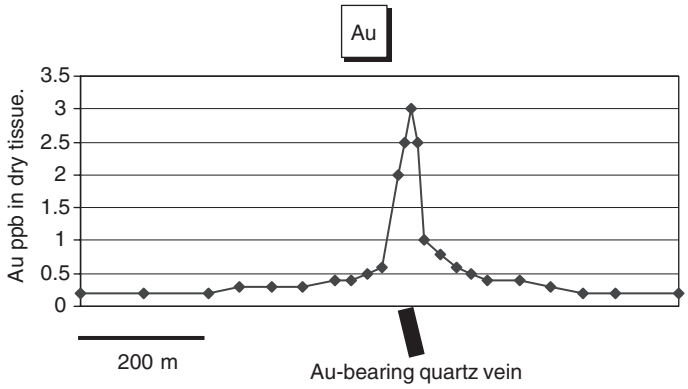

Fig. 5-10. Idealized profile over Au-bearing quartz vein. Sample spacing at 10 m intervals over vein, increasing outward to 25 m, then 50 m and finally 100 m.

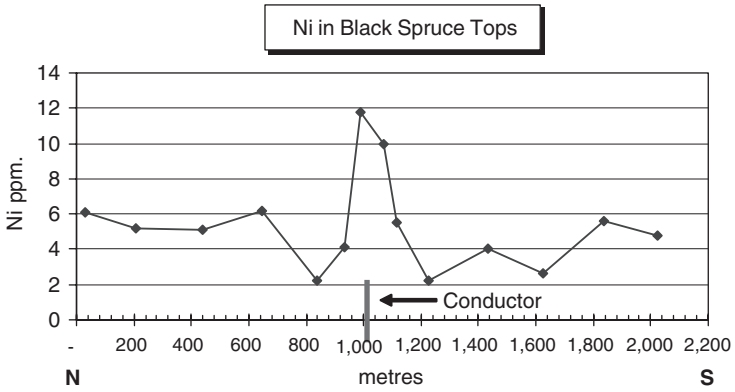

Fig. 5-11. Ni in spruce crowns – concentrations in dry stems (most recent three years of growth). Analysis by ICP-MS on an aqua regia digestion.

anomaly. Moving outward from the vein four samples on either side at 25 m intervals are followed by another four at 50 m intervals and finally two sites at 100 m intervals. The slight right skew to the anomaly is indicative of the dip of the vein.

A similar sampling protocol can be employed over geophysical conductors in order to determine the elemental composition of the conducting body. Whereas the idealized case shown for Au in Fig. 5-10 is from an artificial dataset, the profile shown in Fig. 5-11 is from a real survey designed to differentiate between Ni-bearing and Ni-deficient geophysical conductors (massive sulphides) that had not been drilled. In the example shown, sample spacing was at 200 m, closing to approximately 100 m over the conductor. The results clearly indicate that the massive sulphide that generated the geophysical anomaly was Ni-bearing.

Another orientation survey design that is particularly appropriate for circular targets, such as kimberlites, is to collect samples along two lines that cross in the middle of a prescribed (e.g., geophysical) anomaly, generating a 'cross-hairs' pattern. Figure 5-12 shows an example of sample sites over a kimberlite in the Ekati area of Canada's Northwest Territories. The area surveyed was tundra with little relief and a number of boggy depressions. The dominant plant species was dwarf birch. Over the circular geophysical target of about 100 m in diameter and for 100 m to either side the selected sample spacing was 25 m, increasing to 50 m for two sites moving outward from the anomaly, and finally three sites at 100 m spacing. This proved to be an effective pattern for defining the biogeochemical response to the concealed kimberlite (more details in section on kimberlites – Chapter 11).

COMPARISONS WITH OTHER TYPES OF GEOCHEMICAL SURVEY

A question often asked is 'How do results from a biogeochemical survey compare with data obtained from other sample media?' As a first response, the reader is referred

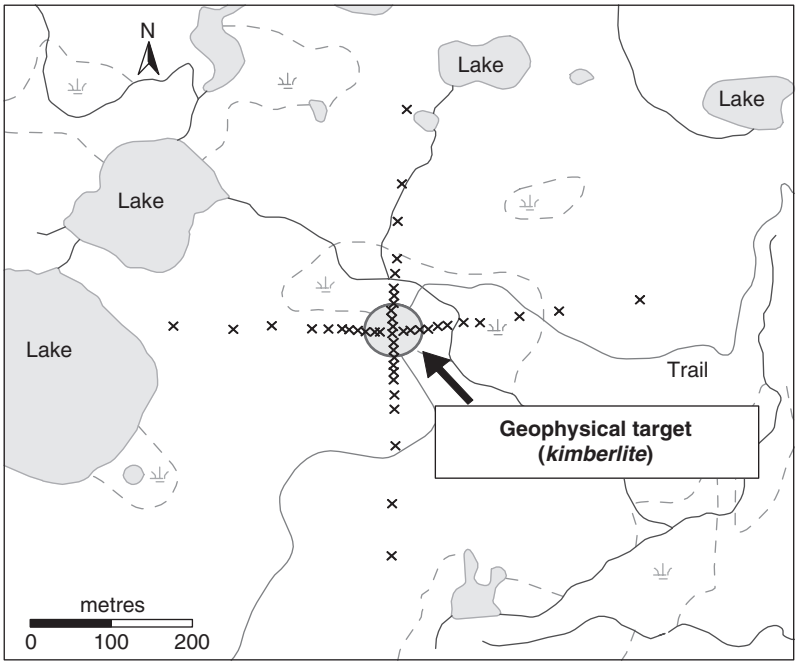

Fig. 5-12. 'Cross-hairs' sample design over circular geophysical target. Sample survey pattern over the Bighorn kimberlite, Ekati area, Northwest Territories, Canada (courtesy of Dr. W.B. Coker, BHP Billiton Ltd.).

to the discussions in Chapter 4 where it is noted that the roots from a large tree commonly penetrate all soil horizons and integrate the geochemical signature of several cubic metres of soil. As a result, a similar geochemical response in vegetation and soils should only be expected when elements are quite evenly distributed throughout a large volume of soil.

Vegetation versus Soils

In areas where there is either glacial dispersal or down-slope movement of soils, typically a biogeochemical response is more directly centred over a zone of mineralization than is a soil response. At Imperial Metals' Mount Polley Cu/Au porphyry open pit mine in central British Columbia, there are several nearby undisturbed zones of mineralization. The geochemical responses of some, such as the Boundary Zone, were investigated prior to trenching and drilling. Comparisons of metals in cedar foliage (*Thuja plicata*) with concentrations in B-horizon soils demonstrate this spatial relationship of elements in the two media. Figure 5-13 shows an outline of the geological contacts, and two plots showing concentrations of Hg in dry foliage from the cedar and in the –80 mesh fraction of B-horizon soil. Dots are shown as five

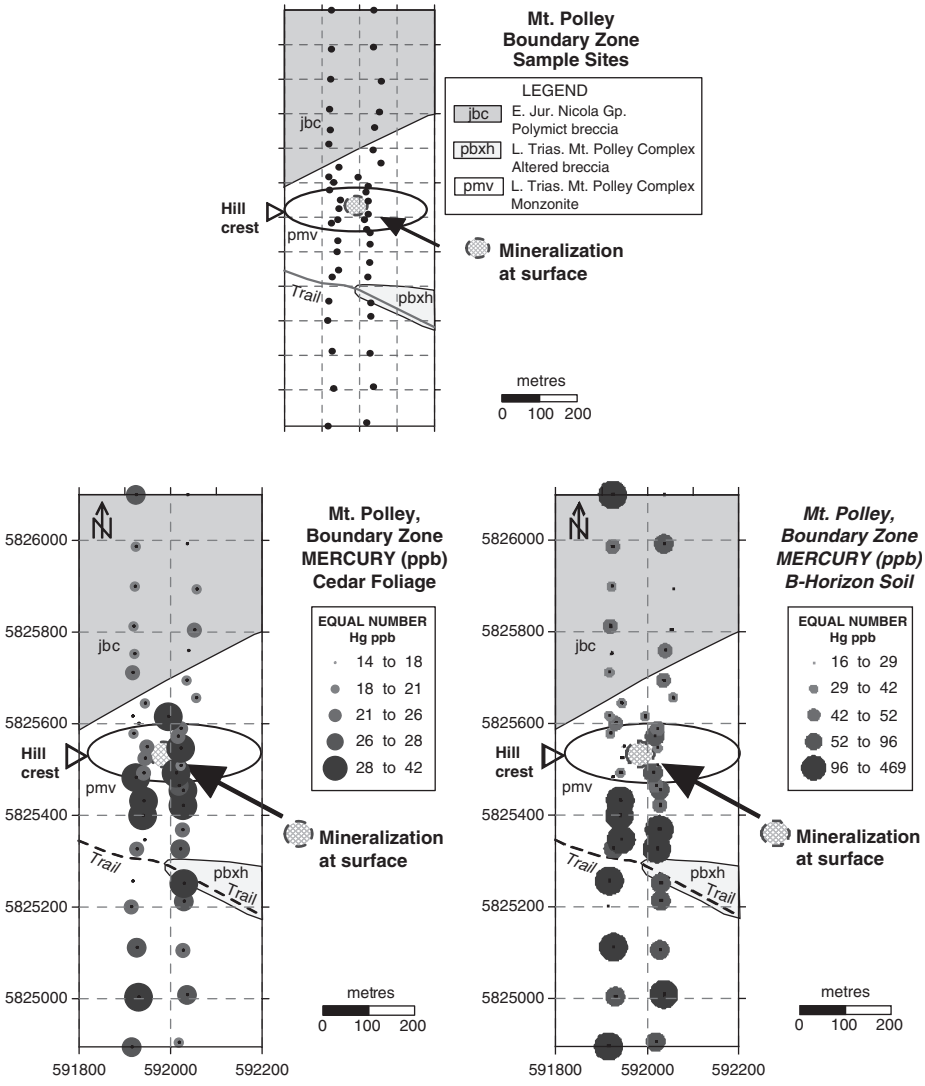

Fig. 5-13. Mount Polley Cu/Au/Mo porphyry, central British Columbia. Sketch of geological map with and locations of (1) B-horizon soil and cedar samples and (2) concentrations of Hg in –80 mesh soil and dry cedar foliage (from Dunn et al., 2006a,b).

classes of 10 samples (50 samples), with the dots proportional in size to increasing element concentrations. The sketch shows that the highest concentrations in the soil are down slope to the south from the mineralized hillcrest, whereas the foliage has highest concentrations directly over mineralization. This pattern of a biogeochemical anomaly being located more directly over mineralization than a soil anomaly is characteristic of many sites with undulating relief and/or glaciated terrain. In flat arid

environments upon which residual soils are developed, soil and vegetation anomalies are commonly more spatially coincident.

Vegetation versus Glacial Tills

Mount Milligan is located on the Nechako Plateau in central British Columbia at an elevation of 1508 m. Eight kilometres to the northwest are some significant Cu/Au porphyry deposits that have yet to be developed. The Mount Milligan deposits are centred on Early Jurassic crowded plagioclase-porphyritic monzonite intrusions known as the MBX and Southern Star stocks. These, and numerous smaller stocks, intrude Upper Triassic Takla Group augite (+/– plagioclase) porphyry agglomerate, trachyte breccias and flows, and bedded epiclastic sediments of the Witch Lake Formation. The last glacial event in the region occurred during the Late Wisconsinan (Fraser Glaciation) and involved ice movement primarily to the east–northeast. Till was deposited during the last glacial episode and is commonly hummocky and forms drumlins. Drift thickness is highly variable, ranging from less than 1 m on rocky highlands to over 80 m. In the survey area shown in Fig. 5-14, the thinnest till occurs at the MBX and Southern Star deposits and thickens to the east.

A biogeochemical survey (Dunn et al., 1996b) involved the collection of outer bark from 134 mature lodgepole pines (*Pinus contorta*), whereas a simultaneous till survey involved digging pits at most of these same sites (Sibbick et al., 1996). The bark samples were reduced to ash by controlled ignition at 475°C, and the tills were sieved for the –80 mesh fraction to be submitted for analysis. Analyses were by INAA, and ICP-ES on an aqua regia digestion of each sample medium.

Figure 5-14 shows the results for Au, with lodgepole pine on the left and till on the right. Both media exhibit relative enrichments at both the MBX and Southern Star Zones where the till cover is thin. The bark anomalies are more tightly centred over the mineralization than the till for which some dispersion from ice movement is evident. However, at the Philip Lakes showing, where there is greater thickness of till, the bark anomaly is centred over the showing, but the till anomaly is displaced in a down-ice direction by about 2.5 km.

At the Beaver Dam gold project in Nova Scotia, the Mill Shaft deposit comprises narrow Au-bearing quartz veins in quartzite and argillite of the Goldenville Formation (Lower Palaeozoic Meguma Group). Gold is concentrated within and along the margins of pyrrhotite and chalcopyrite. Samples of B-horizon soil, till and outer bark of red spruce (*Picea rubens*) were collected in a grid pattern at intervals of 50 or 100 m (Dunn et al., 1991). Analysis of the soils (<2 mm fraction) and the heavy mineral concentrates from till indicated dispersal of Au and As in a fan about 100 m wide, that extended about 300 m down-ice from the mineralized zone. The head of the fan was located 50 m to the south of the Au-bearing quartz veins. Outer bark from red spruce exhibited a similar trend for Au and As, coincident with a boulder train of vein quartz on the surface. However, the sites of maximum concentration in

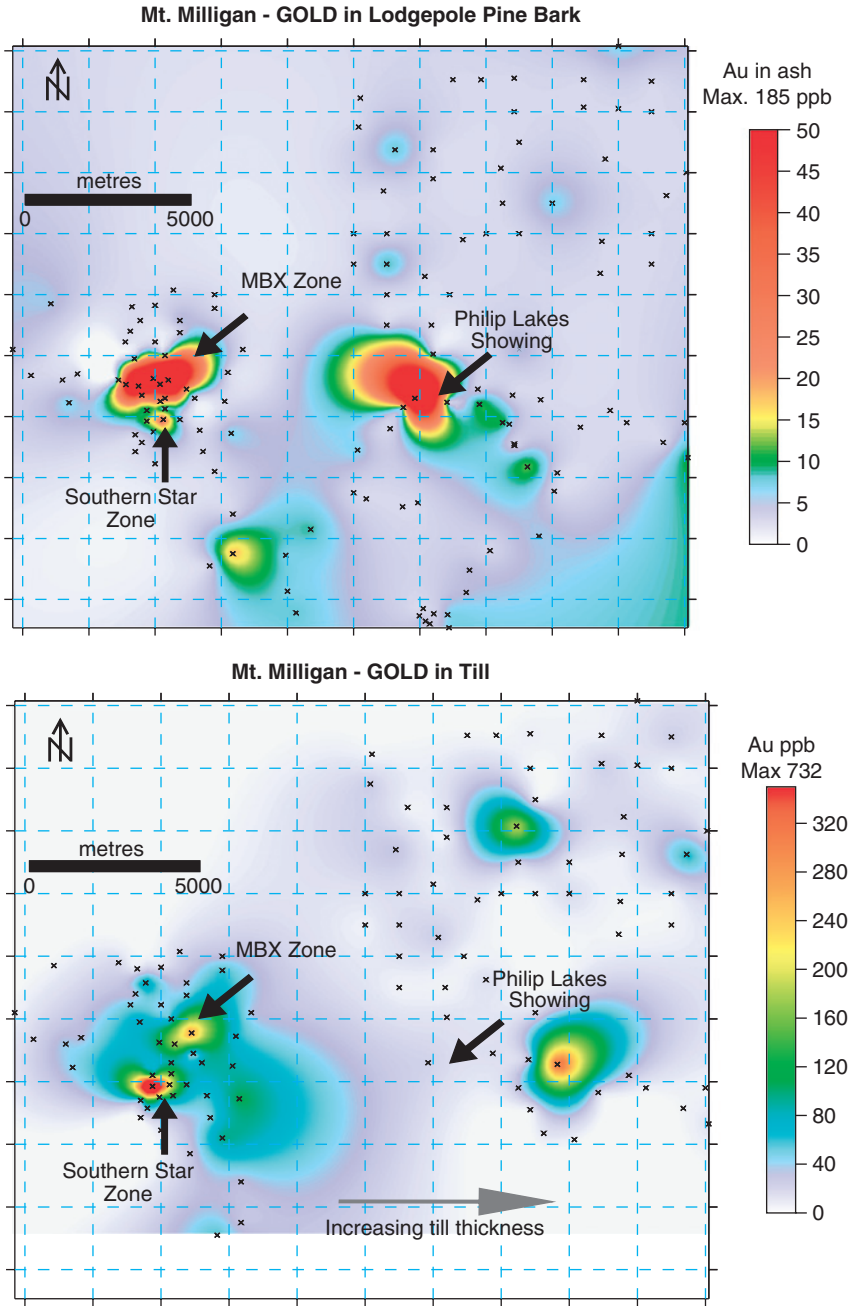

Fig. 5-14. Mount Milligan Au/Cu porphyry, central British Columbia. Gold in the ash of lodgepole pine bark and in the –80 mesh fraction of till. Analysis by INAA.

the bark were more closely confined to the known mineralization, occurring to both the north and south of a W/E trending vein (i.e., a 'rabbit ears' type of anomaly as defined by Govett (1976) and Smee (1983)).

Comparison of multi-media reconnaissance-level surveys using plants, soils, and sediments from lakes and streams

In Nova Scotia comprehensive reconnaissance-level surveys carried out by Rogers (1988, 1989) and Rogers et al. (1984), as well as National Geochemical Reconnaissance surveys conducted by the Geological Survey of Canada in the 1980s, provided substantial databases that allowed comparison of patterns of biogeochemical data from later surveys (e.g., Dunn et al., 1989). A comparison was made of the chemistry of lake sediment and balsam fir twigs (*Abies balsamea*) collected over a 5000 km^2 area of eastern Nova Scotia at a sample density of approximately 1 per 8 km^2. The surveys were conducted 12 years apart. The spatial relationship between areas of metal enrichment in the two sample media was examined by comparing each kriged dataset. Areas where Au and As concentrations were greater than the 70th percentile in both media succeeded in identifying the main gold camps and identified other areas worthy of further investigation (Dunn et al., 1991).

Extensive multi-media reconnaissance-level surveys were conducted in northern Manitoba during the 1990s by a government-sponsored programme entitled 'Operation Superior'. Large databases of analyses from these surveys are available in digital form for statistical interrogation and comparisons (Fedikow et al., 1997a,b, 1998, 1999, 2000, 2002).

In the Cobar area of New South Wales, Australia, Cohen et al. (1998) compared selective extraction soil geochemistry (lag samples and soils from depths of 10–20 cm) with the chemistry of needles from cypress-pine (*Callitris spp.*, also known as white cypress and not a true pine; it is a genus of the coniferous cypress [*Cupressaceae*] family). In addition to a good response to gold mineralization by several of the analytical protocols tested for the various soil components, there was a strong multi-element response in the cypress needles. It was concluded that the cypress may be responding to hydromorphic dispersion patterns at depth and that selective extractions, when integrated with biogeochemistry, offer enhanced potential for detecting mineralized targets buried by significant thickness of transported cover.

A study to determine vegetation and stream sediment patterns in north-eastern New South Wales covered approximately 14,000 km^2 of the Clarence River system (Cohen et al., 1999). Sampling was designed to confine each of 924 sub-catchments to a single lithological group. The composition of the –250 μm fraction of the stream sediments, and the leaves from over 20 genera (dominated by (*Allo-*) *Casuarina*, *Eucalyptus*, *Acacia*, *Callistemon* and *Melaleuca*) was determined by INAA. Results indicated that stream sediment and vegetation geochemistry reflected both hydromorphic and mechanical dispersion within sub-catchments, with regional patterns

dominant over local influences. The vegetation, however, was influenced to a greater extent by hydromorphic dispersion, as indicated by differences in the ratio of leaf to sediment Cr concentrations in sub-catchments draining serpentinites and basalts. A number of Au targets were detected only on the basis of the biogeochemistry, whereas others were reflected only in the stream sediment geochemistry. As with their previous regional study in the Cobar region, Cohen et al. (1999) concluded that differences in the response of the two sampling media suggest their joint use in exploration would maximise the probability of detecting mineralization.

SUMMARY

Biogeochemical surveys can be carried out over a wide range of scales and sample designs, and their formats depend upon the objectives, the target (nature of the mineralization) and various logistical factors. A limitation is that the coverage obtained from a proposed biogeochemical survey can only be as comprehensive as the distribution of the species selected for collection. Consequently, whenever possible, a quick reconnaissance assessment of plant coverage should be made. Information available from local forestry offices and/or botanists at local universities, government agencies, botanical gardens and museums can prove invaluable. In some areas 'biogeoclimatic' maps are available that provide an overview of the distribution of plant species to assist in advance planning.

For detailed surveys, sample spacing should be commensurate with the distribution of the selected plant species. Given the irregular distribution of trees that may occur in undisturbed forest, the geochemist may need to settle for an uneven grid, with perhaps some gaps where a recent clear-cut is encountered or where a change of elevation is sufficient that a different plant assemblage occurs.

As noted earlier, a plant's root system integrates the geochemical signature of several cubic metres of the substrate – all soil horizons, interstitial water, and perhaps penetrates through regolith or glacial deposits into bedrock fractures. As a consequence of the sometimes enormous extent of root systems, a biogeochemical signature is normally a selective mixture of any or all of these components, and that signature is unlikely to be identical to the geochemical signature of any of the individual media penetrated by the roots. Given the complex interactions of physical, chemical and biological dynamics in the natural environment of a forest or scrubland, in most parts of the world it is unrealistic to expect a biogeochemical signature to be the same as that from an underlying soil.

Slight enrichments of metals in soils and tills are unlikely to be detected in the vegetation as weak biogeochemical anomalies. This is especially true in the ppb Au and low ppm ranges of other metals commonly encountered in surface sediments. Some metals may not be present in a chemical form that is available for uptake (e.g., Cr structurally bound in chromite, and therefore not readily released to root systems); some may be excluded from uptake at the roots or only partially absorbed

(because of plant barrier mechanisms); and some may be taken up but dispersed among tree tissues to the extent that inter-site variations are so small that they cannot be detected.

The net result of these factors is that the geochemical information supplied by vegetation is commonly different from that of soil or till, although there may well be a more obvious relationship from areas of residual soils. Just as two methods of geophysical survey will provide totally different information, so will two methods of geochemical survey. A high correlation between distribution patterns of two geochemical sample media is the exception rather than the rule. In geological environments, where there is sufficient concentration of metals to form a mineral deposit, such a 'critical mass' of elements may be sufficient to generate biogeochemical anomalies above (by upward diffusion) or close to (by movement in, for example, electrochemical cells) the mineral source. Soils may have anomalies displaced downslope from mineralization and tills usually have geochemical anomalies displaced down-ice from the mineralized source. Such factors need to be taken into consideration when interpreting geochemical results. Valuable exploration information can be obtained from the analysis of most types of surface geochemical sample media. When this information is coupled with analysis of vegetation samples a powerful combination is provided for assisting in the exploration for a wide variety of mineral deposits.

Chapter 6

SAMPLE PREPARATION AND DECOMPOSITION

INTRODUCTION

The selection of appropriate preparation and decomposition procedures is crucial to the meaningful interpretation and successful outcome of any exploration biogeochemical survey. They require careful consideration prior to introducing vegetation samples into the analytical instrumentation. For many of the elements of interest to the exploration biogeochemist, meticulous experimentation by commercial laboratories has streamlined this process. However, in order to understand the results that can be obtained from an analytical package, and to extract relevant and meaningful information the user should be aware of several procedural options and their respective advantages and limitations.

WASHING

There is on-going debate among those involved in the analysis of plant tissues as to whether or not samples should be washed prior to analysis. In general, in northern forests far from dusty roads, for exploration purposes washing may be redundant or even deleterious to survey results. In arid to semi-arid regions where winds constantly coat samples with dust, it is commonly better to *rinse* samples prior to drying and separating tissues. A judgement call based upon the experience of the biogeochemist needs to be made in the field, that takes into account the degree of dust coating of vegetation samples and the nature of the dust (i.e., is it from a mining operation or smelter, and therefore might metal-rich particulates be coating the plants?).

Thorough washing using solvents or dispersants

Wyttenbach et al. (1987) and Wyttenbach and Tobler (1988) advise that washing should be undertaken with a mixture of toluene and tetrahydrofuran accompanied by mechanical vibration to remove wax and adhering aerosol particles from the surface of needles, because for

> many elements … quantities on the needle(s) surface are greater that those within the needle … The treatment used does not remove elements from within the needle.

Whereas some contend that washing should be carried out as a matter of course, regardless of local conditions, washing with these organic solvents would be onerous, expensive, potentially hazardous for some toxic solvents such as toluene, and impractical to apply to all samples from a large biogeochemical survey. Furthermore, it can be argued that by removing the surface waxes (see Figs. 3-3 and 4-1 – SEM of twig surface) an integral part of the plant structure is removed that may contain elements secreted from within the plant as well as the less desirable aerosol particulates. The term 'less desirable' is used because in addition to distant sources of contaminants (i.e., allochthonous) the aerosols may contain particulates from the microenvironment around a plant (autochthonous) that could be useful for exploration purposes. Gaseous emanations from an orebody (e.g., Hg, F, Br, I) that are not entirely introduced into a plant via its roots, but de-gas through fractured rock and from soil, could precipitate on plant surfaces and enhance the biogeochemical signature of these elements. Therefore, an added signature from *locally derived* aerosol particulates (the 'autochthonous' microenvironment) is not necessarily unwanted, and can provide geochemical information of value to a survey seeking to delineate concealed mineral deposits.

Usually, an unknown factor is the degree of contamination from distant sources. Certainly for samples from around a smelter or other industrial activity the airborne source needs to be minimized, and a thorough cleaning of the type recommended by Wyttenbach et al. (1987) and Wyttenbach and Tobler (1988) or one of the other methods listed below would be warranted.

Chloroform is another organic solvent that can be used for removing surface waxes. Table 6-I shows analyses of leaves from three samples of mountain alder (*Alnus crispa*) that were prepared in the field at the collection site. Each sample was divided into two portions. One-half was prepared for analysis with no pre-treatment; the second was stripped of its waxes by swirling in a jar of chloroform, and then air-dried to evaporate the solvent. The two splits of each sample were analysed by INAA for 35 elements, of which those recording losses from the chloroform treatment, although not consistent for the three sites, are shown in Table 6-I. The data imply that losses were those portions of each element associated with the surface waxes and cuticles, confirming the results of Wyttenbach and Tobler (1988). Similar tests run on twigs of balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) did not indicate loss of these elements, because they comprise woody tissue devoid of significant amounts of surface wax.

A simpler alternative to the use of organic solvents is to wash samples in distilled water to which a little detergent (e.g., Calgon, or an ultrapure dispersant such as Photoflow^{TM} by Kodak) is added to reduce surface tension of adhering particulates. However, there exists the potential problem that such a procedure may remove elements of exploration interest, because any rigorous swirling of samples can break down the outer cells of the plant surfaces and release some elements into the washing medium. Many surface outgrowths are also the location of various crystals (druses) and removing these structures could dilute the natural biogeochemical signature.

TABLE 6-I

Analyses of leaves from mountain alder (*Alnus crispa*) from the La Ronge belt of Saskatchewan – portions untreated, and rinsed with chloroform ('treated') to extract waxes and associated elements. Concentrations in dry tissue determined by INAA

	Alder Leaves (*Alnus crispa*)					
	Site 1		Site 2		Site 3	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Ca (%)	0.46	0.46	0.50	0.42	0.54	0.49
Ba (ppm)	15	13	20	8	9	13
Zn (ppm)	27	23	18	21	27	22
Fe (%)	0.04	0.02	0.04	0.03	0.02	0.02
Na (ppm)	214	160	245	155	109	97
Cr (ppm)	0.8	0.6	1.0	0.7	0.5	0.3
Th (ppm)	0.11	0.05	0.10	0.10	0.06	0.04
La (ppm)	0.30	0.20	0.37	0.22	0.24	0.22

Prolonged washing can damage plant tissue, allowing cell cytoplasm to become hydrated and leak into solution and, because it is a differential leaching (cell walls remain intact) the signature generates a false bias.

A study by Larry Cook at the University of Idaho (personal communication) has examined several washing procedures of big sagebrush (*Artemisia tridentata*) using the usually 'conservative' (immobile) element, titanium. Washing consisted of immersing each sagebrush sample (twigs with leaves) 20 times into the treatment solution and then rinsing with deionized water. Controls were unwashed plants. Averages for the controls were derived from six replicates and there were three replicates for each of the washing treatments (Fig. 6-1). The Tween20 and Tween80 products are non-ionic surface wetting/emulsifying agents with differing surfactant properties. SDS is sodium lauryl sulphate – another wetting agent. The results were similar for other species that were treated in the same manner, and no one method emerged as superior in removing soil from plant tissue and none were effective in removing all adhering particles.

Microscopic examination of big sagebrush shows that it has felted leaves that are capable of readily trapping airborne particles (Fig. 6-2) and, since the Idaho study site was described as extremely dusty, the washing removed some, but not all of the adhering inorganic particulate material. Results of the various washing treatments show a reduction in Ti content by 20–40%, depending upon the solution. However, most treatments fell within a close range of about 150 ppm $+/-10\%$, with significant over-lapping of error bars. In summary, although washing removed some dust, no method was successful in removing all of the inorganic material.

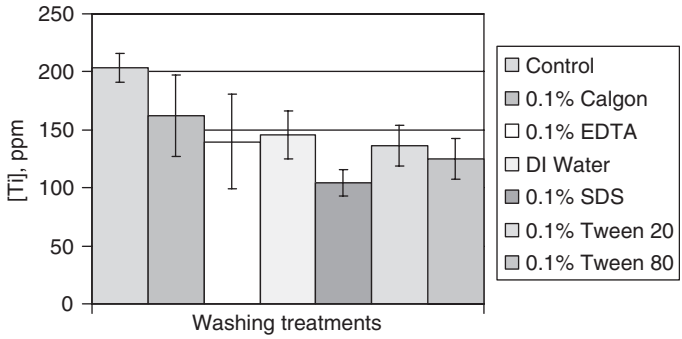

Fig. 6-1. Results of washing leaf and twig tissues of big sagebrush (*Artemisia tridentata*) using 6 different cleaning agents (courtesy of Larry Cook, University of Idaho, 2005). Error bars represent one standard deviation.

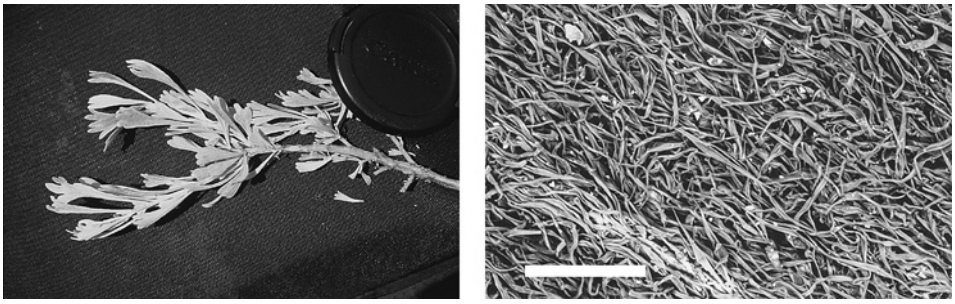

Fig. 6-2. Big sagebrush (*Artemisia tridentata*). Left: typical foliage. Right: SEM showing felted texture of leaf trapping dust particles in its fibres (scale bar is 200 μm).

In another study on plant washing Azcue and Mudroch (1994) reviewed the literature, and conducted some experimental work involving 10 trace elements that compared washing with distilled water, a detergent (Alconox^{TM} [1%]), and 1% HCl. They concluded that the best results were obtained with distilled water.

Washing in water

It is as well to discriminate between rinsing and washing. Rinsing involves simply passing the sample through a stream of water. Washing is defined here as placing the sample into a path of liquid where it is submerged for a specified period and/or agitated. One concern with regard to washing is that the process may lack consistency. For routine washing under controlled conditions, a PreVac^{TM} washer could be used. This controls the quantity and duration of the water treatment and the washing container is pressurized in a controlled manner. The addition of any other substance

(wetting agent, dispersant, solvent) to the wash might introduce unknown contamination. Commercial soaps can have various contaminants that could enhance a natural biogeochemical signature.

Tests conducted on combined stems and leaves of big sagebrush and on pine bark involved analysis of washed and unwashed portions of the same sample (Dunn et al., 1993a). The sample site was near the Nickel Plate gold skarn open pit mining operation in southern British Columbia. The high Au and As concentrations are a reflection of this mineralization (Table 6-II). Washing was extremely rigorous in that portions to be washed were placed in deionized water in an ultrasonic bath for 1 h. After drying, the samples were reduced to ash by controlled ignition at 475 °C and concentrations were determined by INAA. Results of the 35-element analysis showed loss of Fe from the woody tissues (twigs and bark), but the only element to exhibit an appreciable and consistent difference was K. Values for all other elements fell within the narrow range of analytical precision typically obtained by INAA.

In a test that involved some extremely dusty outer bark of Ponderosa pine (*Pinus ponderosa*), unwashed and washed samples were analysed as well as the dust that settled out from the distilled water used for the washing. Samples were put in a beaker containing deinonized water that was placed on an ultrasonic shaker for 30 min. The results (Table 6-III) showed that concentrations of most of the trace elements were higher *after* washing, suggesting that adhering dust (mostly silicates) *diluted* the trace element composition of the bark. This was confirmed from examination of the analysis of the dust removed by the washing. Elements yielding lower concentrations after washing were the major nutrients P and K, and traces of Mo.

A study of very dusty samples from the Ballarat East goldfield of Victoria, Australia, found that washing failed to remove the contaminants. It was concluded that

TABLE 6-II

Effects of thorough washing in distilled water (1 h in ultrasonic bath) on the chemical composition of different plant tissues. Concentrations in ash determined by INAA

	Sagebrush twig		Sagebrush leaf		Lodgepole pine bark	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Au (ppb)	270	294	279	267	293	298
As (ppm)	100	95	50	64	150	160
Ba (ppm)	330	300	140	150	590	590
Co (ppm)	4	4	2	2	11	10
Fe (ppm)	6300	5500	2500	2800	17,600	17,200
K (%)	26.3	24.3	17.4	13.2	3.2	1.5
Mo (ppm)	11	10	9	11	2	3
Sb (ppm)	1.7	1.5	0.7	1.1	4.2	4.3
Zn (ppm)	570	550	530	610	1300	1400

TABLE 6-III

Analysis of Ponderosa pine bark: dusty (unwashed), washed and the derived dust. Samples collected several kilometres from an open pit exposing copper and gold-rich mineralization (Iron Mask batholith, near Kamloops, British Columbia). Washing was for 30 min in distilled water on an ultrasonic shaker. Concentrations are in ash, determined by INAA (As, Au, Sb) and ICP-ES for the remaining elements

	Unwashed	Washed	Dust washed from the bark
Ash yield (%)	1.07	1.05	–
Cu (ppm)	3265	3642	291
Au (ppb)	67	140	19
As (ppm)	39	39	3.4
Sb (ppm)	4.4	5	0.5
Pb (ppm)	239	259	22
Zn (ppm)	1172	1328	44
Cd (ppm)	37	39	<1
Mo (ppm)	18	15	<1
P (%)	1.05	0.85	0.6
K (%)	3.31	2.46	0.06

the rough outer bark of some *Eucalyptus* species should be avoided in areas of potential dust generation (Arne et al., 1999).

Thanks to developments in ICP-MS that now permit determinations of ultra-trace levels of a broad range of elements in dry tissues, further insight and quantification of the elements that are removed during washing can now be more readily obtained than was previously practical. Figure 6-3 shows results of tests on dry leaves (with a felted texture, not unlike that of sagebrush) and stems from a genus of the persimmon family (*Diospyros spp.*) from Africa. Washed samples are shown as diamonds and unwashed samples as open triangles. Analyses of stems are shown on the left half of each chart, followed by plots for the leaves from these stems. On the far right of each chart the average values of washed versus unwashed are shown, and for most elements these are almost identical.

Figure 6-3 shows that patterns of washed versus unwashed samples are similar for Cd, Fe, Ti and Mo, and concentrations are mostly within the usual levels for analytical precision for these elements at the concentrations present (see Chapter 8). In the plot of Zn, the profile for the twigs indicates that washing may have removed particulates that were slightly diluting the Zn signature, because the unwashed twig samples yielded marginally lower concentrations than their washed counterparts; however, this difference is not apparent for the leaves. Conversely, the last plot shows that washing removed some of the K from the leaves. This finding is in accord with the results of vigorous washing illustrated in Tables 6-II and 6-III.

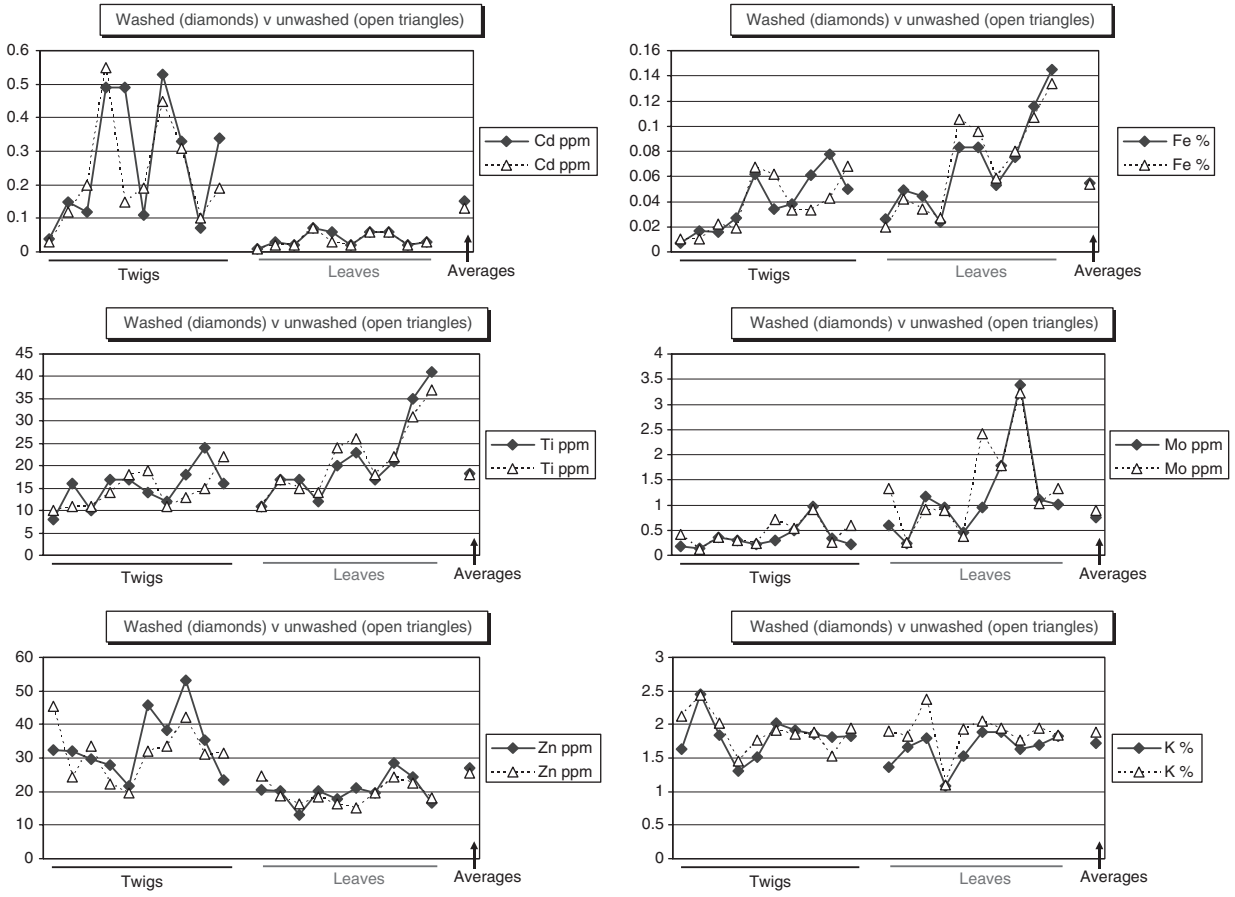

Fig. 6-3. Comparison of element concentrations in washed versus unwashed twigs and leaves of *Diospyros spp.*

Of the elements determined in the study of *Diospyros*, in both leaves and twigs almost identical patterns were derived from the washed and unwashed portions for Ag, Al, Bi, Ca, Cd, Co, Cs, Fe, Ga, K, Mg, Mo, Na, Ni, P, Pb, S, Sc, Se, Th, Ti, Tl, U and V. Leaves generally had more consistent relationships between washed and unwashed portions than did the twigs; additional elements with similar profiles from the leaf tissues (washed and unwashed) were Au, Ba, Cr, Cu, Mn and Zn. No element yielded a washed versus unwashed *profile* of concentrations that was substantially different. It is noteworthy that elements actively used in physiological processes (B, K, Ca, Cu, Fe, Mg, Mn, Mo, Na, P, S, Zn) were more homogenously distributed between the leaves and twigs than the non-essential elements that constitute the remainder of the list, e.g., Ag, Al, As, Bi, etc., of which some were higher in twigs, and some higher in leaves.

A study of vegetation samples from close to the Giant gold mine near Yellowknife (NWT, Canada) and from a site some 1.5 km distant served to determine differences in anthropogenic and natural sources (Dunn et al., 2002) and the effects of washing samples. Among the concluding remarks the following was noted.

> The environment of the sample sites is atypical of the boreal forest in general, in that the vegetation samples were collected from close to a significant source of metals derived from the Giant mine and mill. A measure of the amount of particulates adhering to the plant surfaces can be obtained from determining the ash yield of unwashed and washed samples, but washing does not remove all of the particulate material because some is contained [entrapped] within the plant structure (particularly in the bark). Thus, in this environment, washing does not give an accurate estimate of the element uptake purely from natural sources, and an anthropogenic component is dominant in all of the samples tested. Some of the anthropogenic component falls to the ground and is subsequently dissolved in groundwater and taken up by the plant roots then sequestered in the tree and shrub tissues.

To summarize the many options described above, for exploration purposes the addition to the sampling/preparation/analytical process of the extra step of washing creates another possible source of error. During sampling and preparation there are several potent potential sources of error that include sample identification, cross-contamination and external contamination. Each can contribute to masking the natural biogeochemical signature. If a sample drops to the ground during sampling, another sample should be collected. If samples are obviously dusty, they should be *rinsed* in running water. However, in most situations, sample washing is not a requirement. The spatial patterns derived from unwashed samples are typically robust and are likely to be similar to, if not exactly the same as, those derived from washed samples. Potentially, because of the high solubility of some elements, a relevant subtle biogeochemical signature could be washed away by vigorous washing. A recurring theme in this book is to remember to ask 'are the data fit for the purpose?' In general, a small amount of background dust contamination (but not from a highly enriched source such as a smelter) can be tolerated without compromising the integrity of a survey.

PARTICLE AND SAMPLE SIZE

If the selected analytical protocol is to digest dry samples completely in acids, it should not make any difference whether they are coarsely ground or milled to a fine powder. Usually, each commercial laboratory applies standard acid concentrations and digest durations in preparing samples for rapid instrumental multi-element analysis. As a result, if samples are coarsely ground, there may be some particulate residue that does not go completely into solution in the prescribed time. Certainly, the finer the powder the more readily a sample will go into solution.

Simple coffee-mill types of equipment with rotary blades are adequate for reducing soft to moderately hard tissues to a fine powder. However, cones, twigs greater than 5 mm in diameter, and twigs of hard woods such as *Acacia* are difficult to grind in anything other than a Wiley or Retsch-type of mill that has a shearing action (Fig. 6-4).

In the latter mills, adjustable hard tool steel knives are bolted to a removable rotor. These knives work against stationary knives, independently adjustable, that are bolted into a steel frame. The sheared particles pass through a hardened steel mesh to ensure consistent particle size. Typically, a mesh with 1 mm apertures is used, although optional sizes range from 0.5 to 6 mm. It takes considerably longer for material to pass through the fine-aperture mesh screens, thereby increasing sample preparation costs. If very fine particles are required, samples can be passed through a mill that grinds by high-speed rolling of the sample against an inner durable grinding surface and then passes it through a fine mesh screen. These grinding mills are rapid and effective, but for multi-element exploration purposes the user must be aware of potential contamination from the grinding surface, because it is usually tungsten

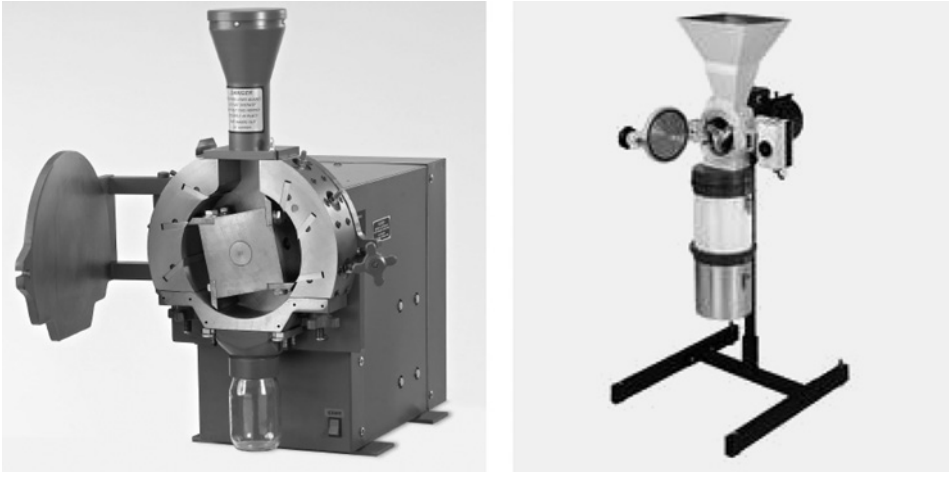

Fig. 6-4. Left: Model 4 Wiley mill (available from Thomas Scientific); right: Model SM 100 mill (available from Retsch™ GmbH).

carbide that has a cobalt-bearing binding agent. Table 6-IV compares elements for which analyses of samples milled in a shear-type of mill were significantly different from those passed through a tungsten carbide-bearing rotary-plate grinding mill. Data for all other elements determined (53) were comparable.

The scientific literature offers several suggestions with regard to the optimum particle size to which vegetation tissues should be milled. Vien and Fry (1988) recommend milling to a median size less than 20 μm, whereas Carrion et al. (1988) determined that for pine needles the fraction between 106 and 160 μm is preferable for obtaining optimum analytical precision.

Figure 6-5 shows results of some Douglas-fir outer bark that was coarsely ground (particle sizes of less than approximately 3 mm) compared to the same material that was ground to a fine powder (<0.5 mm). There is a very good correlation between the datasets obtained for each element; for some elements the concentrations were the same (within the limits of analytical precision), but the finer grinding commonly released slightly higher levels of elements. The amount of Fe and Hg released was appreciably higher in the finely ground fraction. This indicates that particle size can be important, and for exploration purposes the key to obtaining meaningful results rests with being consistent in the particle sizes of milled samples. Of lesser importance is the desirability of selecting a particle size that provides the highest concentrations, because the underlying tenet for exploration is that the primary objective is to establish contrasting spatial relationships among carefully acquired biogeochemical data.

TABLE 6-IV

Elements exhibiting different concentrations from passing two separate wood samples through a shear-type mill compared to a rotary disc-type of mill

	Sample 1		Sample 2	
	Very fine	Fine	Very fine	Fine
	Disc grinder	Shear mill	Disc grinder	Shear mill
Bi (ppm)	0.25	0.05	0.11	0.02
Ce (ppm)	0.05	−0.01	0.03	0.01
Co (ppm)	0.06	0.04	0.03	0.02
Cu (ppm)	1.33	0.73	1.68	1
Fe (%)	0.006	0.001	0.005	0.001
Ni (ppm)	20.3	0.1	10.5	0.7
Pb (ppm)	0.6	0.2	0.51	0.2
W (ppm)	12.9	−0.1	6.7	0.5
Y (ppm)	0.006	−0.001	0.004	0.001
Zr (ppm)	0.08	0.03	0.06	0.02

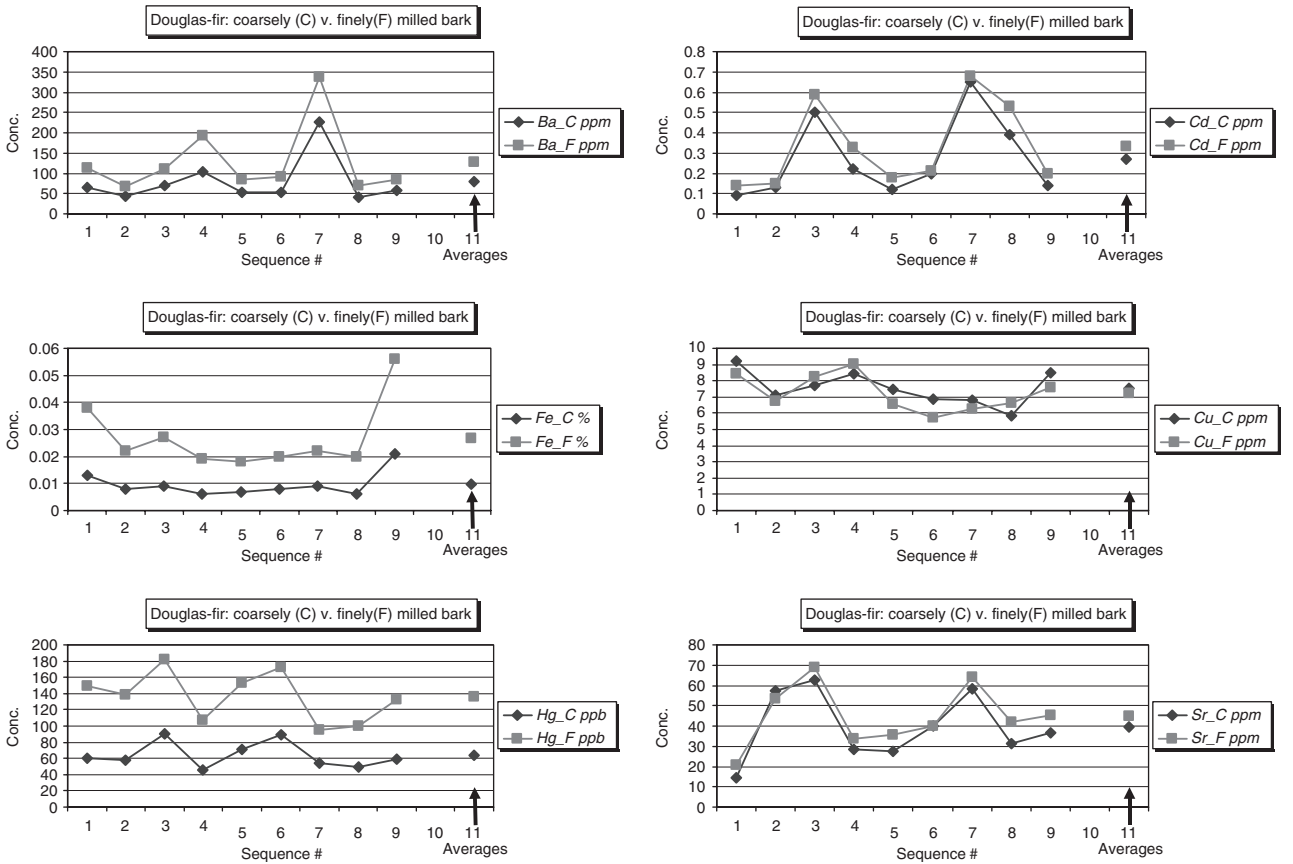

Fig. 6-5. Douglas-fir bark: analysis of coarsely milled material compared to finely ground. One-gram samples digested in HNO_3 then aqua regia with ICP-MS finish.

As a second example, Table 6-V shows the effect of different grinding times, hence fineness of particle size, on the analysis of *Acacia* stems from samples collected in central Africa.

In Table 6-V gold shows lack of homogeneity at the sub-ppb levels. This is typical for one-gram samples of milled tissue, although gold is more evenly distributed in tissues of some species than in others. Gold has an acropetal tendency in leaves – i.e., it tends to migrate toward leaf tips. Consequently, the fine fraction of milled leaves is

TABLE 6-V

Stems of *Acacia* milled for different durations in a grinder with rotary cutting blades. One-gram samples digested in HNO_3 then aqua regia with ICP-MS finish

	20 s grind	40 s grind	60 s grind		
	Coarse	Medium	Sieved fines	Coarse residue	Fine + coarse
Ag (ppb)	2	4	4	3	4
Au (ppb)	<0.2	0.6	0.3	0.2	0.7
B (ppm)	9	11	19	7	14
Ba (ppm)	28	29	62	23	44
Ca (%)	0.99	1.05	2.2	0.9	1.47
Ce (ppm)	0.03	0.02	0.07	0.03	0.05
Co (ppm)	0.01	0.01	0.02	0.01	0.03
Cr (ppm)	1.37	1.55	1.92	1.52	1.68
Cu (ppm)	3.98	4.78	6.57	3.88	5.1
Fe (%)	0.002	0.002	0.004	0.002	0.003
K (%)	0.99	1.12	1.57	1.02	1.33
La (ppm)	0.06	0.04	0.11	0.06	0.09
Li (ppm)	0.02	0.01	0.04	0.03	0.06
Mg (%)	0.089	0.106	0.223	0.087	0.139
Mn (ppm)	11	12	20	13	13
Mo (ppm)	0.47	0.33	0.66	0.43	0.6
Na (%)	0.007	0.008	0.016	0.01	0.01
Nb (ppm)	<0.001	0.001	0.009	0.002	0.006
Ni (ppm)	1	1.3	1.8	0.9	1.6
P (%)	0.094	0.106	0.185	0.084	0.132
Pb (ppm)	0.02	0.07	0.02	0.08	0.07
Rb (ppm)	12.4	14.3	19.3	12.1	15.8
S (%)	0.1	0.1	0.17	0.08	0.15
Sb (ppm)	0.05	0.08	0.12	<0.02	0.12
Se (ppm)	0.5	0.4	0.6	0.3	0.6
Sr (ppm)	209	219	443	197	271
Y (ppm)	0.01	0.006	0.017	0.007	0.015
Zn (ppm)	10.5	11.6	22.3	12.4	16

likely to contain the relatively soft and fragile leaf tips, whereas the coarser residue is made up of the harder vein tissues which typically contain less gold. The result is that higher gold values tend to be obtained from fine fractions. This relationship holds, too, for other tissue types and other elements. Pickering et al. (2000, 2003) used a Synchrotron to study the distribution and chemical speciation of Se in leaves of the Se hyperaccumulator plant *Astragalus bisulcatus* (two-grooved poison or milk vetch). This fundamental investigation showed, among other observations, that selenate is located almost exclusively in the soft tissue of mature leaves, rather than the veins of the leaves (Fig. 6-6).

SAMPLE DECOMPOSITION

Once samples have been prepared for analysis, the next consideration becomes whether to analyse dry tissue directly or first reduce tissues to ash. If low and acceptable detection levels can be obtained from the direct analysis of dry tissue, and the selected method (e.g., ICP-MS, INAA) can generate high-quality data (good precision and accuracy) for all elements of interest, then reducing samples to ash is an unnecessary step. However, plant tissues contain only minute traces of many elements, and so from the direct analysis of dry tissue by such methods as AAS and ICP-ES many values, particularly some of those of relevance to mineral exploration, are recorded as 'below detection' and ashing may be warranted. The various analytical methods and their merits are discussed in the next chapter, in which discussions centre on the sophisticated analytical instruments that are becoming increasingly available for determining 'ultra-trace' levels of almost all elements. Consequently, the multi-element analysis of dry tissues is steadily gaining a foothold as the method of choice.

Historically, no single analytical instrument was sufficiently sensitive to provide data for a comprehensive range of elements of relevance to mineral exploration. The non-destructive methods of INAA and XRF require no sample decomposition and provide excellent data for many elements. However, the main limitations for INAA are (a) the requirement of access to a nuclear reactor; (b) the inability to provide data for certain elements; and (c) the need for special irradiations to obtain data for certain elements. Included in the list of elements that are problematic or not possible to determine by INAA are Be, Bi, Cd, Cl, Cu, Ga, Ge, I, In, Li, Mg, Mn, P, Pb, Pd, Pt, Re, S, Te, Ti, Tl and V. Other elements have detection levels that are too high for most vegetation samples – e.g., Ag, Ni and Sr. Yet others may require substantial corrections for fission products, so if a sample has a high U content, then it may be impossible to report values for Ba, Mo, Te and some of the rare-earth elements (REE) (Hoffman, 1992). Furthermore, high levels of Na or Br in halophyte species necessitate additional corrections and therefore higher detection levels. Similarly, XRF has its limitations. These non-destructive methods are discussed in the next

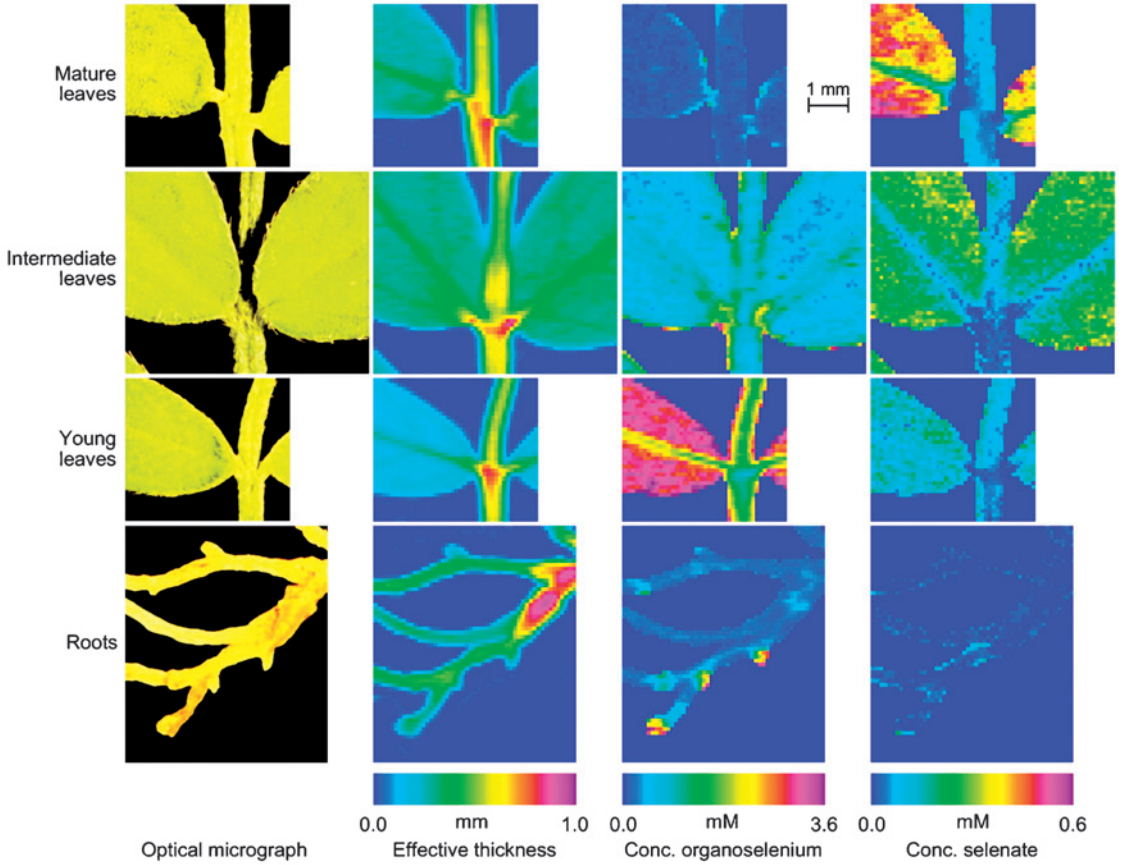

Fig. 6-6. Selenium concentration images of different parts of *Astragalus bisulcatus*. From left to right: optical micrograph; effective tissue thickness; concentration of organoselenium; concentration of selenate. Top to bottom: mature, intermediate and young leaves and roots. The mature leaves, young leaves and roots were taken from the same plant, the mature leaves from the lowest shoot and the young leaves from the highest shoot of the same plant branch. Reproduced with permission from National Academy of Sciences, USA, Ref. www.pnas.org, Pickering et al. (2000).

chapter, and the remainder of this section deals with procedures required to decompose plant tissues prior to their introduction into analytical instruments.

A number of the elements listed above can be determined by other techniques (e.g., ICP-ES or AAS), but without careful pre-concentration procedures multi-element scans generate data that are mostly below the level of detection. Many analytical methods have been developed that can address these problems, but they involve costs that are too high for the implementation of a biogeochemical exploration programme for minerals.

A solution to the problem of plant tissues having very low levels of many elements of exploration interest is to pre-concentrate them. This can be done by first reducing samples to ash by controlled ignition to drive off organic compounds, so that only the inorganic constituents remain for analysis. This 'ashing', as it will be referred to here, substantially reduces the volume of the plant material so that a sample that has, for example 1 ppm Ni in twig tissue, will have 50 ppm Ni in ash, because the ash yield of the twig is only 2% (i.e., a 50-fold concentration). From an exploration point of view, it is more comforting to see a value of 50 ppb Au (in ash) than 1 ppb Au (in dry material). In practical exploration terms, it is more appealing to an exploration manager to see an 'anomaly' of 50 ppb Au than the same 'anomaly' at 1 ppb Au in dry-weight equivalent.

Prior to discussing the merits of analysis of 'dry' versus 'ash', it is worth noting that modern analytical equipment (ICP-MS) can now provide remarkably good multi-element data from the analysis of just 1 g of dry tissue, and so the need to ash samples is diminishing. However, for the reasons discussed above, results of past surveys were quite commonly reported as concentrations in ash and, since the expensive state-of-the-art instrumentation will not be universally available for some years to come, the ashing process warrants discussion.

As is noted repeatedly in this book, in biogeochemical exploration, the absolute concentrations of elements are of lesser importance than their distribution patterns. Provided there is a direct relationship between the element content of dry tissue and the element content of ashed tissue, it makes no difference to the spatial distribution of anomalous zones of concentrations, and the data therefore provide the same focus for defining exploration targets.

DRY ASHING

Element volatilization

The most important consideration in dry ashing is the potential loss of some elements due to volatilization. For Hg, Br, Cl, F and I only a small percentage, if any, remains in ash after controlled ignition at 475 °C. Although there is partial loss of many elements during ashing the critical factor for exploration is that, for a given plant species, the amount of an element lost remains constant. The question is, if, for

example, twigs from species 'A' are found to lose 20% of their As at one sample station, do they also lose 20% of their As at another? Provided this loss remains constant, then the relative concentrations remain the same and it becomes possible to generate realistic plots of As distribution patterns. These plots of relative concentrations are critical to interpreting biogeochemical exploration data, because they may define stratigraphic, lithological and structural trends, in addition to zones of mineralization.

Volatilization depends on the chemical form of the element as well as the composition of the matrix (i.e., the type of plant tissue). Lead remains in ash if it is present as its sulphate, nitrate or oxide, but significant loss occurs if it is present as the chloride (Hall, 1995). Furthermore, during heating to decompose vegetation tissues an element may react with an organic or inorganic constituent to produce a volatile chemical species. Various ashing aids can be added to control chemical reactions. The complexity of potential chemical reactions during the heating phase is enormous and chemists can point to many combinations of physico-chemical parameters that could take place to render the ashing process an untenable option. Some of these are summarized in Hall et al. (1991) and Hall (1995). Fortunately, as will be shown in the following pages, the actual losses of elements are surprisingly consistent and quantifiable (for most species), confirming that ashing is a viable option. Consistency in the approach to ashing is, like so many situations in science, the key to obtaining meaningful results that the exploration biogeochemist can plot to reveal structure in the data that can be attributed to underlying geological conditions.

In order to minimize and standardize elemental losses, the ashing parameters need to be closely controlled. Ashing should take place by slowly ramping up the temperature of a muffle furnace (or kiln), dedicated to vegetation, to about 475 °C. It rarely makes any difference if a temperature of 500 °C is selected, but it is as well to be consistent, and 475 °C is about the lowest temperature at which vegetation will be reduced to ash without a long period of charring. If the temperature is maintained at 450 °C, most types of plant tissue go through a long phase as charcoal before all of the organic components are released. Increasing the temperature by just 25 °C to 475 °C greatly accelerates the ashing process.

Borosilicate glass beakers are suitable for ashing vegetation samples; although after repeated usage the glass becomes etched from reaction with the hot ash and a few ppm B is transferred from the borosilicate to the ashed samples. However, since B is commonly present in plant ash in hundreds of ppm, the slight addition of boron from the beakers does not usually significantly affect natural distribution patterns of boron.

Quartz vessels are reported to be unsatisfactory for dry-ashing of biological materials, because Zn (and perhaps other metals) has been found to penetrate the surface and cannot be readily extracted with acid (Spitzy and Dosudil, 1962). An alternative is to use large aluminium trays, since they are suitable for ashing moderately large samples (50–100 g) of dry tissue. They can be rinsed between batches of samples and they can be re-used for many years. Dry tissues of plants commonly

used in biogeochemical exploration contain tens to hundreds ppm Al. On reduction to ash, these concentrations are magnified 30- to 100-fold, depending on the type of tissue, so that the content of ash is frequently in the range of 0.1–1% Al. Thus, as in the case of B, the potential addition to samples of a few or tens ppm Al from contact with the Al trays is not significant for most purposes. Of course, other vessels should be used if low-level Al determinations are required.

Temperature control can be particularly important for a number of reasons, not least of which is that aluminium trays melt at around 550 °C. Also, at around this temperature, the carbonate component of the ash may start to dissociate releasing CO_2 (and possibly some higher temperature phases of metals contained within the ash), especially if the ash contains a magnesite component. Kovalevsky (1987) notes that the chemical composition of plant ash approximates that of dolomitized carbonate rocks and so plant ash can be treated as if it is a carbonate.

Tests on dry black spruce samples show that between 100 and 475 °C there is a weight loss of approximately 98% for twigs and outer bark scales and 97% for needles; between 475 and 700 °C there is a further loss from the 475 °C ash weight of 15–20%. As the temperature increase continues, additional weight losses occur as some elemental oxide bonds break down (e.g., PdO), and by the time 900 °C is reached all of the carbonate has dissociated releasing CO_2, and for some tissues a fused pellet has formed. Elements that show significantly higher concentrations in ash heated to 900 °C, mostly due to breaking of oxide bonds, include Al, Au, Ba, Eu, Ga, Ge, Li, Na, Pb, Pd, Yb and W. If ceramic crucibles are used for high-temperature ashing, some tissues are sufficiently reactive at 900 °C for the remaining ash to further complicate the situation by fusing with the crucible itself, introducing contaminants from the crucible.

The door to the muffle furnace or kiln should remain closed throughout the ashing process, because if it is opened a rush of fresh oxygen can cause partially decomposed samples to ignite. This sudden and localized increase in heat can cause differential losses of elements among the samples (because of differing temperatures of element dissociation) with the result that analytical results cannot be effectively compared. Furthermore, flash fires in the furnace can cause partial or even complete melting of some aluminium trays. In the former Soviet Union large scale biogeochemical exploration programmes sometimes used open fires to rapidly reduce samples to ash in the field, claiming that they were able to treat 200–1000 samples per shift and that the temperature of ashing ranged from 400 to 700 °C with ashing time ranging from 20 min to 4 h (Kovalevsky, 1987). Kovalevsky noted that

> the percentage losses of volatile elements for background and mineralized samples under standard ashing conditions are similar and have no effect on the main results of exploration: i.e., the shape, degree of contrast, and intensity of biogeochemical anomalies and haloes.

Given what is now known about element volatilization at increasing temperatures, these conditions, although expeditious in the field, are rather too semi-quantitative for

some elements and probably too much valuable multi-element information is lost by not closely controlling the ashing conditions.

Nonaka et al. (1981) determined losses of elements during dry ashing of standard reference material (SRM) orchard leaves and bamboo leaves, at stepwise temperature increases from 200 to 800 °C. Their principal findings were [with added comments in square parentheses] as follows.

- Loss of Hg began at 110 °C and increased steadily thereafter [studies using pine twigs yielded similar results, see Table 6-VI].
- [Partial] losses of As and Sb occurred at 200 °C.
- There was a sharp loss of Br, Cl, Cr and Se at 200 °C and again above 500 °C [this provides further strength to the argument that 475 °C should be a preferred temperature for ashing].
- There were no losses of alkaline earths, REE, or Al, Co, Fe, Mn, V and Zn.
- Losses of alkali elements were dependent upon the crucible material (e.g., severe in a silica dish) and sample species.

Once samples have been reduced to ash, care should be taken not to expose them to highly volatile elements such as Br, Cl, F, Hg, I and S, because ash is a good absorbent of gases (Kovalevsky, 1987). This phenomenon explains why some multi-element ICP-MS determinations of ash occasionally yield a few ppb Hg.

On occasion a perceived ashing loss can actually be an inadequate digestion of the ash, or the formation of poorly soluble oxide bonds that require high temperatures to dissociate. Also, apparent 'element losses' can be the result of element incorporation into the insoluble residue, which is dominated by silica in some plant species (Hoenig and de Borger, 1983), and the HCl and HNO_3 normally used to digest the ash do not dissolve silica nor the elements associated with it.

TABLE 6-VI

Loss of Hg from pine twigs (*Pinus banksiana*) on heating for 24 h at progressively higher temperatures (material comprising control V6). Analysis by ICP-MS after aqua regia digestion

Temperature (°C)	Hg (ppb)
Air-dried	40
70	40
80	40
110	30
150	30
200	<3

Although the ramping rate does not appear to be critical, an increase in temperature of about 100 °C per hour has proved to be appropriate and, depending on the amount and nature of the material to be ashed, the furnace is held at 475 °C for about 24 h. For small samples of leaf tissue 12 h is sufficient. For cones or large chunks of wood the ashing may take up to 48 h.

These examples serve to demonstrate the complexity of problems that can exist during reduction of plant tissues to ash at various temperatures. However, to reiterate a point made earlier 'in order to minimize and standardize elemental losses, the ashing parameters need to be closely controlled'.

Dry ashing – the realities

The last section outlined many of the potential problems that can arise from ashing samples. They appear sufficient in number and complexity that the explorationist might dismiss the idea of ashing. In hindsight, it is fortuitous that ashing was once the only feasible approach for determining concentrations of many elements, until the commercialization in the 1980s of multi-element INAA of pelletized dry tissues and, more importantly, the introduction by commercial laboratories over the past 10 years or so of multi-element analysis of small samples of dry tissue by ICP-MS. The historic necessity 'to ash' provided a large amount of information, and showed that, provided ashing takes place under controlled conditions, the biogeochemical signatures are remarkably robust. Modern comparisons of 'ash' versus 'dry' analyses of the same samples permit evaluation of concentrations (and attendant losses) of a wide array of elements.

Figures 6-7, 6-8 and 6-9 provide some examples of various *Acacia* tissues from Western Australia that were analysed both as 50 g samples reduced to ash (from which a 0.25 g portion was taken for analysis) and a 1 g sample of milled dry tissue. Both were digested in aqua regia with an ICP-MS finish. The data from analysis of the ash have been normalized to a dry weight basis by taking the concentration in ash, dividing by 100 and multiplying by the ash yield. These data are shown on the accompanying CD as Table 6-ID permitting the user to examine the relationship of ash versus dry for all 51 elements that were determined. The digital table also permits sorting into relative concentrations of different plant tissues. In the examples illustrated here, samples are sorted from lowest to highest concentrations, with the average for the dataset ($n = 53$) plotted as single symbols on the right of each chart. They illustrate three features.

- Figure 6-7 shows examples of elements that exhibit little or no loss from controlled ignition to 475 °C. The profiles are almost identical. Gold gives somewhat erratic results, partly because it is not always all dissolved from dry tissue. This is discussed in the section on data quality.
- Figure 6-8 shows examples of elements that partially volatilize during ashing.
- Figure 6-9 shows elements for which data derived from the analysis of dry tissue are too close to the detection limit for data structure to be determined. By reducing

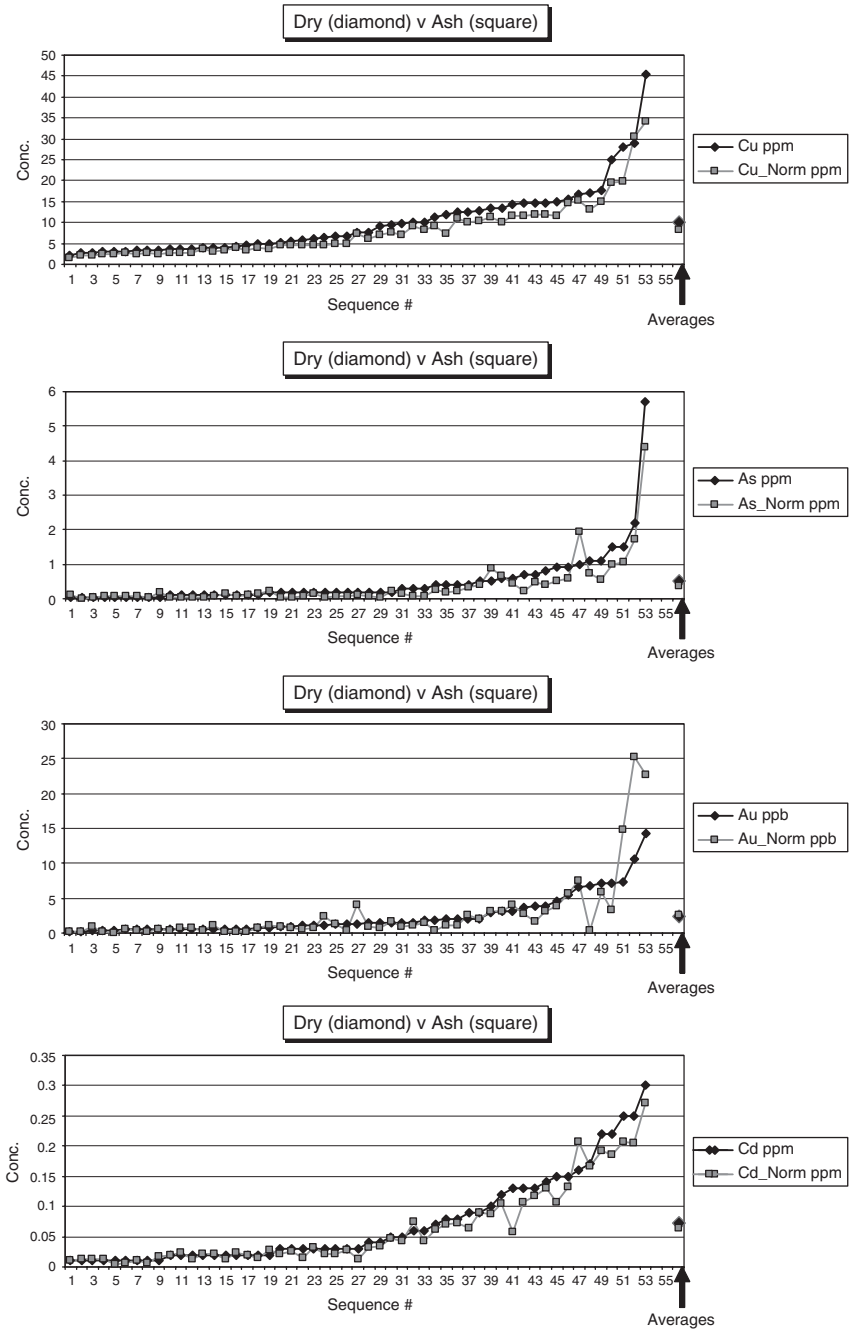

Fig. 6-7. Examples of elements showing little or no loss on ignition. Comparison of element concentrations determined by ICP-MS (aqua regia digestion) in dry *Acacia* tissues compared to ashed portions of the same samples. Data normalized to dry weight basis (details on CD – Table 6-ID).

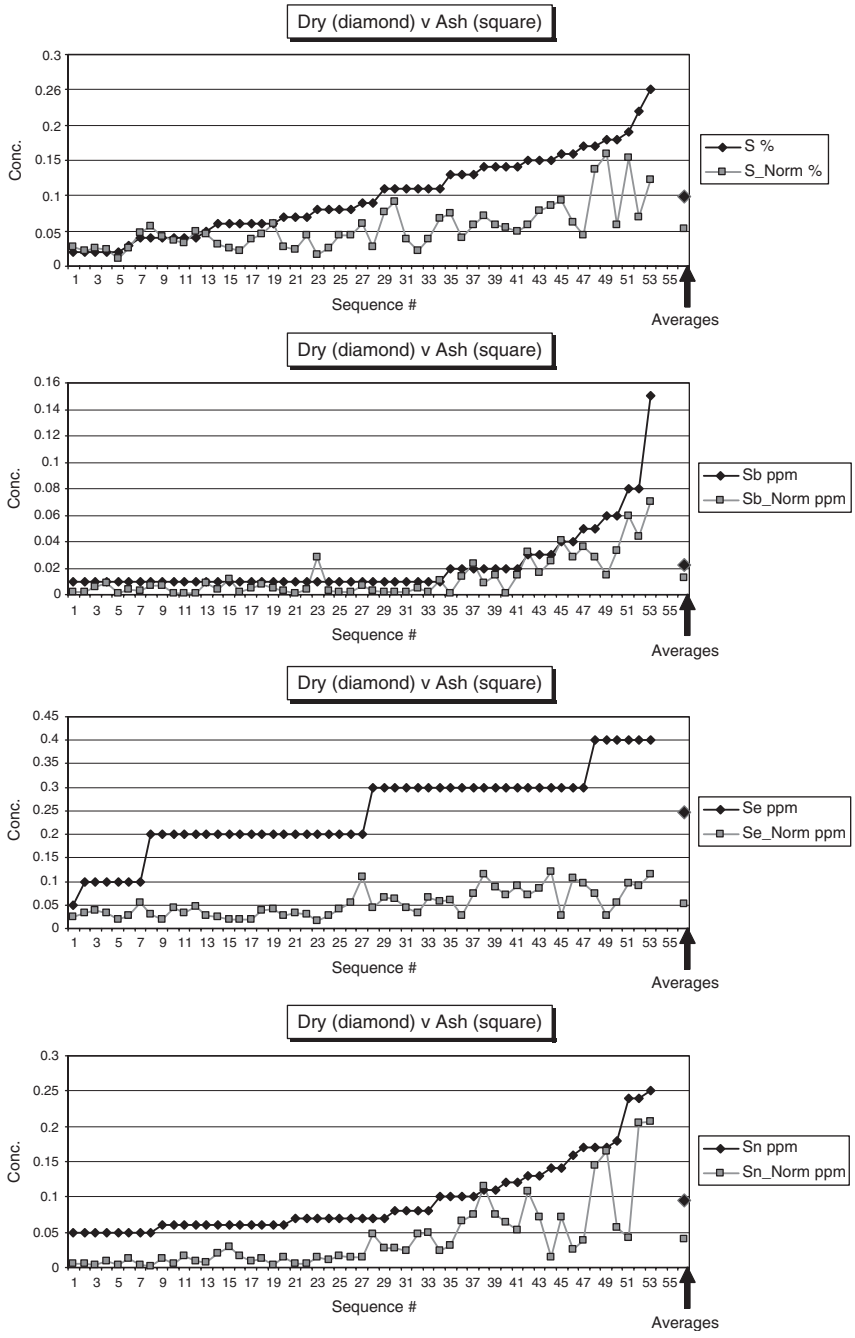

Fig. 6-8. Examples of elements showing moderate to significant loss on ignition. Comparison of element concentrations determined by ICP-MS (aqua reiga digestion) in dry *Acacia* tissues compared to ashed portions of the same samples. Data normalized to dry weight basis (details on CD – Table 6-ID).

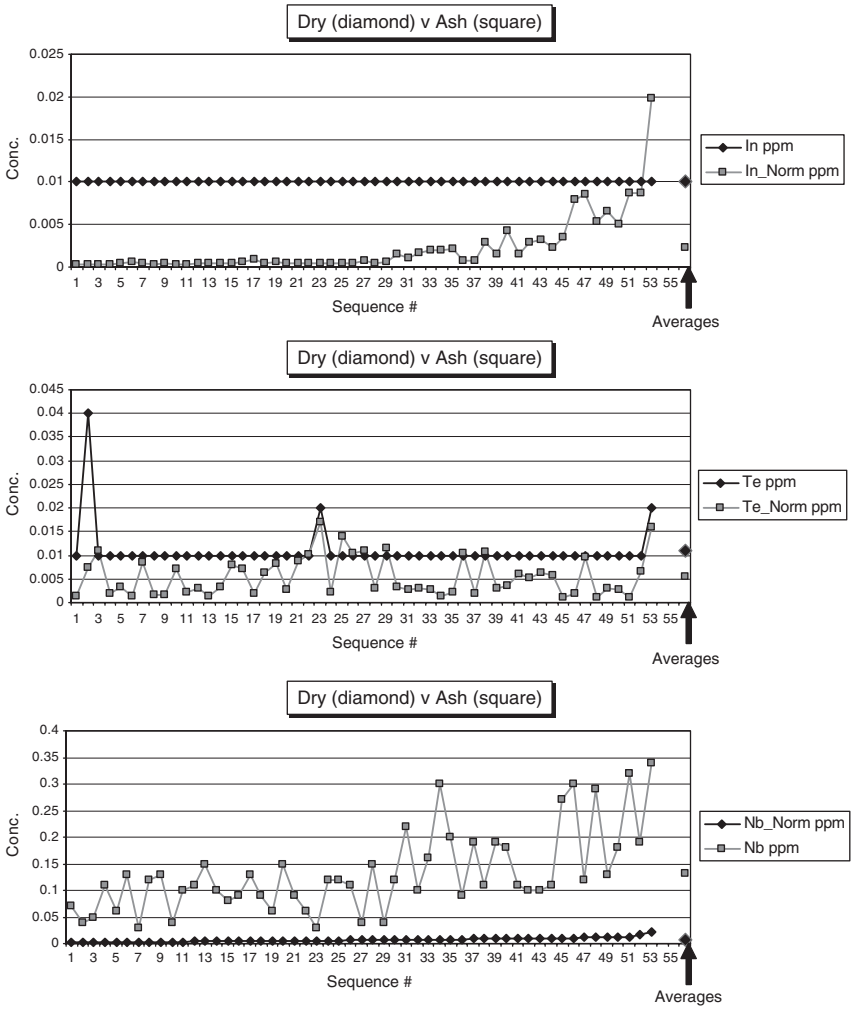

Fig. 6-9. Examples of elements best determined on ash. Comparison of element concentrations determined by ICP-MS (aqua regia digestion) in dry *Acacia* tissues compared to ashed portions of the same samples. Concentrations in dry tissue (diamond symbol) are either all or mostly below the detection limit, plotted here at half the detection limit. Ash data are normalized to dry weight basis (detailed spreadsheet on CD – Table 6-ID).

the samples to ash prior to analysis, elements are concentrated to levels above the detection limit and the data structure can be observed. Commonly, this exercise reveals data distribution patterns that can be helpful for focusing on exploration targets. Elements that are typically at or close to the detection limit by ICP-MS, and for which ashing may provide valuable insight, include Be, Bi, Ga, Ge, In, Nb, Pd, Pt, Re, Se, Te, Tl, U, V and W. Gold and As data can be quite imprecise at low levels in dry vegetation; therefore these two elements can be added to this list.

Losses of elements during ashing may vary according to the plant species. Girling and Petersen (1978) showed that in plants containing cyanogenic glycosides (e.g., those of the prunus and rose families) the Au volatilizes as Au cyanide well before the normal temperature of ashing is attained. Most plants used in biogeochemical prospecting do not contain cyanogenic compounds.

Ashing to 475°C results in the loss of some Cr in species such as *Acacia* (Table 6-ID on CD), whereas many plants of the boreal and temperate forests lose much of their Cr content. Figure 6-10 shows a comparison of Cr in pine bark samples (*Pinus contorta*) from British Columbia. More than 70% of the Cr volatilized during ashing, yet the sites of enrichment are the same, and in fact the correlation coefficient (r) between the two datasets is 0.965 ($n = 55$). Fortunately for prospecting, this strong relationship between patterns derived from the analysis of ash and those from the analysis of dried tissue are typically very similar for most elements, attesting to the robustness of the biogeochemical method.

Much of the advice given by Kovalevsky (1987) on ashing and analysis was based upon his many years experience and tens of thousands of samples, and remains sound and well worth heeding. However, it is 20 years since his last book in English was published, during which time considerable advances in our understanding have been made as multi-element analytical technology has surged forward. In fact Kovalevsky (1987) states that

> it should be appreciated that analytical methods are improving rapidly … analyses of unashed plant material are at present used to a small extent due to insufficient sensitivity for determining background and minimum anomalous contents of most indicator elements.

The exploration biogeochemist now has available the technology to determine most elements in dry tissue, and only a few ultra-trace elements still need to be determined on ash.

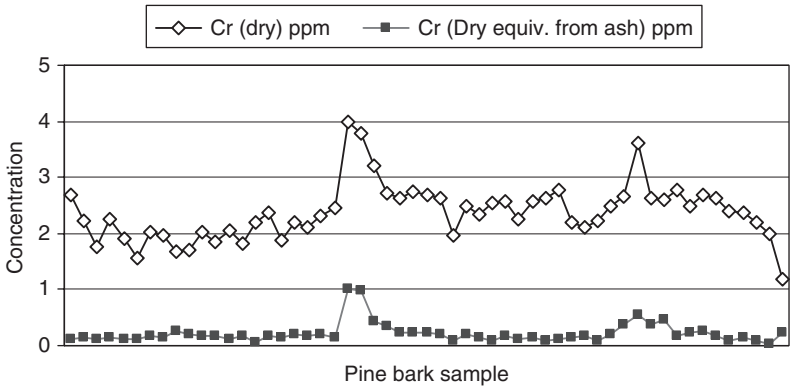

Fig. 6-10. Chromium in dry lodgepole pine bark (*Pinus contorta*) compared to bark ash normalized to dry weight.

A SPECIAL CASE: VEGETATION FROM SITES NEAR SMELTERS OR OTHER SITES OF POINT-SOURCE METAL EMISSIONS

As already stressed, when using ashed plant tissues for biogeochemical exploration it is important that, for meaningful interpretation of survey data, there are fairly consistent losses of each element. Near mine sites, smelters and other point-source sites of metal emissions, when compared to areas remote from such locations, these losses on ignition are greater. This may be because during roasting of ores the relatively volatile fractions dominate the emissions from the stack and settle on the ground and vegetation. During reduction to ash of plant tissues from such an environment, the surface particulates that may be complexed as relatively volatile compounds are released more readily than elements absorbed through root systems (Dunn et al., 2002).

Two sites were studied near the Giant Mine, located in an area of discontinuous permafrost, in Yellowknife, Northwest Territories, Canada. From 1948 until 2004 when it was decommissioned, the Giant Mine produced 200,000 kg of gold. The refractory ore, containing arsenopyrite, was mined from underground and roasted to facilitate the recovery of gold. Over 50 years of operation resulted in the accumulation of more than 265,000 tons of arsenic trioxide-bearing dust from the roasting process which was stored in underground chambers (Thompson and Schultz, 2001). These authors note that the As_2O_3 dust included the following.

- Arsenic: 36–67%;
- Antimony: 0.30–2.13%;
- Iron: 0.78–2.62%;
- Gold: 2–80 (ppm), averaging 15 ppm Au.

Dr. D. Kerr at the Geological Survey of Canada (GSC) initiated a project to assess the effects of the mine operations on the local environment and bulk samples were collected from two sites in accord with established protocols (Dunn et al., 2002). Previously, Dave Nickerson (personal communication) had established the extent of As and Au enrichments down-wind from the mine (Fig. 6-11).

Two sites for the GSC study, both underlain by metavolcanic rocks, were selected for the collection of bulk samples of vegetation. Site A was located 1.5 km north of the Giant Mine mill north of Yellowknife and Site B was approximately 250 m north of the mill. At each site collections comprised two of the most common plant species that occur in the boreal forests – black spruce (*Picea mariana*) and Labrador tea (*Ledum groenlandicum*). Each collection consisted of 1 kg of outer bark scales from several black spruce trees and 1 kg of Labrador tea stems from several shrubs. These were sent to Activation Laboratories Ltd. (Ancaster, ON, Canada) for sample preparation, processing and analysis for 35 elements by INAA of dried and ashed tissue, washed and unwashed. The analytical scheme is summarized in Table 6-VII.

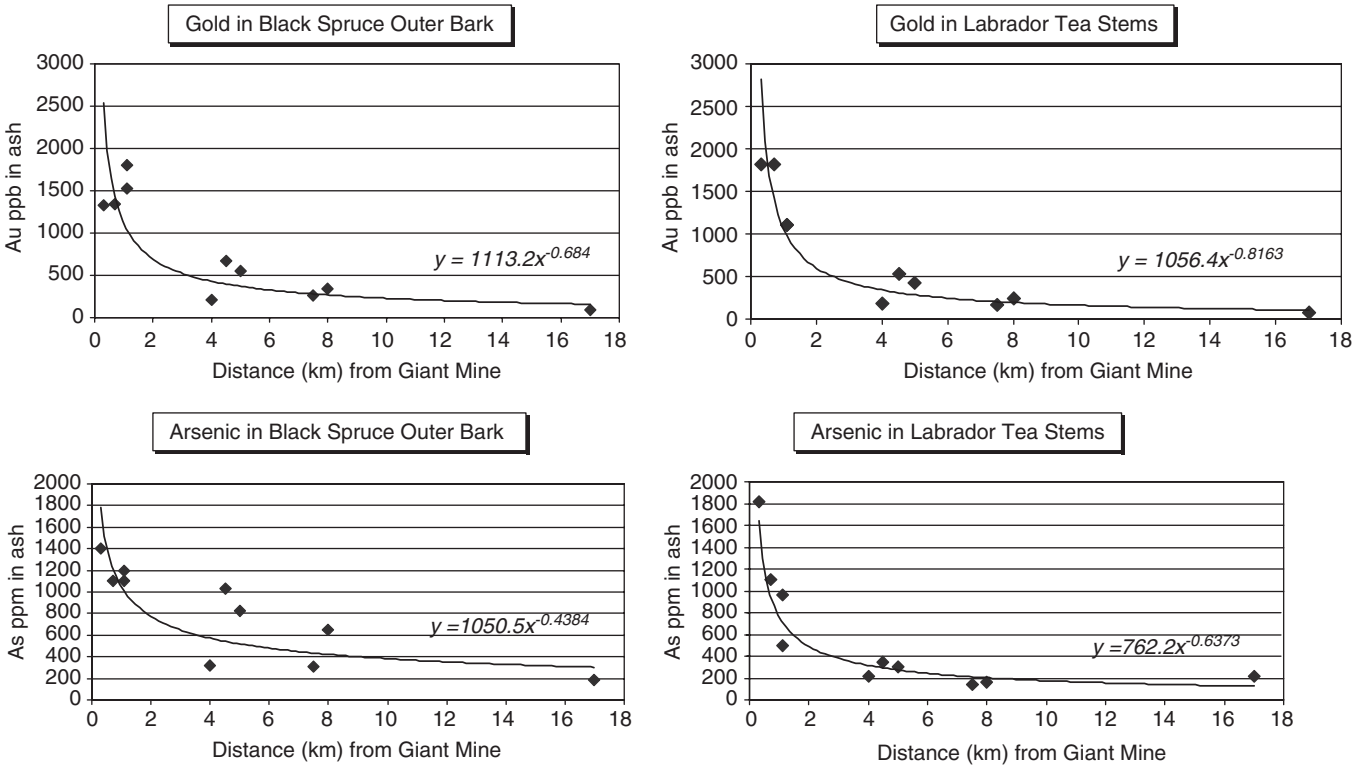

Fig. 6-11. Gold and As in spruce bark and Labrador tea stems. Changes in content with increasing distance from Giant Gold Mine and mill.

TABLE 6-VII

Protocols for preparation and analysis of samples by INAA (Giant Mine)

	Step 1 (subdivided)	Step 2	Step 3	Step 4 INAA ($n = 5$ sub-samples of each prepared sample)
Site A (1.5 km north of mill)				
Black spruce bark	For washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
	No washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
Labrador tea stems	For washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
	No washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
Site B (~250 m north of mill)				
Black spruce bark	For washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
	No washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
Labrador tea stems	For washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash
	No washing	Milled	Direct analysis	15 g pellets (dry)
	Milled		Reduced to ash	1 g ash

The data obtained from these samples provided insight into elemental losses during sample washing and ashing, from sites affected by more than half a century of mining. Among the observations, some trends were apparent with respect to the principal elements associated with the As_2O_3. These are summarized in Table 6-VIII.

From the data in Table 6-VIII there are several features of note.

- There is considerable enrichment of Au, As, Fe and Sb in all samples.
- As would be expected, samples from close to the mine yielded higher concentrations than those from a distance of 1500 m.
- Unwashed samples yielded greater losses of Au, As and Fe from ashed tissue near the mine than from a distance of 1500 m, implying that surface particulates derived from the mine are relatively volatile.
- Washed samples showed a similar trend of more As loss from ashed tissue near the mine than from the more distant site; however, Au losses were similar at both sites.

In summarizing the Giant Mine study it was noted that during ashing Hg volatilizes completely and some 80–90% of Br is lost. For many elements the amount of loss is within the general range of 20–40%, with a few elements (Ba, Ca, Rb, Sr

TABLE 6-VIII

Metal losses from black spruce outer bark (*Picea mariana*) at two sites near the Giant Au mine, NWT, Canada. Each value is the mean of five determinations by INAA

	250 m N of mine			1500 m N of mine		
	Mean (dry)	Ash recalc. to dry	% loss	Mean (dry)	Ash recalc. to dry	% loss
Unwashed						
Ash yield (%)		6.40%			5.5	
Au (ppb)	210	122	42	97	68	30
As (ppm)	644	294	54	122	77	37
Fe (%)	0.366	0.21	42	0.252	0.17	34
Sb (ppm)	81	53	35	16.8	10.5	38
Washed						
Ash yield (%)		4.80%			5%	
Au (ppb)	111	79	28.7	80	56	29.8
As (ppm)	412	235	43	126	83	33.9
Fe (%)	0.193	0.15	23	0.217	0.15	32
Sb (ppm)	71	46	36	17.2	9.7	43.5

and Zn) indicating apparent slight gains that are may be attributable to some imprecision related to instrumental calibration of the two methods of INAA – dry pellets and ash.

The implications for biogeochemical exploration are that close to sites of mining activities, especially where ore-roasting or smelting has taken place, the natural biogeochemical signature from a mineral deposit may not be discernible from the superimposed imprint of an anthropogenic signature. Consequently, the signature from any undiscovered mineral deposit may be masked. In the case of the Giant Mine, no biogeochemical study was undertaken prior to mining operations, and so the extent of any naturally occurring anomalous concentrations of metals is unknown. A survey area should be widely reviewed for past and present activities before a decision is made to undertake a biogeochemical exploration survey. It might be possible to devise a procedure for separating out the natural signature, but this is rarely a simple and reliable exercise. Procedures such as normalizing biogeochemical data to 'conservative' elements, as can be done with lithogeochemical data, are invariably too imprecise, because many of the lithogeochemically conservative elements can be absorbed to varying degrees by certain plant tissues. It is not sufficient to invoke, for example, any of the high field strength elements (HFSE – e.g., Hf, Nb, REE, Ta, Ti, Zr) against which to normalize data for dust contamination, because many studies have indicated that these elements can on occasion be absorbed and even utilized by some plants (notably REE in ferns). They are not, therefore,

'immobile' in the lithogeochemical sense of the word. That said, an examination of the HFSE content can provide an indication of whether a biogeochemical dataset might be contaminated from dust particulates, but this is usually difficult to quantify.

The study emphasizes the importance of washing samples from close to point-source areas of metal emissions, always bearing in mind the caveats expressed in the section on 'washing' in that some of the natural signature may be removed. It shows, too, that the relatively high loss on ignition of elements associated with such point-source emissions suggests that they are present in the environment as the volatile phases. During ore-roasting the volatile phases dominate the particulates that eventually precipitate on the ground and plant surfaces. Since they are mobile, they are the first components to be absorbed and/or adsorbed by plant tissues and subsequently they are the first to be released during ashing.

WET DECOMPOSITION

Hall (1995) provided a detailed and comprehensive account of methods of wet chemical decomposition and the remainder of this chapter leans heavily upon the information that she supplied, supplemented with data from personal observations and data compilations. Additional information has been extracted from Gorsuch (1970), Bock (1979), Fletcher (1981) and Batley (1989). Hall (1995) notes that, following ashing, a sample is normally digested in a dilute acid such as HCl or HNO_3 or, more vigorously, in aqua regia. The dissolution of silicate material (which is a structural component of some plants such as horsetails (*Equisetum spp.*), bamboo and grasses) requires evaporation with HF whereupon SiF_4 volatilizes. Hot aqua regia is an effective solvent for numerous sulphides, arsenides, selenides, sulphosalts, simple oxides and their hydrates, Ca phosphates and most sulphates (except barite). Evaporation to dryness with HCl converts salts to chlorides, ready for final solubilization in dilute (0.5–1 M) mineral acid which is compatible with the analytical technique. In the previous section it was noted that the process of ashing changes the forms of the elements and, rather than *facilitate* subsequent solubilization, it can *hinder* it (e.g., Pd). This explains why some element determinations by a 'total' method (XRF or INAA) may yield higher results than by an acid digestion from which some elements may be only partially dissolved.

If an exploration programme requires total dissolution of all elements, a stronger oxidant can assist in this process. Those most commonly used are high purity hydrogen peroxide (H_2O_2) or perchloric acid ($HClO_4$). Hydrofluoric acid (HF) is required for bringing the silica component of plant tissues into solution, but care must also be taken to avoid losses by volatilization when evaporating off acids and chemical species such as SiF_4. The valency in which the element is present plays a role; for example, the halides of As (III) are much more volatile than those of As (V) and thus pre-oxidation in the acid mixture prior to evaporation is necessary. Low recoveries

may sometimes be explained by adsorption or coprecipitation of the analyte on to the solid material remaining after digestion.

For non-exploration oriented decomposition of organic matter, nitric acid is the most widely used as it reacts readily with both aromatic and aliphatic groups. The normal acid boils at about 120 °C; a factor which assists in its removal after oxidation but which correspondingly limits its effectiveness. Thus, nitric acid is used in the presence of sulphuric acid which partially degrades the more resistant material, or with perchloric acid (boiling point 203°C) which continues the oxidation after nitric acid has been removed. Although sulphuric has similar properties to perchloric acid, it has not found such widespread application probably due to the interference effects created by SO_4 in AAS and to the low solubilities of alkaline earth and Pb sulphates. Care must be exercised in using perchloric acid as it is such a powerful oxidising agent and explosions have occurred. Arafat and Glooschenko (1981) reported complete recovery for As, Al, Fe, Zn, Cr and Cu in various plant tissues using 0.5 g samples and a mixture of 10 ml of HNO_3, 5 ml of H_2SO_4 and 2 ml of 70% H_2O_2. Haas and Krivan (1984) successfully determined As, Bi, Cd, Cr, Hg, Pb, Sb, Se and Tl in lichens, pine needles and grasses using a mixture of HNO_3, HCl and H_2O_2.

Adeloju (1989) compared the efficacy of wet digestion and dry ashing methods for voltammetric analysis of leaves. He found the direct dry ashing method without an ashing aid proved to be the most suitable for the determination of Bi, Cd, Co, Cu, Pb, Ni, V and Zn, but As and Se required the incorporation of an ashing aid or wet digestion with HNO_3 and H_2SO_4.

MICROWAVE DIGESTION

It needs only a brief review of the literature on methods to decompose plant tissues to realise that the chemistry involving wet decomposition can be complex and fraught with potential problems. Pressure digestion in closed or semi-closed vessels placed in industrial quality microwave ovens lowers the risks of contamination from the atmosphere and of volatilization losses in addition to decreasing the amount of reagents necessary (hence lower blank levels and better detection limits). Decomposition times are reduced to minutes as reactions carried out at elevated pressures and temperatures require considerably less time to reach completion. Moreover, substances that ordinarily would not be decomposed by these acids at their normal boiling points react at elevated temperature and pressure. Some of the pioneering work in this field was reported by Kingston and Jassie (1986), Matusiewicz and Sturgeon (1989), Matusiewicz et al. (1989), Bettinelli et al. (1989) and Sah and Miller (1992). A brief review and details of procedures are given in Miller (1998).

Despite the encouraging results of these early workers and subsequent researchers, especially using PTFE (Teflon) 'bombs', microwave ovens are still not in

common use for digesting exploration-oriented biogeochemical samples. The primary reasons seem to be

- Cost: the pressure resistant vessels are expensive.
- The limited capacity of most ovens compared to the many beakers which can be placed on a hot plate. This becomes a critical consideration for time-sensitive exploration activities, since a quick turn-around from sample collection (commonly many hundreds of samples) to results for follow-up work can be of paramount importance.

Microwave decomposition has distinct advantages for the dissolution of dry vegetation where maintaining both a minimum sample-to-reagent volume ratio and contamination level to optimize detection capability is particularly advantageous when elements of interest are present at low levels.

Many tests have been conducted on the decomposition of dry plant tissues, resulting in voluminous literature in chemical journals and textbooks on the preferred techniques to be employed in research laboratories. Now that modern analytical instrumentation is capable of detecting very small amounts of many elements in dry plant tissue from a single digestion, tests on ashed tissue are largely, but not entirely, of academic interest. There remains the suite of elements discussed above, present at sufficiently low levels that reduction to ash is required to determine 'relief' in the geochemical data. These elements include Be, Bi, Ga, Ge, In, Re, Te, Tl, U, V, W, many of the REE and all of the platinum-group elements.

SELECTIVE LEACHING

> In order to pursue the use of biogeochemical sampling in mineral exploration to the fullest extent, we must attempt to understand the mechanisms of accumulation and translocation taking place in the species under investigation … We should be designing experiments to answer such questions as: (1) What form(s) of element X is preferentially taken up by this species? (2) Where does the element accumulate in the plant? (3) How does the element exist in the plant, in which forms in which organs? (Hall, 1995)

Examples of some of the early attempts to address these questions for issues affecting biogeochemical exploration include the following.

- Girling and Peterson (1978) examined the effect of the form of Au on its uptake in a range of species (e.g., *Phacelia sericea*, *Hordeum vulgare*) and found the cyanide complex to be absorbed to a much greater degree than the chloride or thiosulphate form. Autoradiography was employed to identify the location of Au accumulation within the plant and the study established the acropetal tendency (movement toward growing tips) of Au with enrichments occurring toward leaf tips.

- A study using electrophoresis examined the influence of humates on the Au uptake by perennial ryegrass (Jones and Peterson, 1989). Both complexed and ionic forms of Au were found to exist in the humic acid solution and the uptake of this Au depended upon (1) humic acid concentration; (2) pH; and (3) size fraction (e.g., more uptake from unfiltered solutions). Far less Au was absorbed from the humic solutions than from solutions of $AuCl_4^-$.
- Nickel in hyperaccumulator plants is bound to citric, malic and malonic acids and their derivatives. This association was found by Brooks (1983) in a study of Ni in *Alyssum serpyllifolium* from the Iberian Peninsula; more than half the Ni was soluble in water and dilute acid, indicating its presence also as polar complexes.

There remains a gargantuan task that is beyond the scope of this book to discuss the many answers provided, yet many questions still remaining, since Hall's suggestions were made. Progress is steadily being made at many institutions, and key results are published in many professional journals (e.g., Biogeochemistry; Chemosphere; Science of the Total Environment, to mention but a few). Adriano (2001) provides a comprehensive review of trace elements in terrestrial environments, and the publication 'Biogeochemistry' (Schlesinger, 2005) delves deeply into the biogeochemistry of organic compounds, and every two years since 1990, a wealth of research is summarized as extended abstracts in the Proceedings volumes of the International Conference on the Biogeochemistry of Trace Elements (ICOBTE). The web page www.isteb.org/default.asp?id = 14&lid = 2 provides details of these conferences and their respective multitude of abstracts, some of which are directly relevant to biogeochemical exploration, but many deal with the fine points of biogeochemical processes in many environments – including plants, animals, soils, waters, atmospheric combustion and bio-solids in general.

A comparison of vegetation samples leached by aqua regia (AR), pH 7-controlled ammonium acetate (AAc7) and distilled water provides further insight to the degree by which elements are bound within plant tissues (Dunn et al., 2006a). Whereas the amount of each element extracted by the aqua regia leach is for most elements, as would be expected, considerably greater than from the weak leaches, the relative amounts of each element extracted remain much the same, such that the *patterns* are consistent. Again, it is the *relative* concentrations that are of interest. In the case of Ni (Fig. 6-12) the patterns are almost identical.

Highly soluble elements, such as Rb are mostly extracted by just a simple water leach. Figure 6-13 shows that more than 80% Rb is removed by the water leach.

Another selective leach test compared three weak leaches – distilled water, dilute [2%] nitric acid and pH 7-controlled ammonium acetate. Two types of tissue from the same location were tested – Douglas-fir needles, and lodgepole pine outer bark. For some elements (e.g., Rb – Fig. 6-14) the amount extracted by each leachate was virtually identical.

In general there is little difference in the amount of each element extracted by a water leach and a dilute (2%) nitric acid leach. The AAc7 leach extracts higher

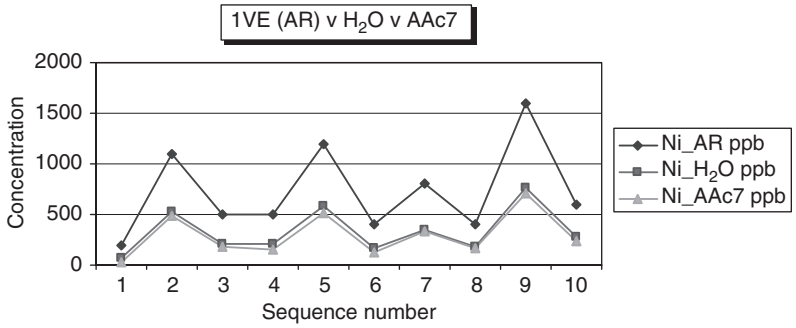

Fig. 6-12. Nickel in dry fir needles determined by ICP-MS after three types of leach – one strong acid digestion (nitric acid/aqua regia) and two weak digestions (water and pH 7-controlled ammonium acetate).

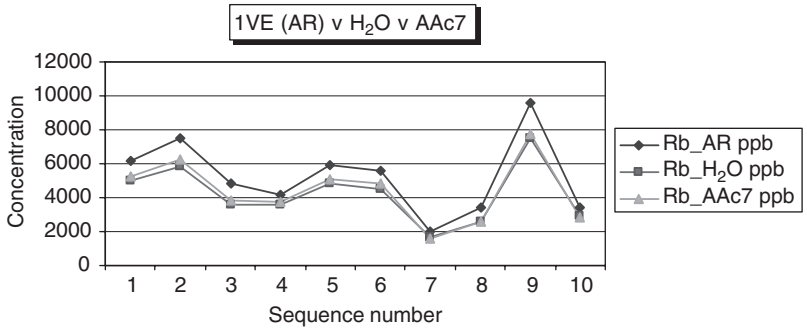

Fig. 6-13. Rubidium in dry fir needles determined by ICP-MS after three types of leach – one strong acid digestion (nitric acid/aqua regia) and two weak digestions (water and pH 7-controlled ammonium acetate).

quantities of some elements but less of others. For each leach, the detection limit varies. Figure 6-15 shows similar amounts of Cu extracted by the three leaches, but with the AAc7 leach extracting a little more from the fir needles than from the pine bark. Comparative data for these leaches are presented for approximately 50 elements on the accompanying CD in the 'Halogen Study'.

This observation demonstrates that for optimal metal extraction by weak leaches, there is the need to select an appropriate leach for each individual plant species and plant tissue. However, also of importance for biogeochemical exploration is the fact that the relative amounts of each element extracted remain much the same – i.e., the patterns of relative concentrations are very similar. This is evident from the plot of zinc in the two tissue types by the three leaches (Fig. 6-16), with the AAc7 yielding more Zn than the other two leachates, but with appreciably more extracted from the bark than from the needles. Note, however, that the element profiles across the

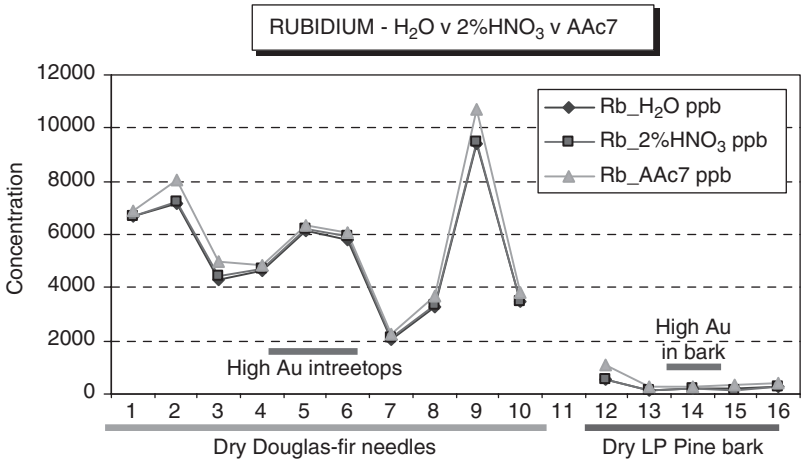

Fig. 6-14. Rubidium extracted from fir needles and pine bark by three weak leachates – water, dilute nitric acid and ammonium acetate (pH 7). Determinations by ICP-MS. Note the virtually identical amounts extracted by each dilute leach.

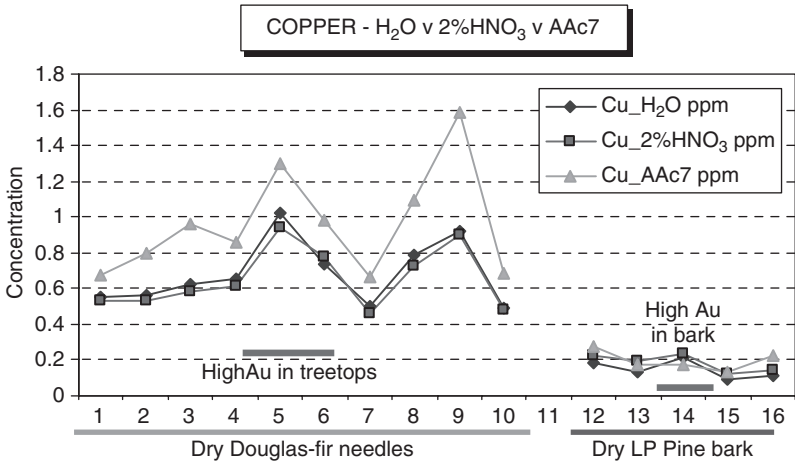

Fig. 6-15. Copper in fir needles (left) and pine bark (right) extracted by three weak leachates. Determinations by ICP-MS.

sampling profile remain almost identical, so any of the leaches would provide the same patterns of element distributions, but different absolute concentrations.

For other elements, such as potassium, the AAc7 leach extracts proportionally far less K from the needles than from the bark (Fig. 6-17).

Each element has its own characteristics, and there is a wealth of information that can be extracted from the digital data comprising part of the 'Halogen Study' on the CD that accompanies this book.

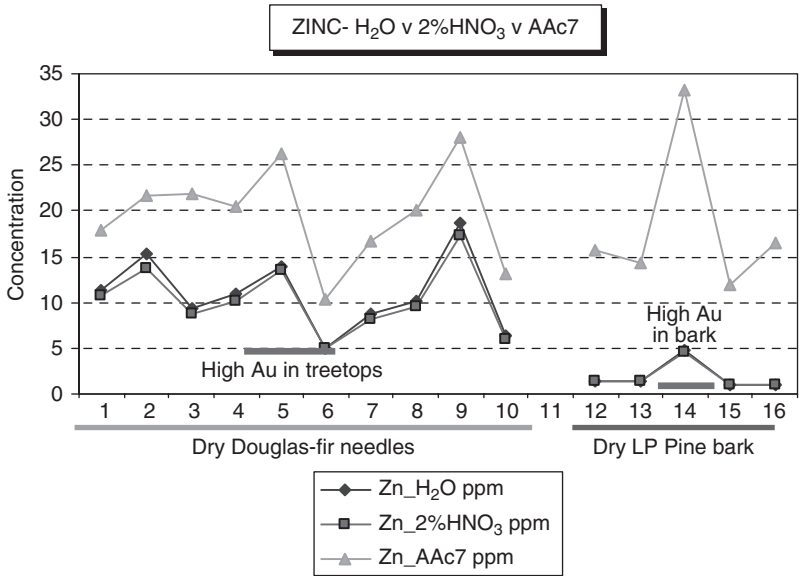

Fig. 6-16. Zinc in fir needles and pine bark by three weak leachates. Determinations by ICP-MS.

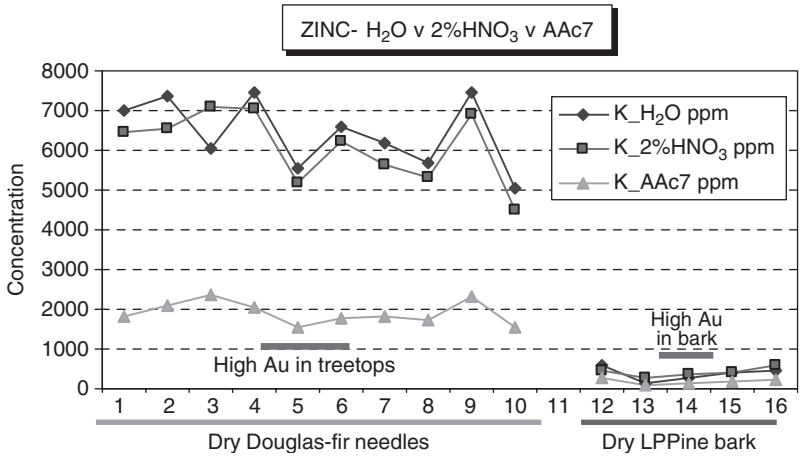

Fig. 6-17. Potassium in fir needles and pine bark extracted by three weak leachates. Determinations by ICP-MS.

The results from these tests have an important bearing on the decision of whether or not samples should be washed prior to drying and analysis. Clearly, intense washing will remove a portion of elements contained within the plant structure. If any washing seems necessary, only gentle *rinsing* should be performed.

FUSIONS

In the exploration for kimberlites, some of the HFSE can be useful 'pathfinder' elements (e.g., Nb, Ta). Some are only partially extracted from an aqua regia leach, and a fusion with a flux such as lithium metaborate is required to facilitate bringing them into solution.

Hall (1995) states that the great efficiency of fusion compared to acid attack is due to the effect of the high temperature (500–1100 °C). Heterogeneous reactions taking place in the melt are of two types: acid–base and oxidation–reduction. Alkaline flux reagents include Na and K carbonate and bicarbonate, Na and K hydroxide, and sodium tetraborate; acid fluxes include Na and K hydrosulphate, Na and K pyrosulphate, boron trioxide and hydrofluoride. Oxidative reagents comprise largely Na_2O_2, KNO_3 and $KClO_3$ while carbonaceous substances such as flour and starch are added to flux mixtures for a reducing action. The drawbacks of a fusion are: (1) the potential addition of contaminants due to the high flux:sample ratio (3:1–10:1); (2) the high salt concentration introduced and subsequent need for a higher dilution factor; and (3) the difficulty in streamlining the operation for high throughput.

Hall et al. (2001) conducted a study using ashed samples to determine the differences found in elemental concentrations when the common HF–$HClO_4$–HNO_3–HCl digestion and lithium metaborate fusion ($LiBO_2$) were applied. The 118 samples for which both datasets are available comprised mostly twigs of paper birch (*Betula papyrifera*), green alder (*Alnus rugosa*), speckled alder (*Alnus crispa*), balsam poplar (*Populus balsamea)*, balsam fir (*Abies balsamea*) and trembling aspen (*Populus tremuloides*). The agreement between the HF and fusion methods was extremely good for Co, Cs, Ga, Rb, Sr, Th, U, V and the REEs represented by La and Ce. Given that sample weights used were 0.5 g and extremely different decompositions were employed in different laboratories with different ICP-MS instruments and procedures, this agreement is quite remarkable. However, Hf and Zr by the HF-acid digestion were significantly low (and noisy), at 0.62 ± 0.68 ppm (*cf* 4.56 ± 0.43 ppm by fusion) and 25.8 ± 25.0 ppm (*cf* 173 ± 24 ppm), respectively.

The two sets of results for the ashed vegetation corroborated the similarities and differences found for the control V6. For Cs and Sr in the mixed tissue vegetation the agreement between the two methods was excellent, with slopes of the regression lines close to unity and R^2 values of 0.80 for Cs and 0.97 for Sr. The data for Nb were rather noisy as many values were close to detection limits, although there was no obvious bias. Results for the ashed vegetation for Hf, Zr and probably Ta were low, and randomly so, by the HF-acid digestion.

The low results by acid digestion for Hf, Zr and Ta were also evident in another project that focused on the use of vegetation for diamond exploration (Seneshen et al., 2005). This low recovery may be due to (1) refractory mineral matter intimately associated with the ash itself, and/or (2) formation of 'insoluble' forms of these elements during the ashing procedure. It is unlikely that Hf and Zr would exist in

'insoluble' forms within the dry vegetation unless they were contained within an inorganic dust component.

STANDARD REFERENCE MATERIALS AND ANALYTICAL CONTROLS

Prior to considering in Chapter 7 the instrumentation available for analysis of plant materials, controls upon analytical quality need to be discussed, because of their vital role in any analytical protocol.

There is an ever-increasing number of standard or certified reference materials (SRMs or CRMs), supplied by a wide array of institutions, which are suitable for assessing the accuracy of biogeochemical data. Ihnat (1998a) lists 16 major producers and suppliers of reference materials for elemental composition quality control in plant analysis, and notes that there are other (unspecified) distributors. In a separate 34 page table, Ihnat (1998b) lists the many elements in reference plant materials for which data are available, with concentrations sorted by increasing order of abundance. Included in Ihnat's lists are the two main suppliers of vegetation controls: (1) NIST (National Institute of Standards and Technology, SRM Program, Gaithersburg, MD 20899, USA), and (2) BCR (Community Bureau of Reference, IRMM [Institute for Reference Materials and Measurements], Brussels, Belgium). Other agencies that offer vegetation controls include the Japanese National Institute for Environmental Study (NIES No. 1 – Pepperbush leaves (*Clethra barbinervis*) NIES No. 7 – tea leaves; NIES No. 8 – seaweed *Sargassum*), and in China the National Research Centre for Certified Reference Materials produces a number of controls (e.g., GBW 07602 and 07603 – bush branches and leaves; GBW 07604 – poplar leaves [*Populus spp.*]).

A comprehensive list of reference control materials was compiled by the IAEA (International Atomic Energy Agency) in 2003, and can be viewed at web page www.naweb.iaea.org/nahu/nmrm/nmrm2003/index.htm. Another web page is www.comar.bam.de/pdf/comarflyer.pdf. Many of the controls that are listed are not of particular use for exploration biogeochemical purposes (e.g., pig kidney or bovine muscle) and many are specific to the food industry (e.g., cabbage, wheat, rice). Controls of particular relevance to biogeochemical exploration include:

- BCR controls such as beech leaves (BCR-CRM-100), spruce needles (BCR-CRM-101), white clover (BCR-CRM-402),
- NIST controls such as pine needles (NIST-SRM-1575a),
- The NIES controls from Japan, listed above,
- The GBW controls from China, listed above, and
- CANMET (Canadian Certified Reference Materials, Ottawa, ON, Canada) controls CLV-1 (black spruce twigs) and CLV-2 (black spruce needles). These two controls are from the uraniferous Cluff Lake area of northern Saskatchewan,

Canada. Although they are somewhat more enriched in a number of trace elements (notably U) than the average black spruce, they are appropriate for many studies in that black spruce is the most common tree species of the boreal forest.

Whenever possible, it is advisable to use an SRM of similar matrix to the sample medium that is the target of a biogeochemical exploration programme. So if leaves are the sample medium, a leaf SRM (e.g., NIST-SRM-1515, apple leaves, or BCR-CRM-100, beech leaves) would be a preferred control; if twigs are collected, a twig SRM (e.g., CANMET CLV-1) would be preferred. However, this is in an ideal world and there simply is not a wide enough array of control materials that have been characterized for their multi-element content. There is not, for example, a control for outer bark from conifers, and so in this case the twig SRM would be preferred to that of a leaf, since the composition of bark is closer to that of twigs than foliage.

There are limitations with regard to using SRMs.

- They are expensive. The cost of including a large number of SRM samples (e.g., one for each 20 survey samples) in a large exploration programme would be prohibitive.
- Each SRM is only 'Certified' for a limited number of elements. Suppliers of SRMs, such as NIST, generally provide 'Reference Values' for some elements, indicating that only a best estimate of the true value is available, but usually a level of uncertainty is assessed. In addition, there are 'Information Values' which are uncertified and have no uncertainty assessed.
- For many SRMs there are relatively few Certified and Reference Values, and some elements, both commodity metals and pathfinder elements, of interest to a mineral exploration programme may have only Information Values published or no values at all. Elements for which there are few or no data include Au, Be, Bi, Hg, PGEs, Re, Sb and Tl. Taking the NIST SRM 1575a pine needles as an example, Certified Values are provided for only nine trace elements and minor constituents; Reference Values are provided for an additional 10 trace elements and Mg; Information Values are given for two trace elements. Values for many additional elements are listed, but the degree of validity of the data is lower than in the above three categories. As an example, the official NIST listings show that five laboratories provided data for Au, all by INAA, yet returned average values (n from 2 to 9) of 0.56–2.6 ppb Au and so this is not a very useful 'control' on analytical data. For some trace elements, e.g., Re and platinum-group elements, no data are provided. Of the commodity and pathfinder short list shown above (Au, Be, Bi, Hg, PGEs, Re, Sb and Tl), only Hg has a Certified Value and only As has a Reference Value, and none fall into the category of Information Value.

Given the various limitations of SRMs with respect to biogeochemical exploration, it becomes necessary to use them sparingly. Published data provide an excellent guide as to the accuracy of the analytical data for a limited number of elements that are acquired from a set of biogeochemical survey samples. For reasons of economy,

SRMs need to be supplemented by a set of secondary reference samples for monitoring quality control (QC). Analytical laboratories typically develop their own bulk sample in-house control materials for all aspects of their analytical protocols. A suitable rock material will be used for monitoring lithogeochemical data; a suitable sediment for a soil or sediment survey; and a suitable vegetation medium for a biogeochemical survey. Some laboratories use, as vegetation analytical controls, such media as peat moss, flour or bulk samples of a common tree species. The laboratories characterize these secondary controls by reference to a few samples of an appropriate SRM. The result is high quality data that are closely controlled.

For an independent assessment of QC by the exploration biogeochemist, a bulk sample of a plant tissue can be collected from an appropriate field location, and, after drying, milling to a fine powder and homogenizing, portions can be inserted as 'blind' controls. The data shown on some of the ensuing pages are compiled from a bulk sample (designated V6) prepared using expedient measures that did not fully conform to the international SRM protocols. The procedure to prepare this material was to first collect a few tens of kilograms (sufficient for several years) of pine twigs from a convenient location near Ottawa, Canada, and pass it through a commercial 'chipper-shredder' of the type that can be used for shredding branches up to 2 cm in diameter. This shredded material was passed through a Retsch^{TM} mill to reduce the particle size to less than 1 mm. It was then homogenized in a proprietary rotating drum at the GSC, quartered several times, mixed at the CANMET laboratories and bottled. It has been used continuously for 15 years and analysed several thousand times – mostly by INAA, ICP-ES and ICP-MS. V6 is not commercially available, but the data obtained by several methods over the years provide an indication of the level of precision that can be expected from an in-house bulk control sample. The level of precision obtained is very close to that for analyses by ICP-MS for NIST 1575a (dry pine needles) inserted in the same batches as the V6 controls, using the same analytical procedures.

Compilations of the data from samples of V6 inserted as blind controls are included on the CD as tables

- Table 6-IID – INAA data on 10 g pellets of dry tissue.
- Table 6-IIID – INAA data on 0.5 g portions of ash.
- Table 6-IVD – ICP-ES (AR) data on 0.5 g portions of ash. The high variability of As exemplifies the low precision typically obtained at concentrations below 10 ppm As; over the years the laboratory changed the detection level for Ag; sensitivity to Pb varied.
- Table 6-VD – ICP-MS (HNO_3 + AR) on 1 g portions of dry tissue.
- Table 6-VID – ICP-MS (HNO_3 + AR) on 0.25 g portions of ash.

In the following chapters consideration of analytical quality is based primarily on analyses of dry V6 by ICP-MS, but with supplementary observations from other controls and analytical duplicates.

SUMMARY

This chapter has discussed the pros and cons of many options involved in the preparation of vegetation samples prior to their introduction into the analytical instrumentation. The literature on these topics is voluminous and the fine points pertain more to the chemists than the exploration biogeochemists. However, it is as well for the explorationist to be aware of these options and the potential pitfalls in order to optimize conditions and provide meaningful interpretation of the results that emerge from an exploration biogeochemical survey.

The wealth of information available can be summarized in just a few recommendations.

- *Washing*: Washing of samples from a non-dusty area creates another possible source of error, and a relevant subtle biogeochemical signature could be removed by vigorous washing. If samples are obviously dusty, they should be *rinsed* in running water. However, in most situations, sample washing is not a requirement.
- *Milling*: Samples should be milled to a fine particle size (at least <1 mm) that is as consistent as is practically attainable.
- *Ashing*: For most elements this is no longer a requirement, because modern analytical instrumentation (e.g., INAA and ICP-MS) is sufficiently sensitive to determine a wide range of elements on small samples of dry tissue. A temperature of 475 °C is recommended. Elements that are typically at or close to detection by ICP-MS, and for which ashing may provide valuable insight, include Be, Bi, Ga, Ge, In, Nb, Pd, Pt, Re, Se, Te, Tl, U, V and W. Gold and As can be added because data are commonly imprecise at low concentrations in dry vegetation. Some laboratories provide at nominal cost an optional ashing 'add-on' to a multi-element package to analyse dry tissue. This is well worth considering.
- *Digestion*: Aqua regia, preferably with a nitric acid pre-wetting, provides data for the greatest range of elements at the lowest cost.

This protocol generates robust data and spatial patterns with an excellent cost-to-benefit ratio. A recurring theme in this book is to remember to ask 'are the data fit for the purpose'? (Bettenay and Stanley, 2001). Given the low concentrations and mostly high precision obtained by modern analytical methods, minor imprecision can be tolerated without compromising the integrity of a survey.

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Chapter 7

PLANT ANALYSIS

This chapter provides a summary account of the principal methods used in the analysis of plant materials, so that the reader has a general understanding of the various options that are offered by commercial laboratories. Emphasis is on what the geologist/geochemist can expect to receive from a low-cost multi-element analysis now available at one of the many commercial laboratories that produce data of outstanding quality and value.

For those seeking information on sophisticated methods using state-of-the-art analytical equipment there is a wealth of literature and some excellent reviews of instrumentation available at 'User Facilities', i.e., state-of-the-art research facilities that have been specifically constructed and operated for use by the general scientific community (Sutton, 2006). This instrumentation is not generally available to the explorationist looking for good to excellent quality data on many elements at low cost. Concise reviews of 'User Facility' locations and instrumentation are given by Brown et al. (2006) and Sutton et al. (2006).

A recent overview by Ayrault (2005) gives descriptive accounts of the more widely used methods.

- Atomic absorption spectrometry (AAS),
- Inductively coupled plasma – atomic emission spectrometry (ICP-AES), sometimes referred to as ICP-ES (or, to the dismay of chemists simply 'ICP', because the ICP component of the description is just the power source, which is the same as that used for the more advanced generation of instrumentation, ICP-MS),
- Inductively coupled plasma-mass spectrometry (ICP-MS),
- Instrumental neutron activation analysis (INAA), and
- X-ray fluorescence *per se* is not described, since the principles are included in the description of Synchrotron X-ray Fluorescence (SXRF).

Ayrault (2005) also includes summaries of some advanced and very expensive instruments, such as SXRF and proton induced X-ray emission (PIXE). SXRF has huge potential for resolving many biogeochemical questions (e.g., results of an intriguing Se speciation study by Pickering et al., 2000), but since access to the synchrotron is not readily available to the explorationist it is not given further consideration in this chapter.

During the 1990s, the Association of Exploration Geochemists (now renamed the Association of Applied Geochemists) sponsored a number of short courses on

biogeochemical methods of exploration and published short course notes that included considerable details on analytical methods and techniques in chapters written by Gwendy Hall (Dunn et al., 1992c, 1993a, 1995a). In the following pages, she has graciously permitted the paraphrasing of many of her insightful publications, including Hall (1992) and a comprehensive chapter in the now out of print book edited by Brooks et al. (1995). In addition, there are useful compilations by Markert (1994, 1995), Kalra (1998) and Ayrault (2005).

Commercial laboratories employ published analytical protocols for many elements, and on request they are usually quite prepared to adopt any particular protocol. There are, amongst many other sources, protocols provided in 'Recommended Guidelines for Measuring Metals in Puget Sound Marine Water, Sediment, and Tissue Samples', published by the US Environmental Protection Agency, Seattle, WA. For those requiring very precise and accurate data, most commercial laboratories can offer such data commonly charged by single element, but the user should be prepared for a substantially larger bill than for the multi-element 'packages' that are offered. For most exploration purposes this extra charge is not warranted, since in the terms of the economist 'diminishing returns' set in.

On a point of terminology, 'analytical technique' refers to the analytical instrumentation used in the analysis of a substance, whereas 'analytical method' describes the complete scheme used to obtain the results, including the sample decomposition, any associated procedures and the instrumentation.

In addition to descriptions of the techniques, commentary is provided on personal compilations of many results obtained from the commercial laboratories that offer extraordinarily good value for multi-element analysis, using what has become the most valuable tool in exploration biogeochemistry – ICP-MS.

'FIT FOR PURPOSE'

In a way, this section is putting the 'cart before the horse' by posing this question prior to consideration of the various available analytical techniques. However, it is mentioned earlier in this book and the sooner this concept is entrenched in the philosophy of an exploration programme the better. At an early stage of developing a biogeochemical exploration survey (or any geochemical exploration survey) consideration should be given to the choice of an analytical programme and what is expected from that particular survey. Is there a need to get ultra-trace levels of many elements at the best possible levels of accuracy and precision, regardless of cost? Or can a compromise be made between these important parameters and cost? Analytical protocols are available for individual element determinations, but at huge cost if data for many elements are required.

All too often, the explorationist who submits samples expects to receive data by ICP-MS that are all consistently accurate and precise from a package comprising results for 50–60 elements for a cost of less than $30 from Canadian laboratories in

2006. For many elements the precision and accuracy by such packages are remarkably good and consistent, but for some elements there is greater variability. To keep the cost versus data quality issue in perspective, it is worth reading a thought-provoking article entitled 'Geochemical Data Quality: The 'Fit-for-Purpose' Approach' by Bettenay and Stanley (2001). The following is extracted from that article.

> A (preferred) strategy is to assess data quality in terms of whether it is suitable for the task at hand. This we will call the 'Fit-for-Purpose' approach … we contend that real-world geochemistry is about obtaining adequate and cost-effective results, and these need not necessarily be perfect results. All that we require is data that have sufficient precision and accuracy to enable the confident interpretation of results for the task at hand (e.g., the discrimination of anomalies from background, or the definition of resource grade) through determination of element concentrations to a stated degree of confidence. At the same time, we cannot and must not pay extra for analytical data that are of unnecessarily high quality.
>
> As an example, consider the quality requirements in a routine stream or soil geochemistry [or biogeochemical] survey during mineral exploration. We might typically obtain Pb concentration data to a precision level of +/− 5 ppm (2 standard deviations) at a background level of 50 ppm (i.e., 10% precision at the 95th percentile confidence level). If our geochemical anomalies are >200 ppm, clearly it would not make sense spending extra money on higher quality analyses in this application, say with two standard deviation errors of +/− 1 ppm (i.e., 2% precision). This is because the goal of the survey, namely discriminating anomalous concentrations from background ones, could be easily achieved using the lower quality data, with the exception of a few samples with results near to the threshold
>
> There is no doubt that some laboratories (e.g., geochronology or university research laboratories) could deliver for us a suite of wonderfully accurate and precise lead results to the level of say 50 +/− 0.01 ppm (2 standard deviation precision), but do we need this in the hypothetical example given above? Clearly, we do not. It is highly unlikely that we would gain enough advantage during first-pass exploration to ever justify the cost and time required for such enhanced precision. In fact, our exploration manager would be justified in booting us up the backside for wasting precious time and funds, as well as for failing to appreciate what was required to get the job done.
>
> Of course, it is one thing to seek a pragmatic approach but entirely another to define in measurable terms what constitutes data that are 'Fit-for-purpose'. The experience of the practitioners in comparable surveys enables some predictions of background and anomalous levels, and the importance of orientation surveys in this regard cannot be stressed too highly … . We contend therefore that proficiency testing must provide measures of both precision and accuracy within a framework of the various objectives for which the analyses are being determined – what we have termed here the 'Fit-for-purpose' approach. (Bettenay and Stanley, 2001)

Further to Bettenay and Stanley's sound advice, several charts of analytical data are shown in Fig. 7-1. Each shows the results obtained on an in-house dry vegetation control material designated V6, which will be quoted later in this chapter and extensively in Chapter 9. The study from which these data were extracted involved the analysis of approximately 250 samples that included field duplicates and control V6 inserted randomly within each batch of 20 samples. The laboratory was not informed that any controls were inserted – they received just sequentially numbered packets. Clearly, the reproducibility (i.e., precision) of the data for Zn, Pb, Ni and Hg was excellent. Standard deviations were very small, and the data were accurate with respect to previous batches (penultimate bar marked 'Prev.') and accurate with respect to the 'target' value (last bar). The latter is a value established after many hundreds of analyses and laboratory refinements of analytical techniques.

The data for Au show considerably more variability, and those for As an even greater range of values and a high standard deviation.

The geochemist receiving these data would have no problem with accepting the Zn, Pb, Ni and Hg data. With regard to Au it is necessary to be aware that plants exhibit, to a degree, the same 'nugget effect' that is found in all sample media. In Chapter 2 it was shown that SEM has identified Au phases that have formed within plant structures, thereby providing evidence as to why the nugget effect occurs.

For As, the precision by ICP-MS is inferior to that obtained for Au. The geochemist in receipt of such data should be aware that there are many spectral and/or matrix interferences in the measurement of As by ICP-MS and therefore poor precision is to be expected. Consequently, the Au and As data should be treated with the realization that analysis of 1 g samples of dry vegetation is likely to provide relatively poor precision for Au and As, and no amount of re-analysis of samples will resolve this problem. For those requiring better precision, an alternative analytical method needs to be considered, such as dissolution of a much larger sample (e.g., 15 g for Au), or analysis by another method – e.g., INAA (for Au and As) or fire assay (for Au).

The conclusion from the above is that the geochemist must have some knowledge of the capabilities of the analytical instrumentation and method, and on this basis should be able to decide if the data are sufficiently reproducible to be worthy of consideration – and are they fit for the purposes and objectives of an exploration programme. With this realization firmly in mind, consideration can now be given to the range of analytical techniques that are available, along with their strengths and limitations.

ANALYTICAL TECHNIQUES

Until about 1970, analytical techniques for determining element concentrations in vegetation were dominated by gravimetric, colorimetric and emission spectrographic methods. These were superseded by the introduction of instrumental techniques, such as AAS, XRF, instrumental and radiochemical neutron activation analysis

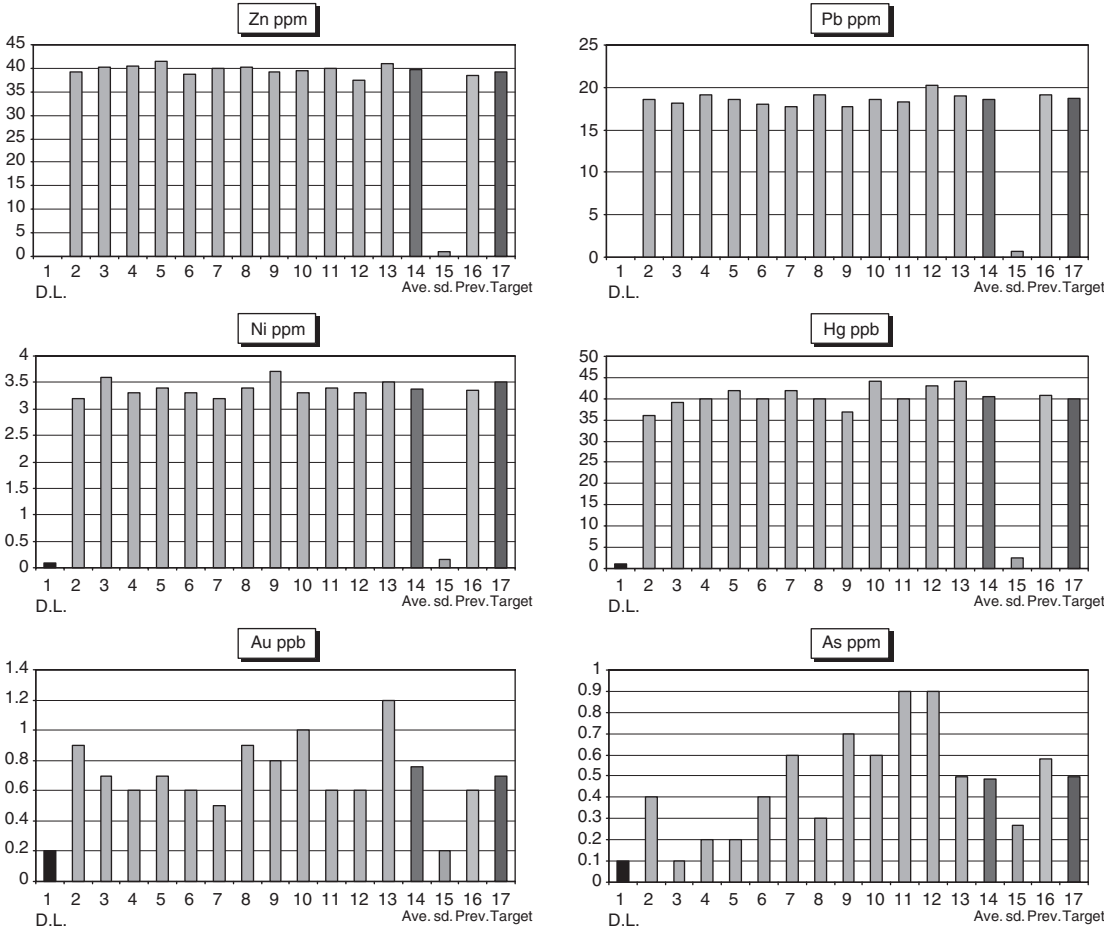

Fig. 7-1. Vegetation control V6 – reproducibility of analytical data.

(INAA and RNAA), ICP-ES and ICP-MS. More recently, high resolution ICP-MS) has become available for even greater insight into the low concentrations of elements in all types of geochemical sample media.

Hall (1995) outlined some of the pertinent questions which the analysts and exploration biogeochemists must consider.

(1) Should the sample be decomposed, or is there a direct analytical technique with sufficient sensitivity?
(2) Should the sample be pre-concentrated by reduction to ash, and, if so, which elements are susceptible to loss via volatilization?
(3) Does the form of the element change during ashing, which alters its properties of ease of dissolution afterwards?
(4) If the procedure is to be based on the unashed sample, is the analytical technique to be employed sufficiently sensitive and is the level of contamination by reagents low enough for adequate accurate and precise measurement of the analytes of interest?
(5) What are the potential interferences to be aware of?
(6) Which reference material(s) (international or in-house) should be inserted in the batch of samples in order to evaluate accuracy?
(7) How much replication is needed to satisfactorily estimate precision?

As in any geochemical work, it is crucial to appreciate that no matter how accurate and precise the measurement step is, the result is only as good as all the preparation that has gone into it before.

ANALYTICAL INSTRUMENTATION

Whereas there are many different analytical instruments, the principal tools currently in use are the five listed on the first page of this chapter – AAS, ICP-ES, ICP-MS, INAA and XRF. Of these, the first three are destructive (tissues or ash are dissolved in acids), whereas the last two are non-destructive – pellets of ground tissue or ash are introduced into the instruments for direct measurement.

Atomic absorption spectrometry

The principle advantages of AAS are its specificity, simplicity, low capital outlay, ruggedness and relative freedom from interferences. The technique was rapidly adapted to geological applications in the early 1960s when commercial instrumentation became available, and it remained the leading technique in the analysis of solutions until the advent of ICP-ES in the mid-1970s. Compared to ICP-ES it has the limitations that:

- only one element at a time can be determined by AAS and hence it cannot compete in speed with multi-element techniques,

- the short linear dynamic range of AAS necessitates dilution for the more highly concentrated analytes which leads to reduced productivity and greater error.

An AAS consists of a light source (typically a hollow cathode lamp) which emits a sharp line of the wavelength for a particular analyte; an atomization cell (flame, furnace, quartz tube); a chopper to eliminate emitted light from the cell; a monochromator to select the line of interest; a photomultiplier detector; and a read-out system. Interferences can be spectral, chemical and ionization or viscosity effects. Background correction is routinely applied for elements such as Pb, Ag, Ni and Co.

The main types of AAS are as follows.

- *Flame AAS (FAAS)* is the most rapid and least complex of AAS procedures. However, detection limits are not sufficiently low for most elements in dry tissue, and generally only suitable for Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Si, Sr and Zn unless there is some pre-concentration procedure, such as ashing. There are potential interferences such as Fe in the determination of Cr under certain conditions.
- *Quartz tube AAS:* Cold-vapour (CV) AAS is an excellent method for Hg.
- *Graphite furnace AAS* is 1–3 times more sensitive than FAAS, but has more complex interferences and lower productivity. Typically, each analysis requires 2–3 min. The improved sensitivity of GFAAS permits measurements of Ag, Cd, In, Sn, Ti and Pb. Unlike FAAS, it is desirable to avoid HC1 in the final sample matrix to avoid Cl vapour phase interferences, and so HNO_3 or H_2SO_4 are preferred. GFAAS can play a valuable role in the analysis of dry vegetation where sensitivity is of utmost importance, but its poor rate of output can be a major limitation. There are many examples in the literature, describing GFAAS analysis of dry vegetation for elements such as Pb, Cd, Ni, Ga, Tl, Se and P at ppb levels. Matrix modification is usually essential to negate suppression or enhancement effects caused by accompanying species in the acid leachates.
- *Slurry AAS* involves the introduction of plant tissue in the form of slurry. This provides a good representation of the sample, and time-consuming digestion is avoided. One drawback of the method is the uncertainty of adequate sample representation since only milligram-sized aliquots are taken. To overcome this problem about five readings of five different slurry preparations are advisable – again making the technique rather too slow for anything other than a research project.

Inductively coupled plasma emission spectrometry (ICP-ES)

The first commercial ICP-ES was introduced in 1975, and in a short period of time replaced DC-arc emission spectrometry as a major producer of trace element data. Among the numerous advantages of ICP-ES are:

- the ability to measure 20–60 elements simultaneously in a cycle time of 2–3 min;
- a long linear dynamic range of 4–6 orders of magnitude so that major, minor and trace elements can be determined in the same solution (i.e., no dilution);

- a superior sensitivity for many elements e.g., B, P, S; the refractory elements Al, Mo, Nb, Ti, Zr and the REE; and
- substantially reduced chemical interferences in the hot environment of the argon plasma.

It is the simultaneous direct reading ICP spectrometer that is a major workhorse in today's geoanalytical laboratory. In this instrument the plasma is a gas in which atoms are present in an ionized state. When a high frequency current flows in an induction coil, it generates a rapidly varying magnetic field within the coil. The interaction or inductive coupling of the oscillating magnetic field with flowing ionized argon gas generates plasma temperatures of 6000–10,000°K. Argon is used because:

- it is inert and likely to suppress chemical interferences;
- it is transparent in the ultraviolet-visible region where most of the ion and atom lines lie;
- it has a high ionization potential, IP (15.75 eV), permitting detection of all elements that can be excited to emit lines in the ultraviolet-visible region; and
- it has a moderately low-thermal conductivity.

The function of the ICP is to vapourize, dissociate, atomize and excite the sample, thereby promoting atomic and ionic line spectra as photons are emitted in energy transfer reactions. The principal weakness of ICP-ES is sample introduction, because the nebulizer delivers only about 2–3% of the sample solution to the ICP. This problem was addressed by a number of researchers in the 1980s using international standard reference materials (SRMs), such as Orchard Leaves (NIST, 1571). These studies yielded some significant advances, but the technology is not generally available from commercial laboratories, not only because of the extra time involved (and therefore the cost) to analyse each sample, but also because of the advent of ICP-MS technology. Nevertheless, ICP-ES remains an extremely valuable method for cost-effective analysis of many elements – notably the base metals. However, for many of the precious and trace 'pathfinder' elements now sought in biogeochemical exploration, the sensitivity of ICP-ES is inadequate for the low concentrations that are typically present. This is especially true for Ag, Au, As, Bi, Hg, Sb, Se, Te, Tl and U. Hydride generation ICP-ES (gaseous rather than liquid introduction) can be used for determination of As, Sb, Se, Te, Bi, Ge and Sn, but this is a method that is no longer readily available from most commercial laboratories. Fortunately, ICP-MS is now available for determining this suite of elements that can be of such great value to exploration programmes.

Detection limits by conventional nebulization ICP-ES in pure solution, calculated as three times the standard deviation of the background signal (3 SD) at the most sensitive emission line, are approximately as follows.

- < 1 ng/mL – Be, Mg, Ca, Sc, Mn, Sr, Y, Ba, Eu, Yb, Lu.
- < 10 ng/mL – Li, B, Na, Si, Ti, V, Cr, Fe, Co, Ni, Cu, Zn, Zr, Mo, Rb, Ag, Cd, La, Hf, Re, Au, Hg, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Th.
- < 100 ng/mL – Al, P, S, K, Ga, Ge, As, Se, Nb, Ru, Pd, In, Sn, Sb, Te, I, Ta, W, Os, Ir, Pt, Tl, Pb, Bi, Ce, Pr, U.
- < 1 μg/mL – Rb.

Practical detection limits are greater than these optimal instrumental limits, by factors generally from 2 to 5 depending on sample type, interferences and conditions of the analysis. Spectral interference from major elements in particular can preclude the use of an analyte's most sensitive line and hence detection power can be further degraded. If 2 g of dry vegetation are digested in an oxidizing mixture of aqua regia or HNO_3–$HClO_4$ and dissolved in a final volume of 20 mL (i.e., 10-fold dilution) for nebulizing into the ICP, in most plant species only about 15 elements can readily be determined. They are, Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, Sr, Ti and Zn. Other elements would require some form of pre-concentration prior to analysis. If the vegetation is ashed, concentrations of many of these elements are raised to high enough levels for easy measurement.

Table 7-I shows concentration levels typically reported by commercial laboratories employing ICP-ES following aqua regia or HF–HNO_3–$HC1O_4$ decomposition. These values are ideal and the presence of high concentrations of certain elements can increase these detection limits substantially.

Over a number of years in the 1990s, ICP-ES data were obtained at the Geological Survey of Canada on an in-house control designated 'V6'. This control was prepared by accumulating a large amount of jack pine twigs (*Pinus banksiana*), with lesser amounts of bark and needles, from forest near Ottawa. Some of this material was reduced to ash by controlled ignition at 475 °C. This ash was used as a guide to the analytical precision obtained for many thousands of samples, mostly by inserting one split of V6 within each batch of 20 samples. The data acquired by ICP-ES determinations of 240 samples of this material has been averaged and the standard deviation computed. This provides a useful guide as to the precision that can be expected from low-cost multi-element determinations by commercial laboratories. The data are summarized in Table 7-II and, for general reference, the equivalent concentrations in dry tissue have been calculated. These are based upon a consistent ash yield of 4.8% for V6.

The reader should be aware that there are a number of sophisticated techniques for digesting plant material and introducing the analyte into the plasma in order to obtain improved precision, accuracy and detection levels for certain elements. However, these are not readily accessible to the exploration geochemists, or they would add considerable expense to an analytical programme. Hall (1995) elaborates on many of these techniques and provides a long list of references. The subject is huge, with more than 4000 papers written up till 1990 on just flow injection

TABLE 7-I

Typical detection limits by ICP-ES

	Detection limit
Ag (ppm)	0.3
Al (%)	0.01
As (ppm)	2
Au (ppm)	2
B (ppm)	3
Ba (ppm)	1
Bi (ppm)	3
Ca (%)	0.01
Cd (ppm)	0.5
Co (ppm)	1
Cr (ppm)	1
Cu (ppm)	1
Fe (%)	0.01
Hg (ppm)	1
K (%)	0.01
La (ppm)	1
Mg (%)	0.01
Mn (ppm)	2
Mo (ppm)	1
Na (%)	0.01
Ni (ppm)	1
P (%)	0.001
Pb (ppm)	3
Sb (ppm)	3
Sr (ppm)	1
Th (ppm)	2
Ti (%)	0.01
Tl (ppm)	5
U (ppm)	8
V (ppm)	1
W (ppm)	2
Zn (ppm)	1

(the technique of injecting a small sample plug into an unsegmented carrier stream) and its applications.

Inductively coupled plasma-mass spectrometry (ICP-MS)

In recent years, ICP-MS has become the most valuable tool for low-cost multi-element analysis of plant tissue. The first commercial ICP-MS instrument was introduced by Sciex in 1983, followed shortly by VG Elemental. Since that time, and especially in the mid-1990s, instrumentation has become significantly more robust and procedures have been steadily improved.

TABLE 7-II

Mean and standard deviations for control 'V6' (reduced to ash) by aqua regia digestion and ICP-ES ($n = 240$). The column 'Dry equivalent' is the equivalent concentration in dry tissue

	Mean	Standard deviation	Dry equivalent
Al (%)	1.17	0.08	0.055
As (ppm)	6.4	5.6	0.3
B (ppm)	174	22	8.2
Ba (ppm)	164	15	7.8
Ca (%)	13.77	1.45	0.65
Cd (ppm)	3.1	0.4	0.15
Co (ppm)	7.8	1.1	0.37
Cr (ppm)	44	9.2	2.06
Cu (ppm)	129	20	6.1
Fe (%)	1.76	0.15	0.083
K (%)	2.06	0.47	0.097
La (ppm)	16	1.9	0.8
Li (ppm)	7.0	3.7	0.33
Mg (%)	2.9	0.55	0.138
Mn (ppm)	751	100	36
Mo (ppm)	6	2.2	0.28
Na (%)	0.19	0.04	0.009
Ni (ppm)	74	16	3.5
P (%)	0.66	0.05	0.03
Pb (ppm)	192	38	9.1
Sr (ppm)	575	45	27
Ti (%)	0.043	0.005	0.002
V (ppm)	28	3.3	1.3
Y (ppm)	7	1.3	0.3
Zn (ppm)	590	52	28

Significant features of ICP-MS are simple spectra, wide linear dynamic range (10^4–10^5), flexibility, the ability to measure isotopes as well as elemental concentrations and excellent detection limits in solution in the range 0.01–0.1 ppb for many elements. Spectral interferences are fewer than in ICP-ES. They are relatively easy to predict and are due to isotopes of another element, oxides (MO^+), doubly charged ions, hydroxides and polyatomic ions formed from matrix elements and the plasma (e.g., ArO^+ on $^{56}Fe^+$). In general, the lighter the analyte and the heavier the interfering element, the greater is the degree of interference – e.g., the effects of the heavy elements such as U and Pb on light elements, such as Li and Be, can be severe. Elements below mass 80 (i.e., the first 35 elements of the periodic table) tend to suffer the most interference, although there are significant exceptions, with Pd being a prime example of an element for which many interferences can occur.

Table 7-III shows the typical detection levels available by ICP-MS from a commercial laboratory, and the mean and standard deviation of the data from 513 determinations of V6 (dry) interspersed among exploration-oriented biogeochemical samples over a two-year period.

The most severe limitations of ICP-MS are polyatomic interferences on the elemental signals, originating from argon and/or the sample matrix. With these limitations in mind, instrument manufacturers such as Thermo Electron Corporation and VG Elemental, UK, have addressed this problem and now offer analysis by magnetic-sector high-resolution ICP-MS (HR-ICP-MS) and multi-collector ICP-MS (MC ICP-MS). Although such sophistication is not a requirement for the exploration biogeochemists, this separation of analyte ions from spectral interferences is a prerequisite for those who require and can afford this extra level of accurate and precise elemental analysis. Furthermore, the HR-ICP-MS provides lower detection levels for a number of elements in dry plant tissues that typically are close to or below the levels of detection by quadrupole ICP-MS – notably Be, Bi, In, Re, Sb, Se, Te, Tl, U, V, W, some of the REEs and all of the PGEs.

Thermo Electron Corporation manufactures the Finnigan ELEMENT2^{TM} HR-ICP-MS and its recent successor the ELEMENT XR^{TM} for even greater sensitivity. To quote from the manufacturer's website www.thermo.com (3 March 2006), features of this instrumentation are as follows.

- Multi-element analysis across the periodic table covering a milligram per litre to sub-picogram per litre concentration range – compatible with inorganic and organic solution matrices and solids.
- High mass resolution to access spectrally interfered isotopes – produces unambiguous elemental spectra.
- A multi-elemental detector for transient signals – for example, CE (capillary electrophoresis), HPLC (high performance liquid chromatography), GC (gas chromatography), FFF (field flow fractionation) and laser ablation.
- High precision isotope ratios – on non-interfered or interfered isotopes.
- Fully automated tuning and analysis – in conjunction with a comprehensive, customizable quality-control system.
- Reliability and robustness to serve as a 24/7-production control tool – highest sample throughput.
- Highest flexibility and accessibility to serve as an advanced research tool.

Through the combination of a single Faraday collector with the SEM, the linear dynamic range of the Finnigan ELEMENT XR can be increased by an additional three orders of magnitude, when compared to the Finnigan ELEMENT2, to over 10^{12}.

Multi-collector ICP-MS instrumentation is not routinely applied to plant analysis, and falls into the realm of the advanced research instrumentation that was mentioned in the opening paragraph to this chapter. It is, therefore, not yet a viable option for biogeochemical exploration.

TABLE 7-III

Multiple analyses of control V6 (dry) by ICP-MS after a nitric acid/aqua regia digestion

	Detection limit	$n = 513$	
		Mean	Standard deviation
Ag (ppb)	2	18	2.6
Al (%)	0.01	0.05	0.01
As (ppm)	0.1	0.6	0.2
Au (ppb)	0.2	0.7	0.4
B (ppm)	1	28	18
Ba (ppm)	0.1	10	0.8
Bi (ppm)	0.02	−0.01	
Ca (%)	0.01	0.75	0.04
Cd (ppm)	0.01	0.24	0.02
Co (ppm)	0.01	0.41	0.04
Cr (ppm)	0.05	4.4	0.3
Cs (ppm)	0.005	0.03	0.003
Cu (ppm)	0.01	8.3	1.2
Fe (%)	0.001	0.08	0.005
Ga (ppm)	0.1	0.15	0.05
Hg (ppb)	1	41	3.8
K (%)	0.01	0.09	0.007
La (ppm)	0.01	0.9	0.08
Mg (%)	0.001	0.13	0.01
Mn (ppm)	1	46	2.6
Mo (ppm)	0.01	0.3	0.02
Na	0.001	0.01	0.002
Ni (ppm)	0.1	3.5	3.4
P	0.001	0.05	0.003
Pb (ppm)	0.01	19	0.9
S	0.01	0.06	0.019
Sb (ppm)	0.02	0.06	0.01
Sc (ppm)	0.1	0.3	0.08
Se (ppm)	0.1	0.2	0.06
Sr (ppm)	0.5	31	2.1
Te (ppm)	0.02	−0.02	
Th (ppm)	0.01	0.1	0.02
Ti (ppm)	1	24	1.9
Tl (ppm)	0.02	−0.02	
U (ppm)	0.01	0.06	0.01
V (ppm)	2	1.3	1.8
W (ppm)	0.1	−0.10	
Zn (ppm)	0.1	40	2.7

Instrumental neutron activation analysis (INAA)

During the 1980s and 1990s, prior to the widespread availability of ICP-MS, INAA was perhaps the best technique for the biogeochemist. Among its numerous advantages are

- multi-element capability,
- high selectivity and sensitivity,
- relative freedom from interferences, and
- the ability to analyse samples of varying weights directly without the need of digestion procedures.

Commercial processing of samples is simple and highly automated. The usual procedure is to activate samples by irradiation with neutrons in a reactor to produce radioactive isotopes by neutron capture-type reactions. In the case of dry vegetation, dry milled material is compressed into a pellet using a conventional XRF briquetting press. The amount of material used is commonly 15 g, but both 8 g and 30 g briquettes are sometimes selected. Although the data obtained on each size are quite comparable, obviously the 30 g briquette is more representative of the original sample (basically the equivalent of one assay tonne), but the 8 g sample, which is slightly cheaper to analyse, provides data that are perfectly adequate for exploration purposes. Even a small vial containing only 2 g of tissue provides comparable and consistent data, but with slightly poorer precision.

In the case of plant tissue that has been reduced to ash, the ash is packed into a small polyethylene vial, and then capped and heat-sealed. An optimal amount of ash has proved to be 0.25 g, although similar results are obtained with 0.5 g samples. Under normal instrumental settings, analytical precision decreases quite substantially when samples smaller than 0.25 g are irradiated. Up to 30 of the briquettes or 120 small vials at a time can be stacked and irradiated.

The instrumentation required to measure a gamma spectrum comprises three parts:

- The detector, invariably a Ge (Li) crystal which becomes ionized by the gamma photons thereby generating an electronic signal;
- electronic amplification; and
- a multi-channel analyser to sort and store detected pulses.

After irradiation, samples are left for a 5–7-day decay period before the simultaneous determination of up to 36 elements (Table 7-IV). This decay, or 'cooling' period as it is sometimes called, is required to allow reduction in unwanted short-lived radioisotope activity (e.g., Na). Specific isotopes are identified by their characteristic gamma-ray energy or energies, normally in the range 60–1600 keV and they are quantified by measurement of the peak area. Calibration is carried out using one or more SRMs that is irradiated and counted under identical conditions.

TABLE 7-IV

Detection limits in humus, dry vegetation and vegetation ash analysed by INA (from Hoffman, 1992)

	Humus	Dry	Ash
Au (ppb)	1	0.1	5
Ag (ppm)	2	0.2	2
As (ppm)	1	0.01	0.5
Ba (ppm)	100	5	10
Br (ppm)	1	0.01	1
Ca (%)	0.01	0.01	0.2
Co (ppm)	1	0.1	1
Cr (ppm)	1	0.3	1
Cs (ppm)	0.5	0.05	0.5
Fe (%)	0.05	0.005	0.05
Hf (ppm)	0.5	0.05	0.5
Hg (ppm)	0.5	0.05	1
Ir (ppb)	5	0.1	2
K (%)		0.01	0.05
Mo (ppm)	0.5	0.05	2
Na (ppm)	100	1	10
Ni (ppm)	10	2	50
Rb (ppm)	20	1	5
Sb (ppm)	0.1	0.005	0.1
Sc (ppm)	0.1	0.01	0.1
Se (ppm)	2	0.1	2
Sr (ppm)	100	10	300
Ta (ppm)	0.5	0.05	0.5
Th (ppm)	0.5	0.1	0.1
U (ppm)	0.1	0.01	0.1
W (ppm)	1	0.05	1
Zn (ppm)	20	2	20
REEs			
La (ppm)	0.1	0.01	0.1
Ce (ppm)	1	0.1	3
Nd (ppm)	3	0.3	5
Sm (ppm)	0.1	0.001	0.1
Eu (ppm)	0.2	0.05	0.01
Tb (ppm)	0.2	0.01	0.5
Yb (ppm)	0.1	0.005	0.05
Lu (ppm)	0.1	0.001	0.05

A separate suite of short-lived isotopes can be measured using epithermal neutrons. These include Al, Cl, Cu, I, In, Mg, Mn, Ti and V. Hoffman (1992) and Hall (1995) both provide detailed accounts of INAA in geo-analysis addressing biogeochemical applications using dry tissue and ash. Table 7-IV shows the various detection levels typically obtained for humus, dry vegetation and vegetation that has been reduced to ash by controlled ignition.

Table 7-V provides an example of the long-term reproducibility of the data obtained by INAA. The data represent results compiled over several years for 272 portions of control V6 (ash), and the average and standard deviation are calculated. Also, data for 19 samples of dry V6 (15 g pellets) were compiled and the same parameters calculated.

INAA is particularly sensitive for As, Co, Cr, Cs, Hf, Ir, Sb, Sc, Ta, Th, U and most of the REEs. It is especially suitable for vegetation, as dry tissue is highly concentrated in such elements as C, N, H and O, which create a very low-background spectrum, and hence cause few interferences.

Whereas there are many advantages to using INAA for determining the trace element content of vegetation samples, the method does have some limitations. For example, when samples have a high U content the correction becomes sufficiently large that data for some elements either cannot be reported (e.g., Mo) or have very high-detection limits. Other potential problems include improper flux monitoring and maintaining consistent sample-to-detector geometry. These last items are technical problems that the analyst must address. However, it is as well for geochemists to be aware of these problems, because it assists in carefully evaluating the data quality. On occasion, data have been released that appear to be incorrect by a factor of five. On questioning the laboratory that provided the data it was found from their investigations that the detector had been set at an incorrect distance from the sample that was being measured – i.e., too far away, so fewer gamma rays were detected. Of course, by inserting appropriate standard samples in the sequence, problems such as this can be identified and readily resolved. The issue of inserting 'blind' (i.e., unidentified) SRMs throughout a sequence of samples cannot be too highly stressed. As will be mentioned time and again in this book, the need to accurately assess data quality is of paramount importance when conducting a geochemical survey – whether using rocks, soils, sediments, waters, or vegetation.

From the exploration biogeochemist's point of view, the drawback of INAA is that not all of the elements of potential interest can be obtained by this method, because they are either impossible or difficult to determine.

- If an exploration programme is oriented toward base metals, several key elements are missing from the suite available from INAA – notably Cd, Cu and Pb.
- Nickel detection limits are high (see Table 7-IV).
- The only platinum group element determined by direct irradiation is iridium. It is highly unusual for Ir to be present in dry tissue above the detection limit of 0.1 ppb Ir. Commonly, values reported at or above the detection limit have proved to be

TABLE 7-V

Analysis of control V6 by INAA using 0.5 g of ash, and 15 g pellets of milled dry tissue

	Ash ($n = 272$)		Dry ($n = 19$)			Ash ($n = 272$)		Dry ($n = 19$)	
	Mean	Standard deviation	Mean	Standard deviation		Mean	Standard deviation	Mean	Standard deviation
Au (ppb)	17	7	0.83	0.4	Sb (ppm)	1.2	0.11	0.07	0.01
Ag (ppm)	<2	–	<0.2	–	Sc (ppm)	4.5	0.31	0.28	0.02
As (ppm)	7.6	0.8	0.51	0.09	Se (ppm)	2	–	<0.1	–
Ba (ppm)	404	37	25	4.4	Sr (ppm)	948	198	31	13
Br (ppm)	12	3.0	2.6	0.1	Ta (ppm)	<0.5	–	<0.05	–
Ca (%)	15.83	1.21	0.94	0.08	Th (ppm)	3	0.34	0.23	0.05
Co (ppm)	8.7	1.0	0.6	0.05	U (ppm)	1.3	0.26	0.06	0.02
Cr (ppm)	68	5.3	4.6	0.5	W (ppm)	<1	–	<0.05	–
Cs (ppm)	1.2	0.6	0.05	0.05	Zn (ppm)	773	67	37	3.7
Fe (%)	1.76	0.13	0.11	0.008	*REEs*				
Hf (ppm)	5.2	0.5	0.3	0.03	La (ppm)	21	1.4	1.1	0.05
Hg (ppm)	<1	–	<0.05	–	Ce (ppm)	42	3.4	2.2	0.16
Ir (ppb)	<2	–	<0.1	–	Nd (ppm)	22	2.6	0.8	0.35
K (%)	3.65	0.6	0.23	0.04	Sm (ppm)	3.1	0.2	0.15	0.009
Mo (ppm)	4.9	1.6	0.3	0.13	Eu (ppm)	0.8	0.10	0.03	0.006
Na (ppm)	11305	662	542	19	Tb (ppm)	<0.5	–	<0.01	–
Ni (ppm)	<50	–	<2	–	Yb (ppm)	1.7	0.18	0.10	0.012
Rb (ppm)	45	7	2	1.8	Lu (ppm)	0.3	0.06	0.01	0.002

unrepeatable, or are a result of a missed correction. INAA is a wonderful technique for PGEs that have first been separated from plant tissue by either chemical procedures or NiS fire assay, but the former is time-consuming and the latter requires a lot of material and is a costly enterprise.
- Whereas INAA is perhaps the best technique for Au in vegetation, some of its pathfinder elements are not determined – e.g., Bi, Te, Tl – and detection limits for others (e.g., Ag, Hg and Se) are a little too high.
- Rhenium and Li each have their place in biogeochemical exploration and data for these elements are not provided by INAA.

No analytical technique is perfect for all elements and so compromises need to be made. A significant advantage of INAA is that it is a non-destructive technique. As a result, if a doubtful or particularly interesting analysis is received, the same sample can be re-analysed to ensure that it is not an analytical problem. Analytical costs are low, and the precision (Table 7-V) and accuracy are excellent for most elements that can be readily determined.

X-ray fluorescence (XRF)

The Swedish Geological Survey has used XRF extensively for the analysis of their 'biogeochemical samples' (stream bank organic material comprised largely of roots). However, nowadays with other techniques available, although it remains a viable approach for some elements, it is not the optimum method for vegetation analysis. Advantages are that analysis is non-destructive, rapid, only a weak X-ray source is required and there are few spectral line interferences. Wilson (1998) indicates that results from the XRF analysis of plant tissue compare favourably with data obtained by AAS and ICP-ES. However, without special concentration procedures, the sensitivity for most elements is inferior to that obtained by INAA or ICP-MS. XRF is of particular use for its very precise measurement of the major elements; for Sr, Rb; and for high field strength elements (HFSE) – Hf, Nb, P, Ti, Y and Zr. It is not practical to determine element concentrations in ash, because 5 g of ash is a usual requirement, and to obtain that amount of ash requires a large original sample – about 100 g of dry foliage, 200 g of twigs or almost 2 kg of conifer trunk wood.

Very much smaller samples are required for Total Reflection XRF (TXRF). In this technique X-rays are guided to impinge on the surface of a sample at a glancing angle (only 4°) such that total reflection occurs. The X-rays excite atoms in the top layers of the material and the fluorescence is detected by a Si (Li) detector placed above the sample. The sample is applied as a thin layer of a dried solution or a slurry and sensitivity is vastly improved with detection limits of a few ppb, but it requires very flat samples. The X-ray source can be an X-ray tube or a synchrotron. An early study of this technique claimed success in determining element distribution patterns in wood, bark, needles and fine roots of healthy and diseased spruce (Berneike et al.,

1987). The consumption of only μL amounts of solution (digestate) by TXRF was an important advantage in the analysis of single fine roots. Results by both ICP-ES and TXRF compared extremely well for Ca, K, Fe, Mn, Sr, Ba, S, Zn and Cu. Aluminium, Mg, Na and P required detection by ICP-ES, while Cd and Pb demanded the low-level detection capability of TXRF.

The new generation of high-resolution XRF instruments (e.g., the Axios [PANanalytical B.V.]) promises to determine sub-ppm levels of trace elements in vegetation. During 2004, PANanalytical introduced a new range of Wavelength Dispersive XRF (WDXRF) spectrometers that included the Axios-Advanced – a system with an enhanced capability for measuring the range of elements commonly analysed in soils. In order to overcome specific problems associated with determining low elemental concentrations, the Axios system is compatible with PANalytical's Pro-Trace module that permits quantification down to sub-ppm levels.

SUMMARY

There are many excellent texts that provide summary tables showing the advantages and disadvantages of the principal analytical techniques used in the analysis of plants, and all provide comments on which elements are best determined by a particular method (e.g., Markert, 1994; Hall, 1992, 1995; Ayrault, 2005). This is valuable information for those seeking to select the optimal method for determining a particular element in plant tissue.

Table 7-VI is a modification of that prepared by Hall (1995), updated to include trends and advances over the past decade, now that ICP-MS has become a more accessible technique and costs are so modest. It does not include the most advanced and expensive techniques that are at present rarely available outside of academic and government institutions.

It is quite remarkable how much valuable and precise analytical information can be obtained from the commercial laboratories for so little cost – typically well under $1 per element determination by HR-ICP-MS and substantially less for the more common quadrupole ICP-MS. Furthermore, the extreme sensitivity of the instrumentation has, at no additional cost, permitted insight into concentrations and distribution patterns of elements that were typically nearly always reported as 'less than' values in vegetation – notably Be, Bi, In, Re, Te and Tl, most of which can be insightful pathfinder elements. This now leaves the biogeochemists of the 21st century in the enviable situation of being able to gain new fundamental information on the multi-element distributions of ultra-low concentrations in large numbers of vegetation samples. There is now a new frontier of insight on elements in plants and their relationships to the underlying substrate.

Furthermore, as far as the exploration biogeochemist is concerned, the complex world of analytical techniques and methods has been greatly simplified, now that ICP-MS covers most of the basic requirements. Of course, many suitable controls on

TABLE 7-VI

Advantages and limitations of the principal techniques applied to the analysis of vegetation

Technique	Advantages	Disadvantages	Best elements
ICP-MS	High Sensitivity	Decomposition required	>50 elements from a single digestion
	Multi-element	Destructive	Good for many ultra-trace elements
	Low detection limits Few spectral interferences	Matrix interferences	
HR ICP-MS	Extremely high sensitivity	Decomposition required	Most elements from a single digestion
	No matrix interferences	Destructive	Only effective method for getting low level PGEs and the halogens (excluding F) in vegetation
		Higher cost of analysis than ICP-MS Limited availability	
ICP-ES	Multi-element	Decomposition required	Al, B, Ba, Ca, Cd, Co, Cu, Fe, K, La, Mn, Mo, Na, Ni, P, Pb, S, Sr, Ti, V, Y, Zn
	Few matrix interferences	Destructive Spectral interferences	Satisfactory for Ag, Bi and Li, but they are rarely above dl in dry vegetation
	Low cost		
AAS	Few interferences	Decomposition required	Same as for ICP-ES, but not B, P, S. Good for Hg by cold-vapour, but not superior to ICP-MS. GFAAS greatly enhances sensitivity, but at extra cost
	Robust and rugged	Destructive One element at a time, therefore high cost for many elements Small linear dynamic range (major and trace	

Continued

TABLE 7-VI *Continued*

Technique	Advantages	Disadvantages	Best elements
		elements cannot be determined from same solution)	
INAA	Direct analysis of dry material (non-destructive)	Nuclear reactor required	Ag, Au, As, Ba, Br, Ca, Co, Sr, Cs, Fe, Hf, K, Mo, Na, Rb, Sb, Sc, Sr, Ta, Th, U, W, Zn
	Multi-element	Decay period cannot be rushed, so potentially slower than by above methods	The REEs La, Ce, Nd, Sm, Eu, Tb, Yb, Lu usually included with above from a single irradiation. High sensitivity for Ir, but Ir rarely above dl. in dry tissue or ash unless near significant PGE mineralization
	High precision and accuracy	Separate irradiation required for short lived isotopes (Al, Cl, Cu, I, In, Mg, Mn, Ti, V)	
	Few matrix interferences	Poor or impossible for the base metals Cd, Cu, Ni, Pb	
	Low cost		
XRF	Direct analysis of dry material (non-destructive)	Matrix interferences	Major elements and Ba, Cl, Cr, Fe, Mn, Nb, S, P, Rb, Sr, Th, Ti, Y and Zr
	Multi-element (Na-U, but lighter elements with HR-XRF [Axios])	Fusion required to avoid matrix effects for some major elements	
	High precision and accuracy	Relatively large sample required for pressed pellets High cost of fused disc	

precision and accuracy should be inserted along with the 'field' samples, and intelligent and informed interpretation is required. The geochemist should also have some appreciation of the data quality that can reasonably be expected – which leads now to Chapter 9 that deals with 'real-world' situations for the individual elements.

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Chapter 8

THE EDEN PROJECT – SOURCE OF A BIOGEOCHEMICAL DATABASE

INTRODUCTION

This chapter describes a valuable source of biogeochemical data obtained from plants growing in immense greenhouses comprising the Eden Project. The complex is located in disused kaolinite quarries cut into Hercynian granites near St. Austell in south-western England. Conceived and built in the 1990s by Tim Smit, the project was designed primarily as an educational and research facility 'to promote the understanding and the responsible management of the vital relationship between plants, people and resources'. In addition, it serves as a unique natural laboratory for comparing the relative uptake of elements by a wide range of plants growing under controlled conditions.

The structures comprise two sets of four giant, translucent, geodesic domes, each emulating a natural biome (an area on the earth with similar climate, plants, and animals), that house plant species from around the world (Fig. 8-1). Collectively they comprise the largest greenhouse structures in the world. The larger biome, covering 15,590 square metres (3.8. acres) sustains the temperature and humidity conditions of a tropical environment; the smaller biome covers 6,540 square meters (1.6 acres) and is regulated to provide a warm temperate, Mediterranean-type environment.

SAMPLES IN THE COLLECTION

Plants from selected areas of the world were collected and planted in these biomes under the temperature and humidity conditions that mimic those from the hot tropical regions (HTB – including the Amazon, West Africa, Malaysia and the Seychelles Islands), and warm temperate regions (WTB – notably the Mediterranean, South Africa and California). To date there are few typical plants from Australasia. A third dome to house plants from desert environments is planned for some future date. There is a strong educational theme to the entire Eden Project; hence most of the plants were selected because of their importance to human needs, including medicine, food, dyes, textiles, fuel, construction materials and the environment in general. Consequently, they are mostly common plants and represent, therefore, good potential candidates to select for mineral exploration.

Among the unique aspects of the Eden Project is the fact that many species of trees, shrubs and smaller plants are growing in controlled conditions. Each biome has

Fig. 8-1. The Eden Project, Cornwall, England. Left: the two biomes. Right: inside the Hot Tropical Biome (HTB).

its own controlled temperature, humidity and soil type. As such, the uniform conditions under which the plants are growing provide an opportunity to evaluate the relative uptake of a wide range of elements by common plants. The chemical analysis of tissues from plants growing under the same conditions provides a guide for selecting optimum species to collect in the biogeochemical exploration for a particular commodity and/or for environmental monitoring. Furthermore, such studies can reveal if any species have the ability to hyper-accumulate metals: such information would be of relevance to any efforts directed at phyto-mining or phyto-remediation. The Eden Project, therefore, provides a unique opportunity to compare and contrast the relative uptake of metals and other elements from diverse areas of the world without the requirement of undertaking lengthy and expensive expeditions to obtain comparable materials, which even then would almost certainly have different substrate compositions making inter-species comparisons uncertain.

SOIL IN THE BIOMES

The soil in the biomes is 1 m deep. The air is kept at temperatures varying between 35 °C and 18 °C, providing a variety of environmental niches in different areas of the biomes. A misting system and a large waterfall provide the high humidity that the species in the HTB require. Conversely, in the WTB the dry, warm and low humidity conditions of Mediterranean climates are maintained. The University of Reading helped devise specific soil recipes for the two biomes and 85,000 tonnes of soil was manufactured from a combination of mine waste (mainly silica sand and lignitic clay) and composted green waste. Inside the HTB, recycled woodchip is used as mulch. The Eden Project also uses other recycled materials on a day-to-day basis, including soil improvers such as composted green waste, bark and forestry wastes.

In both biomes the organic-rich soils have been spread over a base of sand-sized particles obtained from the immediate vicinity of the kaolinite quarries. They are, therefore, granitic in composition. Locally, fertilizers have been applied to provide

the essential nutrients for maintaining the health of certain species – e.g., elevated levels of K and Mg for banana plants (*Musa* spp.). Although there are these local modifications, soils throughout the HTB and WTB are generally similar in overall elemental composition, although the HTB soils contain double the amount of organic matter of that in the WTB (60% compared to 30%). There are some differences between the soils of the two biomes, but the only element that is much more highly concentrated in one biome (the HTB) is Ag. Table 8-I presents average concentrations of 53 elements obtained from analysis of the −80 mesh (ASTM) fraction of soils collected from six widely spaced sites in each biome. To put these data in context, they are compared with 104 soils from a natural environment in central British Columbia – Mount Polley, where Cu–Au porphyry mineralization occurs. All soils were prepared in the same manner by sieving, and were then analyzed at the same laboratory by digesting in aqua regia with an ICP-MS finish.

FOLIAGE COLLECTION FROM THE BIOMES

In order to assess the relative uptake of elements by various species contained within each Eden Project biome, as a first step, representative samples of leaf tissues were collected from a selection of the more common plants. Within a four-hour period, a few leaves from each of 25 species from the WTB and each of 46 species from the HTB were collected, generating a total of 71 samples. These were oven-dried, hermetically sealed, and sent to Canada for milling prior to analysis. One-gram portions of each powdered sample were digested in nitric acid, then aqua regia, with an ICP-MS finish for 51 elements. Tables 8-II and 8-III list the species by biome and geographic area, and provide botanical and common names as well as some noted features.

There are many ways to view the data obtained, hence the complete analysis of each sample is provided on the CD inside the back cover of this book. One perspective is to sort the data with respect to the maximum concentrations of each element and the relative concentration with respect to the mean of the dataset ('anomaly contrast ratio'). Results of this exercise are shown in Table 8-IV. From this table it can be seen that the species concentrating germanium to the greatest degree is bamboo, with a concentration factor (maximum divided by the mean) of 43.6. The 'star cluster' (*Pentas*) has strong relative enrichment of several elements – Co (x40), W (x25), Al (x6.5), Th (x6). Fig (*Ficus*) is enriched with REE (La x34 and Ce x26) and Tl (x14.7). Gold enrichment is the greatest in kapok bush (*Eriocephalus*).

Noted above is the fact that Ag is significantly more enriched in soils of the HTB (average of 16,954 ppb Ag from 6 samples) compared to those in the WTB (average of 162 ppb Ag from 6 samples) It might be expected, therefore, that plants in the HTB would have appreciably higher Ag concentrations than those in the WTB and, in fact, the majority of plants containing the highest levels of Ag occur in the HTB. However, this pattern is not universal. For example, *Acacia nigrescens* (HTB) yielded

TABLE 8-I

Element concentrations in −80 mesh (ASTM) fraction of soils from the two Eden Project biomes, and from over a mineralized zone (Cu–Au porphyry) at Mount Polley in British Columbia. Full analyses of the Eden Project soils are given as Table 8-I D on the CD (back pocket)

	Average		
	HTB ($n = 6$)	WTB ($n = 6$)	Mt.Polley[1] ($n = 104$)
Ag (ppb)	16953	162	274
Al (%)	0.442	0.496	2.038
As (ppm)	9.3	9.9	6.2
Au (ppb)	2.4	4.0	11.0
B (ppm)	16	12	3
Ba (ppm)	64	54	135
Be (ppm)	1.0	0.9	0.5
Bi (ppm)	1.75	2.53	0.16
Ca (%)	0.868	0.527	0.454
Cd (ppm)	0.35	0.22	0.25
Ce (ppm)	12	16	17
Co (ppm)	2.2	2.9	11.5
Cr (ppm)	9.3	8.0	39.7
Cs (ppm)	15.0	18.9	2.6
Cu (ppm)	30	24	92
Fe (%)	0.492	0.543	3.384
Ga (ppm)	3.0	3.0	6.7
Ge (ppm)	–0.1	–0.1	–0.1
Hf (ppm)	0.05	0.07	0.06
Hg (ppb)	129	92	57
In (ppm)	0.06	0.07	0.02
K (%)	0.257	0.207	0.094
La (ppm)	5.8	7.5	9.6
Li (ppm)	61	83	21
Mg (%)	0.093	0.104	0.554
Mn (ppm)	235	253	642
Mo (ppm)	1.48	0.81	1.44
Na (%)	0.031	0.019	0.010
Nb (ppm)	1.36	1.80	0.82
Ni (ppm)	4.9	5.2	22.6
P (%)	0.083	0.053	0.112
Pb (ppm)	37	33	8
Pd (ppb)	–10	–10	–10
Pt (ppb)	–2	–2	–2
Rb (ppm)	51	71	12
Re (ppb)	–1	–1	–1

Continued

TABLE 8-I *Continued*

	Average		
	HTB ($n = 6$)	WTB ($n = 6$)	Mt.Polley[1] ($n = 104$)
S (%)	0.208	0.094	0.007
Sb (ppm)	1.40	1.15	0.28
Sc (ppm)	1.1	1.2	3.8
Se (ppm)	1.0	0.7	0.3
Sn (ppm)	3.6	3.5	0.5
Sr (ppm)	36	24	57
Ta (ppm)	–0.05	–0.05	–0.05
Te (ppm)	–0.02	–0.02	–0.02
Th (ppm)	2.0	3.5	2.0
Ti (%)	0.005	0.006	0.103
Tl (ppm)	0.317	0.397	0.066
U (ppm)	3.4	3.1	0.5
V (ppm)	10	8	97
W (ppm)	2.5	2.3	0.1
Y (ppm)	5.6	6.6	4.0
Zn (ppm)	80	63	98
Zr (ppm)	1.5	2.4	2.8

[1]B-horizon soils from over Cu porphyry mineralization at Mount Polley, British Columbia (from Dunn et al., 2006a,b).

only 85 ppb Ag, whereas the underlying soil contained 74,600 ppb Ag – a plant to soil ratio of 1:878. Conversely, elsewhere in the HTB, rattan (*Calamus*) contained 9,670 ppb Ag (the highest of all the species tested), yet the underlying soil had only 3,800 ppb Ag – a plant to soil ratio of 1:0.39. A similar ratio to the latter occurred in the WTB for the Cape myrtle (*Myrsine africana*) that had 754 ppb Ag, whereas the underlying soil had 447 ppb Ag (a plant to soil ratio of 1:0.59).

Where available, because of its ability to concentrate Ag, the rattan would be a better choice of sample medium than the *Acacia.* Similarly, in warm temperate regimes, the Cape myrtle would be a good choice for optimizing the Ag signature of the underlying substrate, and therefore potentially a useful medium in biogeochemical exploration for Ag-bearing minerals. These examples of Ag serve to demonstrate further the enormous ranges in the capacities of different species to either exclude or absorb and tolerate metals. In the case of *Acacia* leaves, they would not be the optimum sample medium for Ag, but they should not be totally disregarded because over mineralization there may be adequate anomaly to background contrast, such that the patterns of relative enrichment can still be mapped. This reaffirms another point – pattern recognition is of paramount importance in geochemical interpretations of data.

TABLE 8-II

List of samples from the Warm Temperate Biome from which leaves were collected for analysis. Samples from each source area sorted alphabetically by botanical name. Detailed chemical analyses provided on CD in back pocket (Table 8-II D)

Sample #	Botanical name	Family	Common name	Notes on some characteristics
WTB Mediterranean				
MED 7	*Asphodelus fistulosus*	Liliaceae	Onion asphodel; onionweed	Suspected cause of dermatitis in cattle
MED 3	*Ballota acetabulosa*	Labiatae	False dittany	Member of the mint family. Stems once used as wicks for oil lamps
MED 10	*Bougainvillea spp.*	Nyctaginaceae	Bougainvillea	
MED 8	*Chamaerops humilis*	Palmae	Dwarf fan palm	
MED 2	*Cistus laurifolius*	Cistaceae	Rock rose	
MED 1	*Ficus carica*	Moraceae	Fig	The sap and the half-ripe fruits are said to be poisonous. The sap can be a serious eye irritant
MED 4	*Nerium oleander*	Apocynaceae	Oleander; Rose bay	Poisonous sap – glycosides. A single leaf, intensively chewed, has been reported to be lethal
MED 12	*Olea europaea*	Oleaceae	Olive	Leaf properties include antibacterial, antifungal, antiseptic, antiviral, astringent, febrifuge, and tranquilizer
MED 6	*Pinus halepensis*	Pinaceae	Aleppo pine	Resin provides flavour for Retsina wine
MED 9	*Pistacia lentiscus*	Anarcardiaceae	Mastic	Chewed to strengthen the gums, and as a breath sweetener; used as a flavouring in sweets (e.g., 'Turkish delight')
MED 11	*Rhododendron (vireya)*	Ericaceae	Rhododendron	
MED 5	*Tetraclinis articulata*	Cupressus	Arar	Endangered species in Malta and S. Spain

WTB South Africa				
SA 3	*Aloe arborescens*	Asphodelaceae	Krantz aloe	Used in cosmetics and medicinal compounds. Ingesting the plant latex can cause a cathartic action and nephritis
SA 12	*Blechnum tabulare*	Blechnaceae	Table Mountain fern	
SA 2	*Cunonia capensis*	Cunoniaceae	Butterspoon tree; red alder	Member of the lily family
SA 9	*Erica caffra*	Ericaceae	Heather	
SA1	*Eriocephalus africanus*	Compositae	Kapok bush	Linalyl acetate, cymene, 1,8-cineole, many sesquiterpinoids
SA 10	*Leucadendron argenteum*	Proteaceae	Silver tree	
SA 13	*Myrsine africana*	Myrsinaceae	Cape myrtle; African boxwood	Leaves have benzoquinone derivatives, triterpenoids and steroids. Acylated cyanidin glycosides
SA 14	*Nuxia floribunda*	Loganiaceae; (Buddlejaceae)	Kite tree; forest elder	
SA 11	*Protea cynaroides*	Proteaceae	King protea	
SA 6	*Rhumora spp.*	Polypodiaceae	Fern	Probable species is '*adiantiformis*'
SA 5	*Salvia chamelaeagnea*	Labiaceae	Rough blue sage	Leaves produce rosmarinic acid. Medicinal use as a tea for coughs and colds
SA 4	*Sparrmannia africana*	Tiliaceae	African hemp	Proanthocyanidins present; cyanidin. Not cyanogenic
SA 8	*Stoebe plumosa*	Asteraceae	Slangbos	
SA 7	*Tulbaghia fragrans*	Alliaceae	Sweet garlic	

TABLE 8-III

List of samples from the Hot Tropical Biome from which leaves were collected for analysis. Samples from each source area sorted alphabetically by botanical name. Detailed chemical analyses provided on CD in back pocket (Table 8-III D)

Sample #	Botanical name	Family	Common name	Notes on some characteristics
HTB Islands (Seychelles)				
Is 1	*Breynia nivosa*	Euphorbiaceae	Snowbush	
Is 7	*Casuarina cunninghamiana*	Casuarinaceae	River oak: She-oak	Nitrogen-fixing
Is 3	*Chrysobalanus icaco*	Chrysobalanaceae	Cocoplum	Various parts of the plant have been used in folk medicine. It has hypoglycemic effects
Is 5	*Coccoloba uvifera*	Polygonaceae	Sea grape	Salt tolerant
Is 6	*Cocos nucifera*	Palmae	Coconut palm	
Is 10	*Cryptostegia grandiflora*	Asclepiadaceae	Rubber vine	Contains cardiac glycosides
Is 12	*Dendrocalamus asper*	Poaceae	Bamboo	
Is 4	*Hyophorbe lagenicaulis*	Palmae	Bottle palm	
Is 11	*Impatiens spp*	Balsaminaceae	Busy-lizzie	
Is 2	*Mangroves*	Many	Mangrove	About 80 species, 9 in Seychelles. All salt-tolerant and high in tannins. Contain salts, organic acids, carbohydrates, hydrocarbons, benzoquinones, naphthofurans, sesquiterpenes, triterpenes, alkaloids, flavonoids, polymers, and sulphur derivatives
Is 9	*Rothmannia annae*	Rubiaceae	Wright's gardenia	
Is 8	*Terminalia catappa*	Combretaceae	India's almond	Leaves contain agents for chemo-prevention of cancer and probably have anticarcinogenic potential. Antioxidant

HTB Malaysia				
MAL 8	*Calamus spp.*	Arecaceae	Rattan	
MAL 6	*Cananga odorata*	Annonaceae	Ylang-ylang	
MAL 2	*Etlingera elatior*	Zingiberaceae	Torch ginger	
MAL 1	*Ficus auriculata*	Moraceae	Fig	
MAL 5	*Ficus religiosa*	Moraceae	Bo tree	
MAL 3	*Musa spp*	Musaceae	Banana	
MAL 4	*Pogostemon cablin*	Lamiaceae	Patchouli	Oil used to treat colds, headaches, nausea, diarrhea and abdominal pain. Popular aphrodisiac
MAL 7	*Syzygium cumini*	Myrtaceae	Java plum	Antibacterial properties
TP2	*Tectona grandis*	Verbenaceae	Teak	Malaysia
HTB West Africa				
WA 4	*Elaeis guineensis*	Palmae	African oil palm	
TP 1	*Musa spp.*	Musaceae	Banana	
WA 5	*Mussaenda erythrophylla*	Rubiaceae	Flame of the forest	
WA 3	*Nauclea diderrichii*	Rubiaceae	Box wood	
WA 6	*Pentas lanceolata*	Rubiaceae	Star cluster	
WA 1	*Tabernanthe iboga*	Apocynaceae	Leaf of God	Aphrodisiac, CNS-stimulant, hallucinogenic, stimulant, and tonic
WA 2	*Thaumatococcus daniellii*	Marantaceae	African serendipity berry	Natural sweetener 'thaumatin'
WA7	*Thunbergia erecta*	Acanthaceae	King's mantle	
HTB South America				
Sam 9	*Acacia nigrescens*	Fabaceae	Knob thorn	
Sam 15	*Allamanda cathartica*	Apocynaceae	Golden trumpet vine	Poisonous. Fever, swollen lips, thirst, nausea, diarrhea. Skin irritation upon contact with cell sap

Continued

TABLE 8-III *Continued*

Sample #	Botanical name	Family	Common name	Notes on some characteristics
Sam 10	*Annona glabra*	Annonaceae	Pond apple	Leaves contain acetogenins
Sam 8	*Bauhinia thonningii*	Caesalpiniaceae	Camel's foot	Antiseptic properties used for treating wounds
Sam 13	*Cecropia spp.*	Cecropiaceae	Trumpet tree	Caustic latex. Invariably infested with stinging ants
Sam 7	*Cola nitida*	Malvaceae	Bitter cola	Cola nut can mimic malaria-like symptoms; contains caffeine and cyanide
Sam 3	*Costus afer*	Zingiberaceae		Very acidic. Edible flowers. Anti-inflammatory
Sam 12	*'Fern'*	Polypodiaceae	Fern	
Sam 1	*Inga edulis*	Fabaceae	Monkey tail	
Sam 6	*Inga spp.*	Fabaceae	Monkey tail	
Sam 2	*Malvaviscus arboreus*	Malvaceae	Wax mallow	
Sam 4	*Manihot esculenta*	Euphorbiaceae	Cassava	Roots used to make the intoxicant 'cassiri' and tapioca. Rich in cyanide compounds, making it resistant to most insect predators
Sam 5	*Pachira aquatica*	Bombacaceae	Water chestnut; Money tree	
Sam 14	*Persea americana*	Lauraceae	Avocado	
Sam 16	*Psychotria punctata*	Rubiaceae	Wild coffee	
Sam 11	*Tamarindus indica*	Caesalpiniaceae	Tamarind	

TABLE 8-IV

Eden Project – analyses of dry leaves from 71 species. Relative concentrations of each of the 51 elements that were determined. Sorted in order of concentration factor (last column)

Element	Units	Genus	Common name	Biome	Area	Maximum concentration	Average concentration of all samples	Conc. factor relative to all 71 Eden samples
Ge	ppm	*Dendrocalamus*	Bamboo	HTB	Seychelles	10.2	0.23	43.6
Co	ppm	*Pentas*	Star Cluster	HTB	W.Africa	47.2	1.17	40.2
La	ppm	*Ficus*	Fig	WTB	Med.	2.86	0.08	34.2
Nb	ppm	*Leucadendron*	Silver tree	WTB	S.Africa	0.53	0.02	31.6
Ce	ppm	*Ficus*	Fig	WTB	Med.	3.4	0.13	26.1
W	ppm	*Pentas*	Star Cluster	WTB	W.Africa	7.1	0.28	25.3
Ag	ppb	*Calamus*	Rattan	HTB	Malaysia	9670	429	22.6
Bi	ppm	*Cryptostegia*	Rubber vine	HTB	Seychelles	0.52	0.03	20.4
Tl	ppm	*Ficus*	Fig	WTB	Med.	0.82	0.06	14.7
Sb	ppm	*Myrsine*	Cape Myrtle	WTB	S.Africa	7.1	0.49	14.4
As	ppm	*Cecropia*	Trumpet tree	HTB	S.America	28.3	2.0	14.0
Cs	ppm	*Rhumora*	Fern	WTB	S.Africa	16.7	1.23	13.6
Cd	ppm	*Asphodelus*	Onionweed	WTB	Med.	2.2	0.17	12.8
Pb	ppm	*Ballota*	False dittany	WTB	Med.	5.8	0.48	12.2
Mn	ppm	*Tabernanthe*	Leaf of God	HTB	W.Africa	4196	485	8.7
Mo	ppm	*Acacia*	Acacia	HTB	S.America	10.8	1.34	8.1
Y	ppm	*Ficus*	Fig	WTB	Med.	1	0.13	7.8
Re	ppb	*Rothmania*	Gardenia	HTB	Seychelles	149	19	7.8
Na	%	*Impatiens*	Busy-lizzie	HTB	Seychelles	1.6	0.21	7.7
Zn	ppm	*Impatiens*	Busy-lizzie	HTB	Seychelles	455	62	7.4
Ta	ppm	*Tetraclinis*	Arar	WTB	Med.	0.006	0.0008	7.3
Ba	ppm	*Blechnum*	Fern	WTB	S. Africa	115	16	7.1
Au	ppb	*Eriocephalus*	Kapok bush	WTB	S.Africa	33.5	4.8	7.0
Al	%	*Pentas*	Star Cluster	HTB	W.Africa	0.04	0.006	6.5
Th	ppm	*Pentas*	Star Cluster	HTB	Malaysia	0.05	0.008	6.1
Rb	ppm	*Etlingera*	Torch ginger	HTB	Malaysia	216	38	5.7

Continued

TABLE 8-IV *Continued*

Element	Units	Genus	Common name	Biome	Area	Maximum concentration	Average concentration of all samples	Conc. factor relative to all 71 Eden samples
U	ppm	*Tetraclinis*	Arar	WTB	Med.	0.98	0.18	5.5
Be	ppm	*Myrsine*	Cape Myrtle	WTB	S. Africa	0.5	0.09	5.5
Ni	ppm	*Dendrocalamus*	Bamboo	HTB	Seychelles	5.2	1.3	3.9
Cu	ppm	*Cryptostegia*	Rubber vine	HTB	Seychelles	37	9.7	3.8
Te	ppm	*Calamus*	Rattan	HTB	Malaysia	0.04	0.011	3.8
Sn	ppm	*Casuarina*	She-oak	HTB	Seychelles	0.52	0.14	3.6
Zr	ppm	*Pogostemon*	Patchouli	HTB	Malaysia	0.7	0.20	3.6
Hf	ppm	*Pogostemon*	Patchouli	HTB	Malaysia	0.018	0.005	3.5
Li	ppm	*Musa*	Banana	HTB	W.Africa	26	7.4	3.5
Hg	ppb	*Syzygium*	Java plum	HTB	Malaysia	125	36	3.4
B	ppm	*Ficus*	Fig	HTB	Malaysia	277	83	3.3
Cr	ppm	*Dendrocalamus*	Bamboo	HTB	Seychelles	7.2	2.21	3.3
Sr	ppm	*Ficus*	Fig	HTB	Malaysia	115	38	3.0
Ca	%	*Bougainvillea*	Bougainvillea	WTB	Med.	4.71	1.597	2.9
Se	ppm	*Ballota*	False dittany	WTB	Med.	0.6	0.22	2.8
S	%	*Impatiens*	Busy-lizzie	HTB	Seychelles	1.03	0.37	2.8
K	%	*Psychotria*	Coffee	HTB	S.America	5.09	2.10	2.4
P	%	*Ficus*	Fig	HTB	Malaysia	0.577	0.245	2.4
Fe	%	*Musa*	Banana	HTB	Malaysia	0.029	0.013	2.3
Mg	%	*Tabernanthe*	Leaf of God	HTB	W.Africa	0.953	0.412	2.3
Ti	ppm	*Fern*	Fern	HTB	S.America	19	8.6	2.2
Sc	ppm	*Calamus*	Rattan	HTB	Malaysia	0.4	0.22	1.8
Ga	ppm	*Musa*	Banana	HTB	Malaysia	0.1	0.06	1.7
In	ppm					<0.01	<0.01	
V	ppm					<1	<1	

TABLE 8-V

Eden Project – analysis of dry leaves from 71 species. Average concentrations by biome and geographic area, compared to the 'Reference Plant' of Markert (1994)

	Warm temperate biome			Hot tropical biome					Ref. plant
	Mediterranean ($n = 12$)	South Africa ($n = 14$)	All WTB ($n = 26$)	Seychelles ($n = 12$)	Malaysia ($n = 8$)	West Africa ($n = 7$)	South America ($n = 16$)	All HTB ($n = 43$)	Markert (1994)
Ag (ppb)	44	110	79	932	1625	200	145	649	20[1]
Al (%)	0.006	0.005	0.006	0.006	0.005	0.010	0.006	0.007	0.008
As (ppm)	1.0	1.3	1.2	1.8	1.7	3.9	3.0	2.6	0.1
Au (ppb)	4.0	5.9	5.0	5.3	6.9	2.4	3.5	4.5	0.2[1]
B (ppm)	69	49	58	71	121	95	103	96	40
Ba (ppm)	20	21	21	17	13	9	13	13	40
Be (ppm)	0.09	0.12	0.11	0.08	0.08	0.07	0.08	0.08	0.001
Bi (ppm)	0.02	0.02	0.02	0.06	0.02	0.02	0.01	0.03	0.01
Ca (%)	1.80	1.42	1.60	1.86	1.55	0.94	1.68	1.59	1
Cd (ppm)	0.29	0.23	0.26	0.21	0.11	0.18	0.05	0.13	0.05
Ce (ppm)	0.37	0.13	0.24	0.08	0.09	0.07	0.04	0.07	0.5
Co (ppm)	0.12	0.13	0.12	0.24	0.22	7.98	1.20	1.86	0.2
Cr (ppm)	2.09	1.71	1.88	2.72	2.39	2.56	2.14	2.42	1.5
Cs (ppm)	0.59	2.50	1.62	0.50	2.25	0.73	0.97	1.04	0.2
Cu (ppm)	9.4	8.0	8.7	10.3	9.5	11.7	10.0	10.3	10
Fe (%)	0.012	0.015	0.014	0.013	0.014	0.013	0.011	0.012	0.015
Ga (ppm)	0.06	0.05	0.06	0.06	0.06	0.06	0.05	0.06	0.1
Ge (ppm)	0.07	0.01	0.04	0.86	0.24	0.27	0.07	0.36	0.01
Hf (ppm)	0.006	0.006	0.006	0.006	0.007	0.004	0.003	0.005	0.05
Hg (ppb)	21	19	20	53	56	40	40	46	20[1]
In (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.001
K (%)	1.71	1.90	1.81	2.11	2.47	2.98	2.11	2.32	1.9
La (ppm)	0.29	0.07	0.17	0.04	0.04	0.04	0.02	0.03	0.2
Li (ppm)	5.68	9.24	7.59	8.30	7.03	7.80	5.25	6.85	0.2
Mg (%)	0.37	0.32	0.35	0.50	0.46	0.46	0.41	0.45	0.2
Mn (ppm)	379	352	364	632	492	1037	263	535	200
Mo (ppm)	0.79	2.24	1.57	1.58	0.43	1.08	1.43	1.23	0.5

Continued

TABLE 8-V *Continued*

	Warm temperate biome			Hot tropical biome					Ref. plant
	Mediterranean ($n = 12$)	South Africa ($n = 14$)	All WTB ($n = 26$)	Seychelles ($n = 12$)	Malaysia ($n = 8$)	West Africa ($n = 7$)	South America ($n = 16$)	All HTB ($n = 43$)	Markert (1994)
Na (%)	0.127	0.507	0.331	0.352	0.091	0.033	0.058	0.142	0.15
Nb (ppm)	0.013	0.048	0.032	0.008	0.009	0.010	0.007	0.008	0.05
Ni (ppm)	0.8	1.1	1.0	1.5	1.2	2.3	1.5	1.6	1.5
P (%)	0.232	0.214	0.222	0.252	0.284	0.246	0.261	0.260	0.2
Pb (ppm)	1.0	0.4	0.7	0.4	0.5	0.5	0.3	0.4	1
Rb (ppm)	24	42	34	28	61	43	41	41	50
Re (ppb)	9	3	6	37	8	39	24	27	0.1[1]
S (%)	0.36	0.44	0.40	0.51	0.21	0.36	0.31	0.35	0.3
Sb (ppm)	0.53	1.72	1.17	0.11	0.07	0.06	0.13	0.10	0.1
Sc (ppm)	0.19	0.19	0.19	0.23	0.24	0.24	0.23	0.23	0.02
Se (ppm)	0.23	0.16	0.20	0.28	0.18	0.26	0.20	0.23	0.02
Sn (ppm)	0.18	0.08	0.13	0.21	0.14	0.14	0.13	0.16	0.2
Sr (ppm)	47	34	40	44	38	20	38	37	50
Ta (ppm)	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Te (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02[1]
Th (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.005
Ti (ppm)	8	8	8	9	10	9	9	9	5
Tl (ppm)	0.10	0.04	0.07	0.04	0.11	0.04	0.03	0.05	0.02[1]
U (ppm)	0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.01
V (ppm)	<1	<1	<1	<1	<1	<1	<1	<1	0.5
W (ppm)	<0.1	0.29	0.18	<0.1	0.06	1.25	0.30	0.35	0.2
Y (ppm)	0.20	0.16	0.18	0.11	0.13	0.07	0.07	0.09	0.2
Zn (ppm)	69	74	71	80	49	43	50	57	50
Zr (ppm)	0.22	0.23	0.22	0.23	0.27	0.14	0.13	0.18	0.1

[1]Denotes modifications to Markert's compilation.

Another summary of the data shows the average concentrations of each element by geographic region (Table 8-V).

Except for Ag, the average concentrations of elements by region are generally quite similar and provide, therefore, a broad indication of what might be considered the norm for each geographic area. This adds another dimension to Markert's (1994) compilation of data to define a world 'Reference Plant' (see Chapter 1), for which the data are repeated in the last column of Table 8-V and which generally show a close correspondence with the data from the Eden Project biomes. There are some differences, such as relatively high Co and Re in samples from West Africa (which for these purposes includes the Democratic Republic of Congo, DRC). This raises the possibility that plants from West Africa might have developed a tolerance for Co, because of the significant world-class Co deposits in the Katanga region of the DRC.

With respect to higher Au and As levels than in Markert's reference plant, these values from the Eden Project may reflect the 'green waste' that has been added to the soil mix. Elevated levels of Li, B and U probably reflect the composition of the somewhat radioactive and alkali-rich Hercynian granites that make up part of the soil mix.

The Eden Project database in its present prototype form gives a glimpse of its potential for providing comparative baseline information for the many thousands of species currently growing under its domes. However, even in its limited form, it can be used as a first pass for selecting a suitable biogeochemical sample medium. If a particular species that is shown in these tables to accumulate an element of interest proves not to be present in a proposed survey area, given no other baseline information, other species and genera of the same family would be the preferred sample media. Databases for all plants are not available, and so the data provided here can be used just as a general guide. From these tables, a short list of plants can be generated to take to the field or, better still, take to a botanist with the knowledge of the proposed survey area for an opinion on the likelihood of which of the preferred species/genera might be present.

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Chapter 9

BIOGEOCHEMICAL BEHAVIOUR OF THE ELEMENTS

INTRODUCTION

As a general guide, the achievable levels of precision that are currently available from commercial laboratories for analysis by 1CP-MS of 1 g samples of dry vegetation are as follows:

- up to 2 times detection limit +/– 100%,
- 2–5 times detection limit +/– 50%,
- 5–10 times detection limit +/– 25%, and
- >10 times detection limit from about +/– 10 to 15%.

These broad generalizations can be improved upon by analysing larger samples (at greater cost) and these levels of precision vary from one element to another and from one laboratory to another. For example, Au reproducibility at sub-ppb levels is poor to fair. As noted in Chapter 6, the official listings for NIST 1575a (pine needles) show that five laboratories provided data for gold, all by INAA, yet returned average values of 0.56–2.6 ppb Au. Conversely, the precision for Bi close to detection is commonly good. Analyses for Rb, invariably present at levels well above detection, generate precision better than +/– 5% RSD.

Perfect precision for all elements is an unreasonable expectation for a typical analytical dataset generated by currently available low-cost ICP-MS from commercial laboratories. The cost of providing 'assay quality' data for more than 60 elements by ICP-MS would be several hundred dollars per sample instead of the few tens of dollars that are usually charged for a multi-element package. Given these constraints, a certain amount of tolerance should be given to the overall quality of the data that are generated, bearing in mind that for biogeochemical exploration purposes it is *pattern recognition* of element distributions that is of greater importance than perfect precision and accuracy. It is better, therefore, to plot data distributions as percentile ranges rather than rely upon absolute values.

THE IMPORTANCE OF DATA QUALITY

From a purely statistical analysis of data that are close to detection, on occasion an impression might be gained that the overall data quality is poor. Usually, the

'poor' precision is confined to levels very close to detection – and therefore to be expected. At higher levels precision invariably improves substantially. If it does not, then there is a problem that needs to be discussed with the analytical laboratory.

The research chemist can, with meticulous and commonly time-consuming care, obtain highly reproducible analytical data from the analysis of dry vegetation samples. In the 'real world' of commercial analytical laboratories where technicians systematically produce a vast amount of data for a wide range of elements at extraordinarily low cost the question posed by Bettenay and Stanley (2001) needs to be reiterated: 'Are the data fit for the purpose on hand?' That is to say, can meaningful interpretations be extracted from a dataset that is not perfect?

To assess these questions, this chapter provides notes on each element based on experience gained from analysis of tens of thousands of dry plant samples by ICP-MS, and for some elements by INAA, over several years. Interspersed among these samples, at a density of approximately one in twenty, were several thousand control samples, including many hundreds of splits of in-house control 'V6' described in Chapters 6 and 7. The *accuracy* of the data obtained on V6 can be assessed from comparison of values obtained by the same analytical method on 19 samples of the international pine needle control material NIST 1575a (discussed in Chapter 6). The values reported by the National Institute of Standards and Technology for Standard Reference Material 1575a fall into three categories:

- *Certified values*: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated and accounted for by NIST. However, 'certified' values' are available for only twelve elements – Al, Ba, Ca, Cd, Cl, Cu, Fe, Hg, K, P, Rb and Zn.
- *Reference values*: These values are based on results obtained from a single NIST analytical method. They are

  > non-certified values that are the best estimate of the true value; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty.

  Reference values are available for eleven elements – As, B, Co, Cs, Mg, Mn, Na, Ni, Pb, Sc and Se.
- *Information values*: Data for another two elements (Ce, Cr) are provided for information purposes, only. These are non-certified values with no uncertainty assessed.

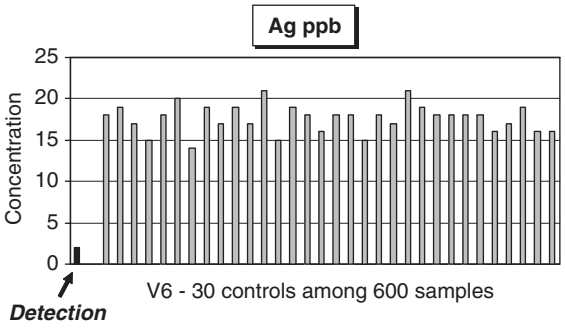
These three categories show that there are reference data available for only 25 elements. In addition, for most elements, the NIST website (www.nist.gov/srm) shows tables of analytical determinations provided by the laboratories that participated in testing NIST 1575a. In the tables shown under each element in the following pages, these categories of data accuracy are combined under the single term of 'target value'. The values listed under NIST 1575a that are in parentheses are those for which data are not certified, indicating uncertainty as to their true

concentrations. For some elements, data are provided in this chapter for which there are no known published reference results (e.g., Ge, Li, Sn, Ti, Tl, Y and Zr).

ELEMENTS IN PLANTS AND THEIR RELEVANCE TO MINERAL EXPLORATION

Elements are listed in alphabetical order by their element name, rather than by chemical symbol. Except for a few elements that are grouped together, such as the platinum group elements (PGE), the rare earth elements (REE) and the halogens (F, Cl, Br, I), they are discussed individually. Discussion of each element is preceded by a table alongside a bar chart designed to demonstrate for that element the typical precision that has been obtained (and can be expected). The first (solid) bar of each histogram shows the detection limit. In most cases, the in-house control V6 has been used as an example, because of the many hundreds of analyses that are now available. A secondary control is V17 (mountain hemlock twigs [*Tsuga heterophylla*]) containing higher concentrations of those few elements that are consistently below detection in V6. The bar charts demonstrate the analytical *precision* (i.e., reproducibility). By way of assessing the analytical *accuracy*, data are provided for NIST 1575a. Each control sample (V6, V17 and NIST 1575a) has statistics computed on determinations of concentrations in 30, 22 and 19 samples, respectively. Figure 9-1 is an annotated example.

	V6**	NIST 1575a**
Target*	18	(12)
Mean	17.6	12
Std. Dev.	1.7	1.5
RSD %	10	12



***Target:** For NIST 1575a 'Target' indicates a certified value, unless it is in parentheses in which case it is a 'reference' or 'information' value, or simply the average value shown in the tables from the NIST website. For V6 the 'Target Value' is derived from the mean of several hundred analyses.

****Mean, Standard Deviation, and Relative Standard Deviation** (RSD) values calculated from:

- The 30 samples of V6 or 22 samples of V17 shown as examples in the bar charts.
- 19 samples of NIST 1575a (not shown as bar charts)

Values for each element are expressed as the same units that are shown in each bar chart (i.e., silver as ppb in this example). RSD% is widely used in analytical chemistry to express the precision of analytical data. This is defined in chapter 10.

Fig. 9-1. Annotated explanation of chart shown in the discussion of each element.

In the following commentaries on each element, unless indicated otherwise, the analytical procedure has involved digestion of 1 g of milled dry tissue in nitric acid, followed by aqua regia, with a quadrupole ICP-MS finish. This is a very economical multi-element analytical procedure that is provided by a number of commercial analytical laboratories. The reader should appreciate that a total analysis (e.g., by INAA) or by other digestions can be requested for specific elements that may present problems with respect to analytical interferences from this digestion (e.g., As, Cr, Pd, S, Se, V). Furthermore, data can be obtained from high-resolution ICP-MS instrumentation (HR-ICP-MS) that provide lower detection limits than those presented here without inter-element interferences. At the time of writing, HR-ICP-MS is not widely available at commercial laboratories and is more costly, but holds excellent potential for future analysis of dry vegetation samples.

Aluminium (Al) (Fig. 9-2)

Aluminium is a common constituent of plants, and in dry tissue it is usually present at concentrations quite close to the detection limit by ICP-MS of 0.01%. At this level, precision is typically +/–100%, but improves considerably at slightly higher levels, such as found in V6 for which RSD is 11%. If an isolated anomalous value occurs in the field data, contamination should first be suspected.

Aluminium is an essential element for many plants, and helps to control colloidal cell properties. However, in soils with a high level of soluble Al compounds, most plant species have developed resistance mechanisms to avoid or tolerate the potentially toxic effects of Al (Foy et al., 1978; Jansen et al., 2003). Tea is somewhat enriched in Al (Owen et al., 1992; Dong et al., 2001), and about 32% of Al-accumulating species belong to the large, mainly tropical, Rubiaceae Family. This family comprises about 450 genera and more than 6500 species of trees and shrubs, including such plants as wild coffee. In the data listings from the Eden Project

	V6	NIST 1575a
Target	0.05	0.06
Mean	0.047	0.06
Std. Dev.	0.1	0.01
RSD %	11	10

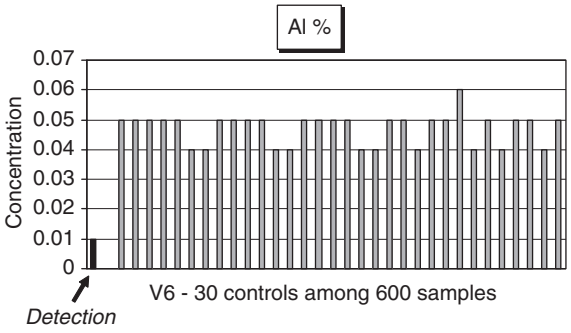


Fig. 9-2. Aluminium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

samples (CD in back pocket), the two plants containing the most Al were from the Rubiaceae Family. Aquatic plants have the capability of accumulating high levels of Al – e.g., *Platyhypnidium ripariodes* (an aquatic moss comprising the SRM BCR-CRM-061) contains in excess of 1% Al in dry tissue. In general, bark of conifers is far more enriched in Al than twigs or foliage of the same species.

In biogeochemical exploration, if plots of Al data indicate trends across a survey area, they may be delineating zones of alteration, because the breakdown of feldspars to clay minerals enhances the capability of roots to absorb Al from the significantly greater surface area offered by fine-grained clay minerals compared to well crystallized feldspars.

Antimony (Sb) (Fig. 9-3)

Precision is fair to poor at the levels of Sb commonly present in plants. Whereas the control data rarely exhibit extreme 'spikes' in the data, values are sometimes erratic. It is usual for precision to be considerably improved when Sb values in survey samples are above 0.2 ppm Sb.

Although Sb can be readily taken up by plants in soluble forms it is considered a non-essential element (Kabata-Pendias, 2001) and it is usually present at low-ppm levels. In plants from the Eden Project, those from the Mediterranean regions have higher concentrations than those from the tropics, reaching a maximum of 7 ppm Sb in leaves from the South African Cape Myrtle and 5 ppm Sb in heather. This is in spite of soils from the two biomes having similar Sb contents.

Mushrooms analysed by INAA contain mostly < 100 ppb Sb in background areas, but up to 0.14% Sb in *Chalciporus piperatus* (Peppery Bolete) is reported in samples from the Přibram mining district (Pb–Zn–Ag–Cu–U) in the Czech Republic (Borovička et al., 2006b).

During the process of reducing plant tissues to ash by controlled ignition at 475°C there is some loss of Sb from many plant species. Typically, this loss is about 20%,

	V6	NIST 1575a
Target	0.06	(0.01)
Mean	0.06	0.02
Std. Dev.	0.01	0.02
RSD %	18	80

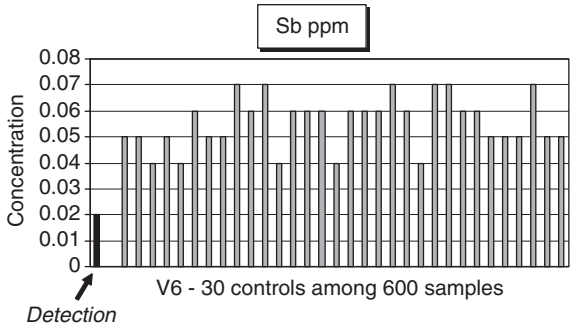


Fig. 9-3. Antimony – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

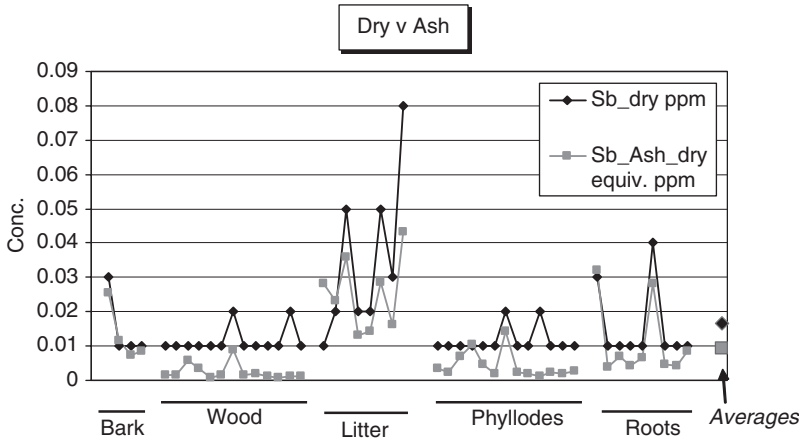


Fig. 9-4. Antimony in *Acacia* tissues and underlying forest litter. Comparisons of losses during ashing. 'Dry equivalent' is the concentration in ash, normalized to a dry-weight basis.

but it can be higher. In Fig. 9-4, losses during ashing of *Acacia* from Australia are shown for different tissues. Except for minimal loss from the bark, other tissues exhibit fairly consistent losses.

Over and near Au–As–Sb mineralization, there is commonly a clear but subtle relationship of Sb in plant tissues to the zone of mineralization. Studies in Bolivia using 'Thola' vegetation (*Baccharis spp.*) have demonstrated a positive response in Sb to mineralization (Viladevall and Queralt, see 'Case History' in Chapter 11).

Arsenic (As) (Fig. 9-5)

Many survey samples yield values close to the detection limit of 0.1 ppm As with the attendant poor precision. V6 has an average of 0.58 ppm, at which level precision is good but values below 1 ppm As should still be treated with caution, because there are a number of analytical interferences that make accurate measurement by ICP-MS difficult. It should be noted that some laboratories declare that low levels of As cannot be accurately measured by ICP-MS, whereas others claim to have circumvented this problem and provide reasonably precise data. At low levels of As, data for field and analytical duplicates should be carefully checked to verify reproducibility of the data.

For As concentrations at higher levels, such as those found typically in Douglas-fir, the analytical reproducibility from two splits of the same samples can be remarkably good. Sixty-four Douglas-fir twigs were re-analysed by ICP-MS, a few weeks after the first set of analytical data were received. The re-analysis was of new splits of the original milled sample, not re-analysis of solutions. Figure 9-6 shows that the *precision* obtained on repeats of 64 samples was almost perfect throughout the

	V6	NIST 1575a
Target	0.6	0.04
Mean	0.6	0.02
Std. Dev.	0.2	0.14
RSD %	33	74

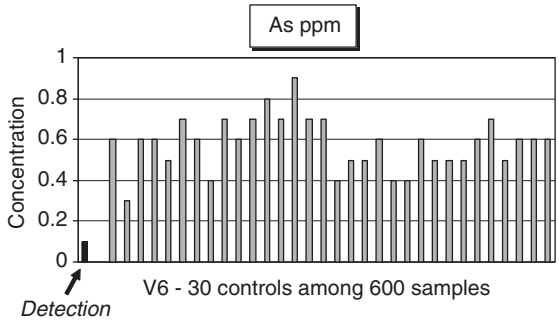


Fig. 9-5. Arsenic – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

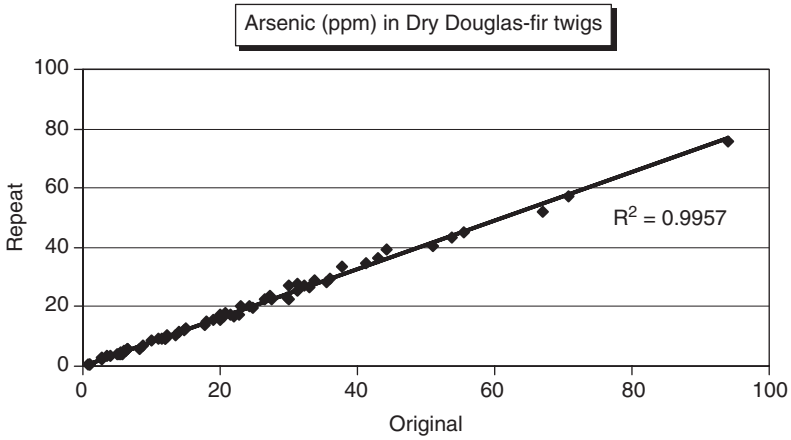


Fig. 9-6. Reproducibility of arsenic data on separate splits after several weeks (dry twigs of Douglas-fir) ($n = 64$).

range of concentrations (1–94 ppm As), although the repeat analysis provided data that were consistently more than 10% lower than the original so there was a problem with the *accuracy* of the data. This emphasizes the importance of inserting controls that will assist in identifying batch-by-batch drift in the analytical data (Fig. 9-6).

Warren et al. (1968) were the first to note the extraordinary ability that Douglas-fir (*Pseudotsuga menziesii*) has to accumulate As, even in areas where there is little As in the ground. The reason for the scavenging of As is unknown, although it is speculated here that, since in the spring As enrichment is the greatest in the growing tips, the tree may be using As as a defence against insects attacking the succulent new growth. Subsequently, it has been found that the coniferous trees western hemlock (*Tsuga heterophylla*) and mountain hemlock (*Tsuga mertensiana*) from British Columbia are also capable of concentrating high levels of As, thus establishing their suitability as sample media for mineral exploration (Dunn, 1995a,b). There is a

wealth of literature on the transfer of As from soils into plants, and these are succinctly summarized by Lombi and Nolan (2005) with additional observations by Anke (2005). It seems that phospholipids can play a role in the metabolism of primitive plants (algae and fungi), partly because As is chemically similar to phosphorus. Since seaweeds are macroalgae, this would account for the relative enrichments of As in brown seaweeds – notably by the common wrack-weed *Fucus* that predominates in many intertidal zones around the world, and by the gulf weed *Sargassum* (Dunn, 1990, 1998a,b,c). Consequently, coastal biogeochemical exploration surveys, especially fjorded coastlines, can take advantage of this phenomenon by collecting brown seaweeds from strategic locations (e.g., drainage into the sea) in inlets in order to identify and prioritize exploration targets. *Fucus* is particularly useful in that it can grow in brackish water. Rugged, highly indented fjord coastlines receive a substantial amount of fresh water from rainfall and melting winter snows, and as a result the dominant brown seaweed is commonly *Fucus*.

Ma et al. (2001) reported that the brake fern (*Pteris vittata*) is a hyperaccumulator of As, concentrating up to 7526 ppm As in dry fronds from samples growing on a site in Florida contaminated with chromated copper arsenate. Further laboratory tests over a six-week period established concentrations of more than 2.2% As in the same species grown in soils spiked with 1500 ppm As. Subsequently, other species of this genus and of other fern genera have exhibited similar ability to concentrate As, but out of the 20,000 or so species of ferns that have been identified this appears to be the exception rather than the rule, and not characteristic of all ferns species. A regional biogeochemical survey of the western Amazon, involving more than 350 samples of tree fern (*Cyathea spp.*), yielded a maximum of 2.9 ppm As against a median value for the dataset of 0.2 ppm As. However, as is repeatedly noted in this book, the absolute As concentrations are not of particular importance, because the regional *patterns* of As enrichment assisted in delineating zones of Au mineralization.

In the exploration for minerals, whether the sample medium is rock, soil, water or vegetation, As can be of great value as a pathfinder element for various types of deposit, including gold, base metals and platinum-group metals. Hence, the data for As should be carefully evaluated and distribution patterns assessed with respect to the geological context of a survey area.

Barium (Ba) (Fig. 9-7)

The detection limit for Ba (0.1 ppm) is well below the typical concentrations in survey samples (several to tens of ppm in dry tissue). V6 has 9 ppm Ba and precision is excellent.

Barium is a bi-valent element that, along with Mg, Ca and Sr, is one of the principal alkaline earths. The remaining elements in this group are the rare Be and Ra. Barium can be toxic to plants, but it can also be highly concentrated in some plants such as the brazil nut (*Bertholletia excelsa*) and Douglas-fir (*Pseudotsuga*

	V6	NIST 1575a
Target	9	6
Mean	9	6
Std. Dev.	0.5	0.5
RSD %	5	8

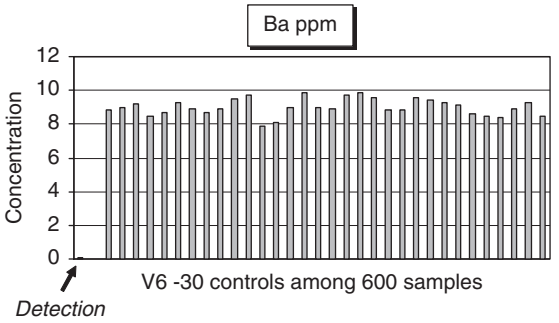


Fig. 9-7. Barium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

menziesii). At the Hoidas Lake REE-rich allanite deposits in northern Saskatchewan, mineralization has associated with it Ba-rich feldspar and some barite. An orientation biogeochemical study (Dunn and Hoffman, 1986) showed that birch, Labrador tea, alder, black spruce and jack pine were all enriched in Ba, with highest concentrations in twigs and bark; cones contained relatively little Ba (Table 9-I). A maximum of 535 ppm Ba was recorded in dry twigs of alder (three years growth).

The Ba values shown in Table 9-I are unusually high for vegetation in general, because of the presence of Ba-rich rocks. However, they serve to show the relative uptake of Ba by a typical range of common plants present in the boreal forests of North America, Europe and Asia. Furthermore, they demonstrate the relative uptake by the different plant tissues.

Patterns of Ba distribution in biogeochemical datasets can assist in delineating carbonate-rich zones, because of the chemical affinities of Ba with, in particular, Sr and Ca both of which are present in carbonates. Kovalevsky (1987) noted that intense biogeochemical anomalies of Pb, Zn and Ba were identified in various species of wormwood (*Artemisia spp.*) over sulphide bodies buried beneath aeolian sand some 10–30 m thick. In light of this observation, Ba anomalies in plant tissues should be sought in areas of suspected sulphide and Irish-type Pb–Zn carbonate mineralization.

At a number of localities Ba enrichment in vegetation has been recorded in a zone peripheral to that of Au enrichment. At one occurrence, Au enrichment in alder twigs and black spruce bark had coincident As and Sb anomalies, with up to 300 ppm Ba in dry bark from an adjacent zone reaching 400 m in width (e.g., Dunn, 1984). This emphasizes that pattern recognition is of paramount importance in successfully interpreting the significance of biogeochemical data.

Beryllium (Be)

Beryllium is rarely concentrated above the detection limit of 0.1 ppm Be in dry plant tissue, including the controls considered in this chapter, and so there is no

TABLE 9-I

Barium in plant tissues from near an outcrop of massive REE-bearing allanite associated with Ba-rich feldspar (hyalophane) and minor barite. Modified after Dunn and Hoffman (1986). Analysis by INA on ashed tissue

Common name	Botanical name	Tissue	Ba in ash (ppm)	Ash yield (%)	Ba in dry tissue (ppm)
Paper birch	*Betula papyrifera*	Twig	13,000	1.82	237
Paper birch	*Betula papyrifera*	Leaf	3000	3.96	119
Paper birch	*Betula papyrifera*	Bark	14,000	2.78	389
Labrador tea	*Ledum groenlandicum*	Root	18,000	0.8	144
Labrador tea	*Ledum groenlandicum*	Stem	1700	1.25	21
Mountain alder	*Alnus crispa*	Twig	22,000	2.43	535
Mountain alder	*Alnus crispa*	Leaf	8300	3.65	303
Mountain alder	*Alnus crispa*	Leaf	8600	3.65	314
Black spruce	*Picea mariana*	Twig	16,000	1.87	299
Black spruce	*Picea mariana*	Twig	18,000	1.71	308
Black spruce	*Picea mariana*	Bark	16,000	2.81	450
Black spruce	*Picea mariana*	Trunkwood	12,000	0.32	38
Black spruce	*Picea mariana*	Cones	2700	0.5	14
Black spruce	*Picea mariana*	Twig	21,000	1.52	319
Jack pine	*Pinus banksiana*	Twig	9900	1.68	166
Jack pine	*Pinus banksiana*	Needles	7800	2.92	228
Jack pine	*Pinus banksiana*	Trunkwood	20,000	0.41	82
Jack pine	*Pinus banksiana*	Bark	15,000	2.03	305
Jack pine	*Pinus banksiana*	Cones	3100	0.26	8

chart for Be. In certain environments (e.g., rare-metal pegmatites, some ultramafic rocks and oxidized black shales) positive Be concentrations are encountered. Duplicates of these samples indicate very good precision by ICP-MS for levels above 0.5 ppm Be.

Kovalevsky (1974, 1978) reported a strong Be anomaly (up to 5000 ppm Be in ash [100 ppm Be dry weight]) in, among other plants, bark of pine in an area with fluorite–phenakite–bertrandite mineralization. This is exceptional, since by comparison pine bark from the Bernic Lake rare-metal pegmatite mine in Manitoba yielded a maximum of 190 ppm Be in ash, equal to 18 ppm Be in dry tissue. Black spruce bark had 6 ppm in dry tissue, whereas twigs of both spruce and bark from the same trees had 2 ppm Be. Birch, poplar and alder had less than 0.2 ppm Be. The Bernic Lake study confirmed Kovalevsky's observation that pine bark concentrates Be to a greater extent than other tissues and other common plants of the boreal forest.

In foliage samples from the Eden Project, Cape myrtle (*Myrsine africana*), containing cyanidin glycosides, concentrated more Be (0.5 ppm) than other species. Also, the bilberry (*Vaccinium myrtillus*), containing anthocyanosides, is reported to concentrate Be up to approximately 5 ppm dry weight (Wedepohl, 1969–1978). These observations imply that plants containing cyanide complexes may preferentially take up Be.

Bismuth (Bi) (Fig. 9-8)

V6 contains almost exactly 0.02 ppm Bi – i.e., the detection limit, yielding typical precision at this level of +/– 100%. However, control V17 has 0.05 ppm Be and data from over 300 analyses inserted among more than 6000 samples during a two-year period demonstrate very good precision.

This good precision is typical for Bi determined by ICP-MS. If concentrations are even slightly above detection, Bi data are usually repeatable, and false anomalies are not observed. Most biogeochemical survey samples yield concentrations below detection, but any positive values should be closely scrutinized to determine their significance,

	V17	NIST 1575a
Target	0.07	No data
Mean	0.05	
Std. Dev.	0.01	
RSD %	27	

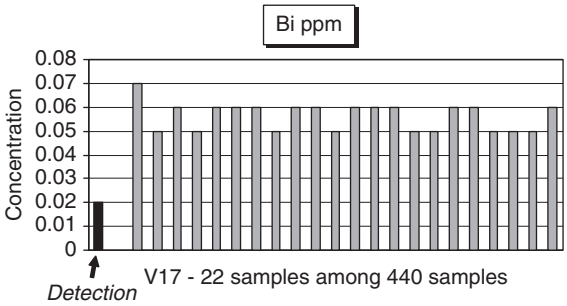


Fig. 9-8. Bismuth – Precision and accuracy on dry tissues – V17 (see Fig. 9-1).

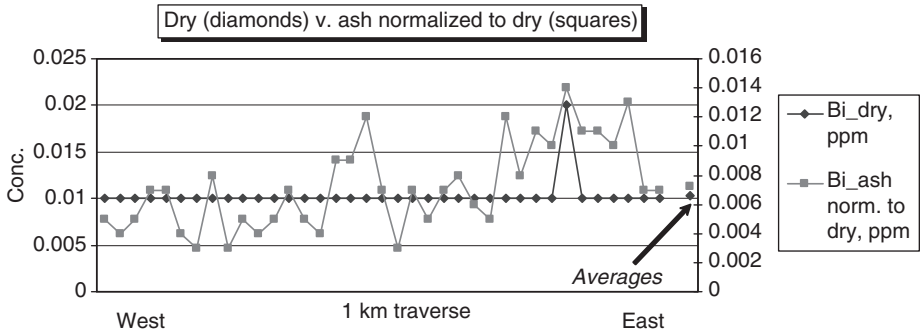


Fig. 9-9. Bismuth in *Acacia* twigs from a precious metal prospect in western Australia. Concentrations in dry tissue versus those in ash, normalized to a dry-weight basis. Analysis by ICP-MS.

especially since Bi is associated with a many different types of mineralization, including a variety of Au deposits – high sulphidation epithermal systems (e.g., Summitville, Colorado), alkalic intrusion-associated Au–Ag (e.g., Cripple Creek, Colorado), many Au-quartz vein systems and skarns (e.g., Nickel Plate in British Columbia). The control V17, which exhibits consistently detectable Bi values, was collected from the site of an undeveloped Au prospect at Mt. Washington on Vancouver Island, where gold-quartz veins with Ag, Cu and As are associated with Tertiary dacitic tuff, breccia and diorite.

In light of the potential use of Bi data for helping to focus exploration activities, the additional procedure of reducing samples to ash prior to analysis can be of value, because ashing significantly concentrates trace elements. Figure 9-9 shows data for *Acacia* twigs from Australia that were analysed for Bi as part of a multi-element package. Note that values in the left scale (concentrations from direct analysis of dry tissue) are higher than those in the right-hand axis (concentrations in ash that have been normalized to a dry-weight basis).

The initial analysis of the dry material yielded only one sample with detectable Bi. Values below detection were plotted at half the detection limit of 0.02 ppm Bi. A 15 g portion of dry material was then reduced to ash, the ash analysed by the same method, and the result normalized to a dry-weight basis. The solitary positive value for Bi in the dry tissue was at the detection limit, and it is coincident with the highest value obtained from the ash (normalized to dry). However, the geochemical 'relief' of the low level Bi data are now apparent, and a second weakly anomalous zone, some 200 m west of the first, is evident (Fig. 9-9).

Boron (B) (Fig. 9-10)

Boron is an essential micronutrient for plant metabolism, and recent developments have contributed to improved understanding of the role of B in plants by

	V17	NIST 1575a
Target	7	9.6
Mean	6.6	10
Std. Dev.	0.8	0.9
RSD %	13	9

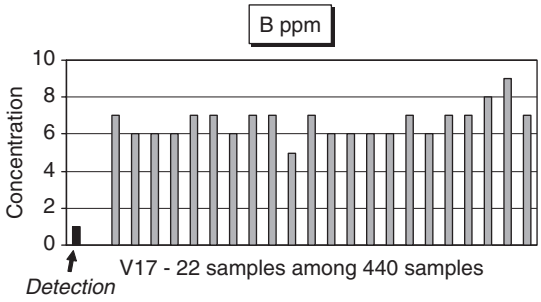


Fig. 9-10. Boron – Precision and accuracy on dry tissues – V17 and NIST 1575a (see Fig. 9-1).

demonstrating their role in binding proteins to cell walls and interfering with Mn-dependent enzymatic reactions (Fig. 9-10) (Blevins and Lukaszewski, 1998).

Concentrations in plants are typically in the tens of ppm B. These levels are well above the detection of 1 ppm B, so precision is good to excellent, and data can be plotted with confidence that they reflect true variations among the field samples. In general, B data do not provide anomalies or distribution patterns of much significance for biogeochemical exploration. However, the data should not be ignored, because there is B enrichment associated with base metal deposits such as the classic Sedex-type of seafloor sulphides constituting mineralization at the former Sullivan mine in British Columbia. At the Sullivan mine, a study of the distribution of elements in a lodgepole pine rooted in tourmalinite demonstrated that top stems of the tree had B concentrations triple those of lower twigs (Dunn, 1995a,b).

Bromine (Br)

See 'Halogens' for detailed discussion of using the labile fraction of Br in mineral exploration.

Total Br content of plants is best determined by INAA for which analytical precision is excellent. Bromine is a volatile element, present in most, if not all terrestrial plants. Seaweeds concentrate Br more than 100 times the average of 4 ppm Br in land plants, but it is not known to be an essential element. Bromine can occur in many forms as complexes within plants. Some Br complexes volatilize during the ashing process, causing losses of up to 90% of the Br contained within the plant tissues. However, in spite of the substantial loss of Br during ashing it has been found that on occasion there is a Au/Br association in plant ash from zones of Au mineralization (Dunn, 1986a). It appears that the chemical species of Br retained in the ash may be related to the presence of mineralization, and it has been speculated that this is because the geochemical halo of a mineral deposit may create physico-chemical conditions that produce differing ratios of Br species within the vegetation (Dunn and Hoffman, 1986). Concentrations in the hundreds of ppm Br in plant ash

sometimes occur, e.g., Manuka (tea tree) shrubs growing around the Au-bearing geothermal fields at Waiotapu and Rotokawa in New Zealand have yielded more than 200 ppm Br in ashed twigs with slightly lower levels in leaves.

Cadmium (Cd) (Fig. 9-11)

The precision of Cd by ICP-MS is excellent. Close to detection (0.01 ppm) Cd data are likely to exhibit the usual +/– 100%, but this precision improves dramatically at concentrations of just 0.05 ppm. Anomalous values are almost always substantiated by repeat analyses and so it is unusual to get 'false positives' that cannot be reproduced.

Cadmium in plants has been studied extensively and a concise summary by Punshon et al. (2005), including almost 200 references, makes excellent reading for those interested in the complexities of Cd transfer from soils to plants and the complex interactions that take place within plants.

There are plants known to hyperaccumulate Cd. The small weed *Biscutella laevigata* is a montane herb of the Brassica Family found primarily in Eastern Europe that is reported to contain more than 200 ppm Cd in its leaves, and an order of magnitude more in its roots (Pielichowska and Wierzbicka, 2004). Although of environmental interest, this plant is unlikely to be of more than local use in mineral exploration.

Other common trees that have relatively high concentrations of Cd, primarily because of its ubiquitous geochemical affinity for Zn, are birch, willow and, to a lesser degree, poplar. Furthermore, a measure of Cd concentrations in most common plants can assist in delineating zones of Zn enrichment, because unlike Zn, Cd is not an essential element and tends to better reflect zones of Zn enrichment than Zn itself. Also, Cd is sometimes associated with Au and can be used as a pathfinder element for Au. Figure 9-12 shows concentrations of Cd and Au in dry spruce needles from a traverse over a till-covered zone of undisturbed Au/Cu/Mo mineralization associated with diorite and mafic volcanics. It is usual for a Cd signature to be spatially broader (i.e., encompass a wider zone) than that of Au (Fig. 9-12).

	V6	NIST 1575a
Target	0.24	0.23
Mean	0.23	0.25
Std. Dev.	*0.02*	0.02
RSD %	7	10

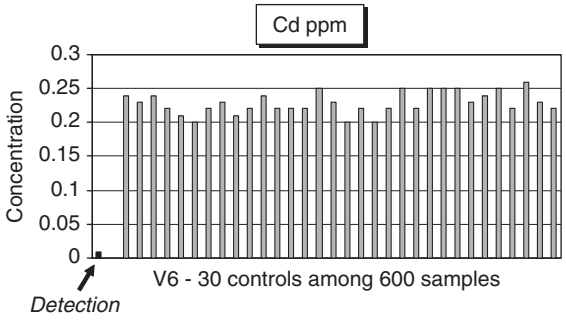


Fig. 9-11. Cadmium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

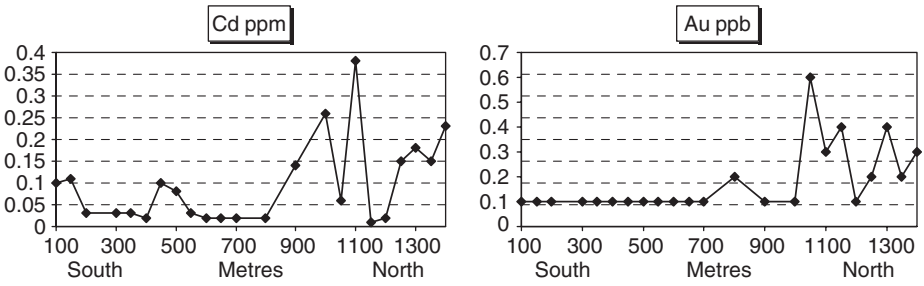


Fig. 9-12. Cadmium and Au in dry needles of Engelmann spruce from the Cariboo Zone in central British Columbia (Dunn et al., 2006a,b).

	V6	NIST 1575a
Target	0.75	0.25
Mean	0.73	0.27
Std. Dev.	0.03	0.01
RSD %	4	5

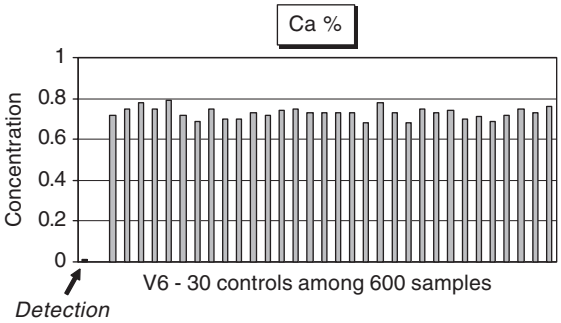


Fig. 9-13. Calcium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Calcium (Ca) (Fig. 9-13)

The precision for Ca is excellent and, because Ca is a fundamental structural element for cell walls and membranes, concentrations are invariably well above the detection limit by ICP-MS of 0.01% Ca. About 1% Ca is present in the average plant, and the range of Ca concentrations is normally between 0.1 and 5% dry weight (Marschner, 1995; see Eden Project data on the CD). Acacias and figs (*Ficus spp.*) have foliage that is relatively enriched in Ca, approaching 5%. Bark and to lesser degree thin twigs have relatively high concentrations of Ca, because of the accumulation of Ca oxalate crystals in these tissues (see Chapter 2). White and Broadley (2003) provide a comprehensive overview of the complex roles that Ca plays in plant structure and metabolism.

In mineral exploration Ca data from plant analyses have limited value, but the data can assist in lithogeochemical mapping, because plants growing on carbonates or other Ca-rich substrates are relatively enriched in Ca. For example, a traverse from granite on to carbonate-rich sediments is reflected in an increase in the Ca

content of the plant tissues. Furthermore, there is commonly visual evidence of such a transition, with trees on granites having relatively thin twigs, whereas on the carbonates twigs are thicker and exhibit more vigorous and robust growth.

Cerium (Ce) (Fig. 9-14)

See Lanthanum and the Rare Earth Elements (REE).

Precision of analytical data for Ce is excellent at concentrations above 1 ppm Ce. The poorer precision exhibited for NIST 1575a is not characteristic of other controls with similar concentrations. A control composed of twigs from mountain hemlock has a similar mean value to the NIST 1575a (0.08 ppm Ce), yet has an RSD of 8.5%. Since concentrations of 0.1 ppm Ce or higher are typical for many types of vegetation, it can be assumed that the data quality is generally very good unless concentrations are close to the detection limit of 0.02 ppm Ce.

Conifer twigs usually have higher concentrations of Ce than their foliage. The reverse seems true for deciduous species. Alder leaves (*Alnus crispa*) from the REE-rich allanite at Hoidas Lake in northern Saskatchewan have yielded 12 ppm Ce and the twigs 8.7 ppm Ce. Black spruce from close to the Rottenstone PGE/Ni/Cu deposit in Saskatchewan have 0.54 ppm Ce in twigs and 0.05 ppm Ce in needles, whereas alder from the same location show the same pattern of Ce enrichment in leaves that was observed at Hoidas Lake.

In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 1.5 ppm Ce adjacent to a kimberlite. Stems from this species had concentrations of Ce that were half to one-third of those in the leaves. Dwarf birch leaves from the Ekati area of kimberlites in the Northwest Territories of Canada yielded average concentrations of 0.08 ppm Ce with a maximum of 0.35 ppm Ce. More discussion on Ce anomalies related to mineralization is presented in the section on lanthanum.

	V6	NIST 1575a
Target	1.6	(0.11)
Mean	1.6	0.07
Std. Dev.	0.1	0.02
RSD %	6	24

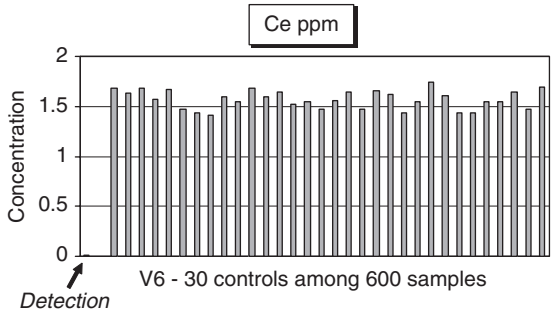


Fig. 9-14. Cerium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Cesium (Cs) (Fig. 9-15)

Biogeochemical survey samples usually contain well in excess of the low detection limit of 5 ppb Cs in dry tissue achieved by ICP-MS. Spikes in the data from survey samples can be interpreted with confidence that they represent true concentrations, since sources of contamination are sparse, and replicate analyses of anomalous levels are nearly always repeatable.

In most geochemical processes, Cs has a strong affinity to K and Rb. In plants this association with K is usually absent or weak, but there is commonly a good correlation between Cs and Rb. On occasion, especially in situations where Cs is associated with some types of mineralization, the Cs/Rb relationship is relatively weak.

An example of modest Cs enrichment spatially related to a Au biogeochemical anomaly is shown in Chapter 5 (Fig. 5-7). This relationship with Au is not too surprising since the Cs bearing mineral 'galkhaite' [$Cs_{0.6}Tl_{0.4}Hg_{3.5}Cu_{1.5}ZnAs_{3.6}Sb_{0.4}S_{12}$] is associated with Au deposits at Hemlo (Ontario) and Getchell (Nevada). High levels of Cs are associated, too, with Au, As and Sb in the areas of geothermal fluid discharge in New Zealand, and some plants from these areas are significantly enriched in several elements, including Cs. Table 9-II shows levels of several elements that are enriched in the 'Tea Tree' Manuka (*Leptospermum scoparium*) growing in the warm mists emanating from Champagne Pool and Sulphur Flats on New Zealand's North Island. In the case of Champagne Pool, the fluids are over-saturated with respect to the As- and Sb-bearing minerals orpiment and stibnite (Pope et al., 2004), so some of the metal enrichments must be directly related to precipitation from the mists that rise from the Pool and swirl around the Manuka.

Ferns and *Ficus* (fig) trees both have a strong propensity to accumulate Cs (Eden Project). In the Chinapintza Au district on the border between Peru and Ecuador (Cordillera del Cóndor), around the low sulphidation epithermal precious metal vein system there are enrichments of more than 20 ppm Cs in dry fronds from tree ferns (*Cyathea spp.*).

	V6	NIST 1575a
Target	0.03	0.28
Mean	0.03	0.29
Std. Dev.	0.003	0.02
RSD %	6	7

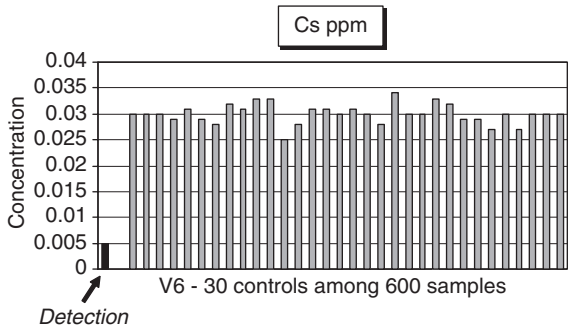


Fig. 9-15. Cesium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

TABLE 9-II

Concentrations of elements significantly enriched in Manuka (tea tree) samples from sites of hot springs on New Zealand's North Island. Concentrations are in ash, determined by INAA. Ash yield data are provided for estimating concentrations in dry tissue

	Waiotapu (Champagne pool)		Rotokawa (Sulphur flats)		Background[1]
	Twig	Leaf	Twig	Leaf	
Au (ppb)	983	1740	79	45	5–10
As (ppm)	86	86	38	54	<5
Br[2] (ppm)	210	180	110	65	<50
Cs (ppm)	140	92	38	36	<1
Rb (ppm)	1500	1100	950	770	<200
Sb[2] (ppm)	270	170	6	4	<1
Ash yield (%)	2.3	3.3	1.9	4.2	

[1]Typical 'background' levels in plant ash.
[2]Up to 90% of Br and 30% of Sb are likely to have volatilized during ashing.

Although a Cs/Au association is not always present in vegetation, this relationship is worth scrutiny in biogeochemical studies designed to delineate Au mineralization, because of the potential of Cs to be used as a spatially associated 'pathfinder' element.

At the Bernic Lake Ta-Li-Cs mine in Manitoba, there are concentrations up to 3600 ppm Cs in ash (358 ppm Cs dry weight) of outer bark from jack pine near the mine site. Maxima in other tissues (with dry-weight values in parentheses) are black spruce outer bark with 2300 ppm Cs (212 ppm), and twigs of both pine and spruce up to 3000 ppm Cs (118 ppm). Concentrations in dry twigs of deciduous species are 5 ppm Cs in birch; 20 ppm Cs in poplar; and mostly greater than 20 ppm Cs in alder. In other areas remote from Cs mineralization alder has shown a propensity to accumulate Cs. The Cs signature at Bernic Lake is no doubt related in part to wind-blown dust from the mining operations. Some of the Cs-bearing particles (mostly contained in the Cs mica pollucite) probably adhere to the vegetation, but most have settled to the ground, then been dissolved by rainwater and subsequently taken up by tree roots. The evidence for this is that analyses of trunk wood yield anomalously high levels of Cs. Whereas background levels in trunk wood would normally be only a few ppm Cs in ash, up to 290 ppm Cs is present.

Chromium (Cr) (Fig. 9-16)

Precision of Cr in V6 is good, largely because concentrations at about 4 ppm Cr are well above the detection limit of 0.05 ppm Cr. The lower precision for NIST 1575a

	V6	NIST 1575a
Target	4.4	(0.4)
Mean	4.3	1.5
Std. Dev.	0.4	0.4
RSD %	9	28

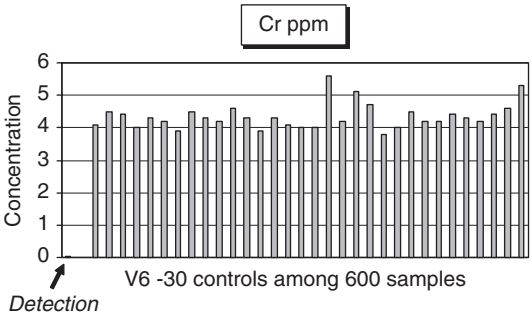


Fig. 9-16. Chromium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

is because it contains less than 1 ppm Cr. Large spikes in field survey data are unusual. When they do occur, they are often due to contamination, and are nearly always accompanied by spikes in Ni (probably reflecting minute metal particles from cutting blades in grinders or mills commonly used for reducing tissues to a fine powder in preparation for analysis). In general, the data are very reliable. Chromium is one of the several elements (including As, S, Se and V) for which some laboratories declare that low levels cannot be accurately measured by ICP-MS, whereas others claim to have circumvented this problem and provide consistently precise data.

Earlier, it was noted that there is a loss of up to 70% Cr from some tissues during reduction of dry material to ash. This factor should be borne in mind in the evaluation and interpretation of any data obtained on the analysis of ash. However, it appears that losses from a particular species are consistent, so that Cr distribution patterns, when plotted on a map, remain much the same whether the data are derived from ash or dry plant tissue.

Chromium is an essential trace element to a wide variety of plants, and they usually absorb Cr in its trivalent state (Cary et al., 1977). Although only usually present at levels in the low-ppm range, elevated levels of Cr can and do occur, especially over ultramafic rocks. Over Co–Cr mineralization contained within ultramafic rocks of the Bou Azzer region of southern Morocco, samples of the goat-root *Ononis natrix* yielded up to 88 ppm Cr in dry stems (Dunn et al., 1996c). This is an unusual enrichment, and probably occurred because Cr mineralization is primarily contained within the relatively soluble Mg–Cr carbonate stichtite (an alteration product of chromite) and Cr–mica (fuchsite).

On occasion, Cr exhibits enrichments in plants over zones of Au mineralization. In Labrador tea (*Ledum groenlandicum*) growing in a bog adjacent to Au mineralization, elevated levels of Au in stems were associated with enrichments of Co, Cr, Th, U and W (Dunn et al., 1990). This site was later developed as the Jasper Au mine in northern Saskatchewan.

In general, the foliage of palms and bamboos contain relatively high concentrations of Cr (Eden Project).

Chlorine (Cl)

See 'Halogens'.

Cobalt (Co) (Fig. 9-17)

Precision for Co is generally good and concentrations in plants commonly collected for biogeochemical surveys are well above the detection limit of 0.01 ppm Co. During uptake by plants Co tends to follow Fe bound to complexing organic compounds (Wiersma and Van Goor, 1979). However, statistical analyses of biogeochemical datasets commonly show that Co follows Ni in geological environments dominated by mafic to ultramafic rocks. In other environments, Co may act independently of other elements. On occasion, there is a spatial relationship between Co and Au deposits, with a zone of Co enrichment peripheral to a central zone of elevated Au values. The epithermal Au/As/Sb deposit at Mount Washington on Vancouver Island exhibits this relationship.

In central Africa, the genus *Haumaniastrum* (a member of the mint Family) has been described as a 'copper flower' because of its ability to hyperaccumulate Cu. It can also accumulate Co, but it appears that this ability is limited to the Shaban Copper Arc of Democratic Republic of Congo (formerly Zaïre) and adjacent parts of the Zambian Copper belt (Paton and Brooks, 1996).

A study of Co in Black gum (also known as Tulepo, *Nyssa sylvatica*) from the United States found that

> Foliar concentrations of stable cobalt increase uniformly until senescence. In late August, foliage accounts for only 9 percent of total tree weight but 57 percent of total tree cobalt. Losses of cobalt from trees occur almost entirely by leaf abscission, and the loss rates of weight and cobalt from decomposing litter are similar. Retention of cobalt in the biologically active soil layers perpetuates zones of cobalt concentration created by this species in woodlands (Williams, 1975).

	V6	NIST 1575a
Target	4.4	0.06
Mean	4.4	0.07
Std. Dev.	0.03	0.02
RSD %	9	23

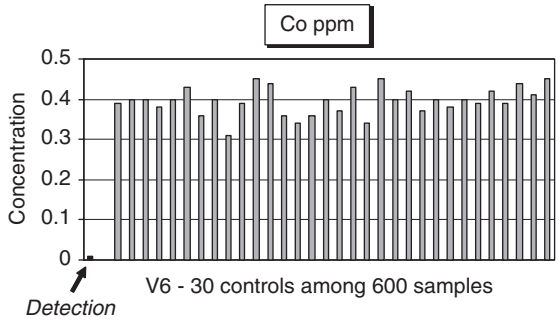


Fig. 9-17. Cobalt – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

These results and conclusions have broad application to understanding biogeochemical cycles, since they demonstrate that biogeochemical enrichment of an element, not just Co, can persist for many years through natural recycling from soil to a plant and back to soil when foliage falls to the ground and decomposes.

Another study of six species of *Nyssa* from the United States and southeast Asia found that they all accumulated Co (Brooks, 1983). Other plants that accumulate Co are from the Rubiaceae ('Bedstraw' family – *Pentas* [Star cluster] with 47 ppm Co in leaves [Eden Project]) and Zingiberaceae (Ginger family – *Costus* with 7 ppm Co in leaves).

In the Co-rich Bou Azzer area of southern Morocco, several plant species growing on serpentinized ultramafic rocks and near Co mineralization (skutterudite, safflorite and birbirite) were found to accumulate Co to modest levels (Dunn et al., 1996c). Noteworthy concentrations in dry tissues were leaves of *Veronica* (24 ppm Co) and *Anvillea garcinii* (12 ppm Co).

Copper (Cu) (Fig. 9-18)

Copper concentrations in vegetation are typically 2–3 orders of magnitude above the detection limit by ICP-MS of 0.01 ppm Cu; hence the precision of Cu is excellent with RSDs better than 10%.

Copper is one of the most thoroughly investigated elements in plants. It is an essential element that plays a significant role in several physiological processes and in disease resistance. Lepp (2005) provides a succinct overview of Cu in plants and indicates that much work remains to be done, in part because of lack of agreement on detailed mechanisms of Cu movement and accumulation.

From an exploration perspective, many of the complexities of Cu can be overlooked without compromising the interpretation of analytical results. No doubt a greater understanding of Cu metabolism will permit finer tuning of data interpretation, but at this time there is sufficient information that can be extracted from

	V17	NIST 1575a
Target	7	2.8
Mean	7	3.3
Std. Dev.	0.2	0.3
RSD %	3	9

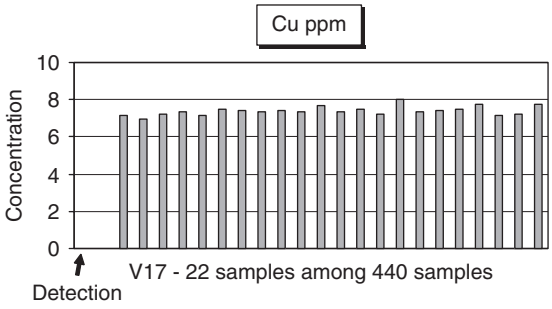


Fig. 9-18. Copper – Precision and accuracy on dry tissues – V17 and NIST 1575a (see Fig. 9-1).

simple data analysis and plotting of Cu from a specific plant species in order to provide the field geochemist with some exploration vectors.

There are plant species capable of accumulating high levels of Cu, notably the 'copper flowers' of Central Africa (e.g., *Becium homblei* and *Haumaniastrum katangense*) – in particular in the Shaba Province of the Democratic Republic of Congo where concentrations in excess of 1% Cu in dry tissue are reported (Brooks, 1998) and in Anhui Province of China (*Commelina communis* – Tang et al., 1997). However, Lepp (2005) considers that there has not been satisfactory demonstration of Cu hyperaccumulation under controlled conditions, with the inference that some of these high accumulations may be attributable to airborne contamination from copper-mining operations.

Markert's (1994) 'Reference Plant' has 10 ppm Cu. Experience with tissues from many different common plants used in biogeochemical exploration indicates that median values are commonly 5–8 ppm Cu, increasing to tens of ppm Cu over mineralization. The rubber vine (*Cryptostegia grandiflora*) from the Eden Project yielded 37 ppm Cu, indicating that it has a propensity to accumulate Cu. Concentrations greater than 50 ppm Cu in uncontaminated areas are exceptional. At the Mt. Polley Cu/Mo/Au porphyry in central British Columbia, concentrations reach over 30 ppm Cu in dry twigs and foliage of several conifers collected from over the original discovery zone (sampled in 1991 – Dunn, 1995a,b), and over undisturbed prospects from the surrounding area sampled in 2005 (Dunn et al., 2006a,b). As a broad, but not definitive, rule of thumb, values in excess of 15 ppm Cu in conifers are worth closer examination.

Dysprosium (Dy)

See Lanthanum and Rare Earth Elements.

Dysprosium has no known biological role. In V6 it exhibits good precision with a mean of 0.09 ppm Dy. Databases are very limited, but it appears that except for near REE deposits and sometimes with other REE near kimberlites Dy is rarely concentrated in plants above its detection limit by ICP-MS of 0.02 ppm Dy. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.42 ppm Dy adjacent to a kimberlite. Stems from this species had lower concentrations of Dy and most were below the detection limit. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada all yielded concentrations below detection.

Erbium (Er)

See Lanthanum and the Rare Earth Elements.

As for the other REE, Er has no known biological role. In V6 it exhibits near perfect precision with a mean of 0.04 ppm Er. Databases are very limited, but except for near REE deposits Er is rarely concentrated in plants above its detection limit by

ICP-MS of 0.02 ppm Er. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.2 ppm Er adjacent to a kimberlite. Stems from this species had lower concentrations of Er and most were below the detection limit. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada all yielded concentrations below detection.

Europium (Eu)

See Lanthanum and the Rare Earth Elements.

Europium has no known role in plant metabolism. In V6 it exhibits very good precision with a mean of 0.025 ppm Eu. Europium is rarely concentrated in plants above its detection limit by ICP-MS of 0.02 ppm Eu, except near REE deposits. Alder leaves (*Alnus crispa*) from the REE-rich allanite at Hoidas Lake in northern Saskatchewan have yielded 0.15 ppm Eu. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.12 ppm Eu adjacent to a kimberlite. Stems and fruits from this species had lower concentrations of Eu and most were below the detection limit. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada all yielded concentrations below detection. More discussion on Eu anomalies related to mineralization is presented in the section on lanthanum.

Fluorine (F)

See 'Halogens'.

Gadolinium (Gd)

See Lanthanum and the Rare Earth Elements.

Gadolinium has no known biological role. It exhibits good precision with a mean of 0.13 ppm Gd in V6. As for most of the other REE, databases for Gd are very limited and it is rarely concentrated in plants above its detection limit by ICP-MS of 0.02 ppm Gd except near REE deposits. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.66 ppm Gd adjacent to a kimberlite. Stems and fruits from this species had lower concentrations of Gd and most were below detection. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada all yielded concentrations below detection.

Gallium (Ga) (Fig. 9-19)

Although Ga is not a particularly rare element (30th in terms of crustal abundance at 19 ppm Ga), concentrations in vegetation tissues are commonly below the

	V6	NIST 1575a
Target	0.15	<1
Mean	0.12	<1
Std. Dev.	0.04	
RSD %	33	

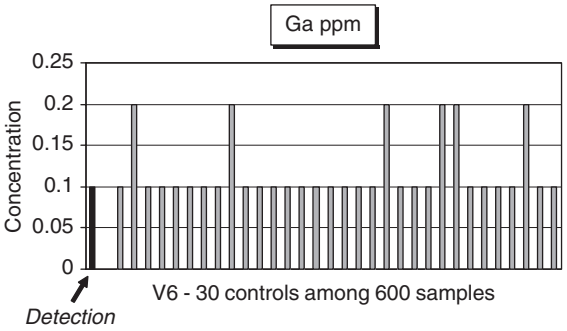


Fig. 9-19. Gallium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

detection limit of 0.1 ppm Ga. V6 has barely detectable concentrations; therefore analytical precision is +/– 100%. Spikes in the data are very rare. Any value of >0.3 ppm Ga should be scrutinized to ascertain its significance, since Ga minerals are extremely rare and it is usually only associated with minerals containing Zn, Cu, Ge and Al. None of the 71 plants analysed from the Eden Project yielded in excess of 0.1 ppm Ga.

A study of *Acacia* in Tanzania (sites in the vicinity of the North Mara gold mine) demonstrated that leaves contain more Ga than twigs, reaching a maximum of only 0.9 ppm Ga. In Western Australia analysis of over 100 *Acacia* tissues revealed that most of the Ga is located in the bark (up to 2.35 ppm Ga), with a maximum of 0.5 ppm Ga in both phyllodes and roots, and only 0.03 ppm Ga in woody branches. In general, it appears that Ga concentrations are higher in foliage than in twigs.

Germanium (Ge) (Fig. 9-20)

Germanium is a rare element that occurs in association with Zn and Cu–Pb–Zn ores, and as the Fe–Cu–Ge sulphide 'germanite' at the Tsumeb mine in Namibia. Analyses by ICP-MS usually return values in plant tissues that are at or below the detection limit of 0.01 ppm Ge. Concentrations in V6 are typically double the detection limit, but precision is poor yielding a RSD of 38%. This lack of precision is similar for Ge concentrations at higher levels (>0.1 ppm), and interpretation of Ge data should be undertaken with due consideration to the poor precision. If positive Ge results are obtained, the field and analytical duplicates should be scrutinized prior to plotting distribution patterns. Markert (1994) indicates that the 'Reference Plant' contains 10 ppb Ge. However, a number of plants, including bamboo (>10 ppm Ge in a sample from the Eden Project) can have Ge enrichments. There are indications in the literature that rice, ginseng, garlic and mushrooms, among others, can contain several hundred ppm Ge. Since Ge has a geochemical affinity for Si, it may be that

	V6	NIST 1575a
Target	0.02	No data
Mean	0.02	<0.01
Std. Dev.	0.01	
RSD %	38	

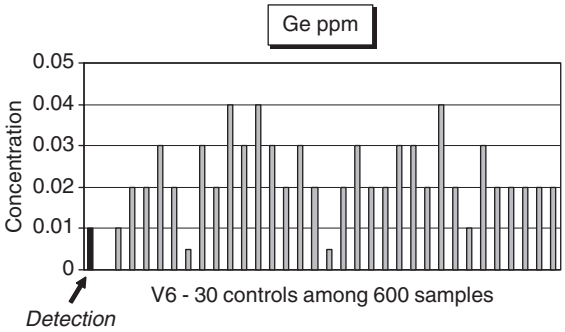


Fig. 9-20. Germanium – Precision and accuracy on dry tissues – V6 (see Fig. 9-1).

there is some substitution of Ge for some Si sites in plant structures, giving rise to Ge enrichments; bamboo is a plant that has a high-silica content to its framework. In general, Ge data have not proved to be of significance in biogeochemical exploration programmes.

Gold (Au) (Fig. 9-21)

It is unfortunate that for biogeochemical surveys seeking gold deposits, Au in vegetation commonly exhibits the classic 'nugget' effect seen in other sample media. The SEM studies presented in Chapter 2 have identified discrete crystals of Au formed in plant tissue, resulting in some extreme 'spikes' in the analytical data. On average, in V6 an extreme value of >3 ppb Au is reported in about 1 sample in 100, and >10 ppb Au in about 1 sample in 300.

Excluding these extreme 'spikes' in the data, the average concentrations are very close to the 'target' value of 0.7 ppm Au in dry tissue, and more than 500 analyses by INA have substantiated the value of 0.7 ppm Au. These data indicate that the 1 g sample typically digested for analysis by ICP-MS is marginally sufficient to obtain consistently repeatable values in control V6. Precision improves a little with a 5 g sample and is considerably better with 30 g, but analysis of such large samples would invoke a much-increased cost.

Further evidence of either inhomogeneity or difficulties in obtaining consistent results at low concentrations comes from data published by the National Institute of Science and Technology on control 1575a. Their data listings show averages of analytical data for Au, all determined by INAA, that were received from five laboratories on 23 samples of this carefully prepared control material (Table 9-III).

From Table 9-III, there is clearly a considerable discrepancy among the laboratories that participated in the determination of Au, with a disconcertingly wide range of average values, even though they all used the same INAA method of analysis. Three laboratories (B, C, D), however, generated average values within the

	V6	NIST 1575a
Target	0.73	(0.25-2.6)
Mean	0.7	0.42
Std. Dev.	0.26	0.27
RSD %	37	64

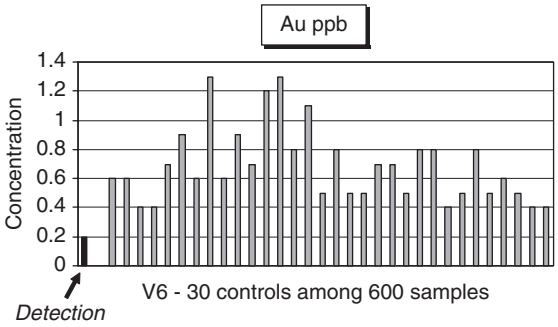


Fig. 9-21. Gold – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

TABLE 9-III

Gold in pine needle control material NIST 1575a (data published by US National Institute of Science and Technology)

Lab	Average (ppb Au)	1 SD	*n*
A	1.5	0.4	3
B	0.7	0.1	9
C	0.26	0.042	3
D	0.56	0.18	2
E	2.6	0.5	6

SD, Standard deviation.

range of 0.26–0.7 ppb Au, and they each had far lower standard deviations (s.d.) than laboratories A and E that provided the highest concentrations.

Data obtained over a four-month period by ICP-MS (personal database) on nineteen 1 g samples of 1575a inserted as 'blind' controls, generated an average value of 0.42 ppb Au, with s.d. of 0.24. This attests to the overall accuracy of the ICP-MS method, but the high RSD that was obtained (64%) indicates that interpretation of low levels of Au in vegetation (<1 ppb Au) should be treated with great caution.

Given this nugget effect and generally poor reproducibility, it is important to be very suspicious of single point Au anomalies, especially if they are not substantiated by pathfinder elements (e.g., As, Sb, Hg, Bi, etc.). If in doubt, it is advisable to request that the analytical laboratory undertakes a repeat analysis of any significant spikes to see if they are reproducible. Experience has shown that repeatable Au anomalies (several to tens of ppb) usually indicate that Au is present in the ground.

A survey in the Bathurst Camp of New Brunswick using needles of balsam fir (*Abies balsamea*) included the comparison of data obtained for gold in dry tissue with

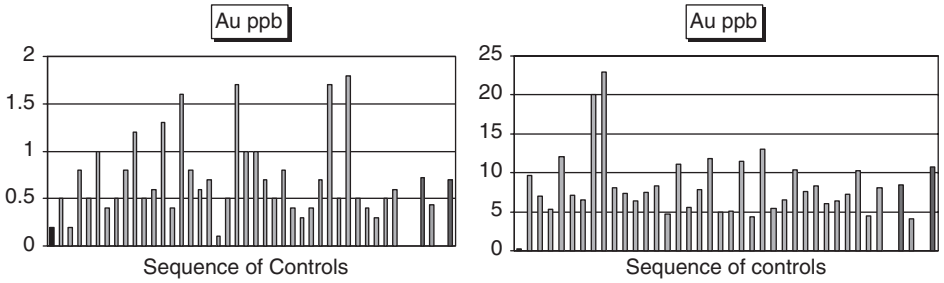


Fig. 9-22. Precision for Au in control V6 – dry tissue (left chart) and ash (right chart).

gold in ash of a 15 g portion of each sample. The analytical precision obtained on the control samples was poor for the dry tissue and fair from analysis of the ash (Fig. 9-22).

On the basis of the poor quality of the control data, it would seem unlikely that any meaningful results might emerge from plotting the data. However, analytical duplicates generated data of quite good precision, and so the data were kriged and plotted as gradational contours. In Fig. 9-23, plots of the data from analysis of the dry tissue are compared with plots of data obtained from analysis of the ash. The principal areas of Au enrichment are outlined as ellipses, and they are seen to persist in data from both methods. Single point anomalies, however, are not reproduced on both plots, and should therefore be treated with caution as to their significance, since they may be simply analytical artefacts. However, this exercise demonstrates the robustness of the biogeochemical method in reproducing *zones* of relative element enrichment, even when control data quality is somewhat lacking.

As always, and with gold in particular, it is important to ensure that an anomaly is not related to airborne particulates or contamination from handling samples. Nobody wearing any gold jewellery should touch a sample, because even if no gold rings are worn, some field and laboratory assistants have the habit of fingering a gold necklace, watch or bracelet. This action is sufficient to add several ppb Au to the analysis of a vegetation sample, and since 1–2 ppb Au is about an order of magnitude above usual background values, a false anomaly can readily be generated.

The background level of Au in plant tissues is sometimes quoted as 1 ppb Au (Markert, 1994), but it is probably about an order of magnitude lower in most environments (Kovalevsky and Kovalevskaya, 1989; Dunn, 1995a). Samples from the Eden Project generated some unusually high concentrations in spite of the soils having only background concentrations of Au. The Kapok bush (also known as wild rosemary) from South Africa yielded 33.5 ppb Au in its foliage, and concentrations greater than 10 ppb Au were recorded for protea, ginger, fig, rattan and several species of vine. Some of these contain cyanide compounds that probably account for the Au being absorbed as the soluble cyanide.

There are several accounts of extreme concentrations of Au in plants. Babička (1943) reported up to 610,000 ppb Au in horsetails (*Equisetum*) from Czechoslovakia,

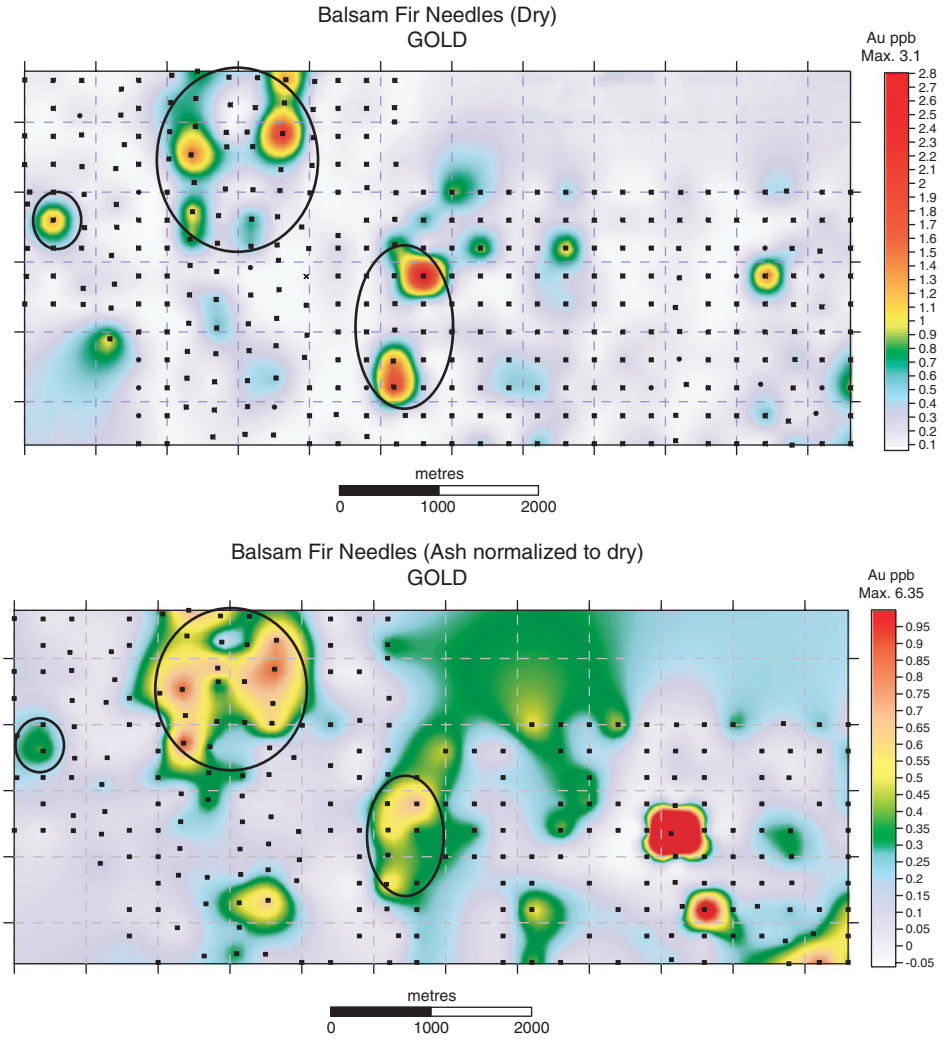


Fig. 9-23. Gold in balsam fir needles. Comparison of distribution patterns from plots of data derived from analysis of dry tissue with those in ash (slightly smaller sample population) normalized to a dry-weight basis.

which was equivalent to 120,000 ppb Au in dry tissue. Subsequent studies have shown that there was an oversight in the analytical procedure (Cannon et al., 1968; Brooks et al., 1981), and therefore these early results have been discredited. Other reports of extremely high concentrations of gold in plants have rarely stood up to close examination, since there has been either contamination or suspect analytical procedures. In Brazil, near former workings by garimpeiros (artisanal miners), the shrub Vassoura de Botão (*Borreria spp.*) yielded 552 ppb Au in its foliage, although

possible contamination from airborne particulates could not be ruled out (Dunn and Angelicá, 2000). It appears that natural concentrations of more than 500 ppb Au in dry tissue rarely occur, although laboratory experiments have succeeded in introducing higher concentrations into plant tissues through absorption by roots of gold-rich salts.

Russian studies report gold particles in the ash of many samples of bark from pine, larch, birch, aspen and willow (Kovalevsky and Prokupchuk, 1978). The particles were determined as scintillations recorded on spectral lines for gold during vaporization of ash in an electric arc (scintillation emission spectroscopy). No gold particles were found in needles or leaves. The authors showed a moderately good correlation between the number of gold particles determined in a 1 g sample and the gold content of the ash, determined by graphite furnace atomic absorption spectrometry. Approximately 30 particles were detected in a sample containing 600 ppb Au, and therefore each particle is extremely small and represents 20 ppb Au.

There is a substantial amount of literature on biogeochemical exploration for Au. Several reviews and compilations of papers have been published, including Brooks (1982), Erdman and Olson (1985), Kovalevsky and Kovalevskaya (1989), Dunn (1992), Kovalevsky (1995a,b), Dunn (1995a) and an up to date review by Anderson (2005).

Hafnium (Hf) (Fig. 9-24)

Precision for Hf is generally poor to fair. Concentrations in many vegetation samples are commonly close to the detection limit, and even when values are quite well above the typical detection limit of 2 ppb Hf (e.g., in V6) the precision remains quite poor, with erratic spikes in the data. Markert provides a value for the 'reference plant' of 50 ppb Hf, but this appears to be higher than usually encountered, since 10–20 ppb Hf is a more common level. Eden Project samples yielded a maximum of 18 ppb Hf. The average value reported for NIST 1575a (NIST website) is 13 ppb Hf, but with a wide range of values from the laboratories that contributed data.

	V6	NIST 1575a
Target	0.01	(0.013)
Mean	0.01	<0.02
Std. Dev.	0.003	
RSD %	33	

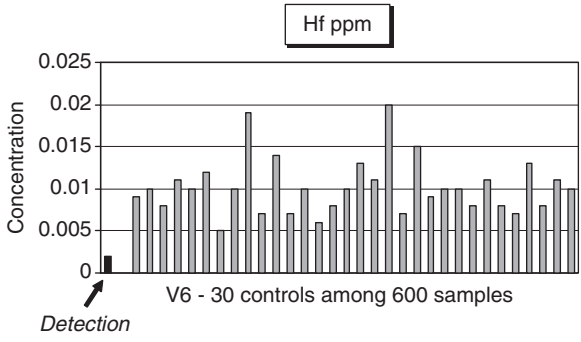


Fig. 9-24. Hafnium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Hafnium is rarely of significance in exploration programmes, and so the data can usually be disregarded or given low emphasis. If unusual levels do occur (e.g., in association with elevated levels of the geochemically associated element Zr) it may be that there is some dust contamination of samples.

Halogens (F, Br, Cl and I)

Discussed in detail on CD.

The halogen elements (F, Cl, Br, I) are commonly associated with the emplacement of mineral deposits. They are contained within the structure of many minerals and in saline fluid inclusions that are typical of a wide range of mineral deposits. Their volatility renders them good candidates to examine as 'pathfinder elements' in surface geochemical media where they may be captured on soil particles and taken up by vegetation. Russian workers (e.g., Trofimov and Rychkov, 2004) have demonstrated the exceptional migrational abilities of I and Br in different geological settings, and found these elements to be highly effective in exploring for ore bodies at depths of up to 1000 m.

Halogens that might be derived from concealed mineralization are likely to be primarily the labile (readily leached) portions that may have emanated from a mineral deposit, and not the halogens structurally bound in crystal lattices (e.g., in apatite, micas and other rock-forming elements). A study designed to determine the optimal analytical procedures concluded that for Br, Cl and I a warm water digestion of milled vegetation, with instrumental finish by high-resolution ICP-MS was the preferred method, with F analysis on the same solution by ion selective electrode (Dunn et al., 2006a,b). By these methods, detection limits are, in ppm, Br (1), Cl (0.2), I (0.001) and F (0.4). These levels are adequate for most vegetation samples to yield values above detection.

The study showed that, from a water leach the halogens exhibit a clear response to most zones of Au and Cu mineralization that were tested. Depths to mineralization varied from a thin veneer to possibly tens of metres of Quaternary and/or volcanic cover. Halogen signatures varied according to the nature of the mineralization: whereas I may provide the best signature in one area, F may be best in another. This indicates that each style of mineralization is likely to generate a different suite of positive halogen responses that have yet to be clearly defined; hence analysis for all four halogens is advisable. Pine bark is the vegetation medium that best concentrates I, and gives good contrast for the other halogens. The full report on this study was presented to the British Columbia research organization 'Geoscience BC' in 2006 (Dunn et al., 2006b), and is reproduced in full on the CD that accompanies this book. Included are full data listings on soils and several types of vegetation for which a 53-element ICP-MS package was obtained, as well as the weak leaches for the halogens.

Holmium (Ho)

See Lanthanum and the Rare Earth Elements.

Holmium has no known biological role. In V6 it is barely detectable at 0.02 ppm Ho. Databases are very limited and Ho is rarely concentrated in plants above its detection limit except for near REE deposit. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.08 ppm Ho adjacent to a kimberlite. At this occurrence the stems from this species had lower concentrations of Ho and all samples yielded concentrations below detection. Similarly, no sample of dwarf birch leaves from the Ekati area of kimberlites of the Northwest Territories of Canada yielded a detectable amount of Ho.

Indium (In)

It is unusual for vegetation tissues to yield In concentrations above the detection limit of 20 ppb. V6 has <20 ppb In. If detected and reproducible, consideration should be given to the presence of Sn and Zn mineralization, or sulphides of Cu, Fe or Pb. To detect the low levels of In in plants, it is usually necessary to first reduce them to ash. The ash of V6 has yielded very good precision with an average of 43 ppb In (standard deviation of 6 ppb, $n = 34$) which represents 2 ppb In in dry tissue. Pine and larch bark from Canada, and all *Acacia* tissues from Australian and African samples have yielded background values of <2 ppb In in all parts of the plants.

Iodine (I)

See 'Halogens'.

Iridium (Ir)

See 'Platinum and the Platinum Group Elements'.

Iron (Fe) (Fig. 9-25)

Concentrations in dry vegetation samples are typically well above detection, resulting in very good precision (RSD 10% or better).

There are many publications on the mechanisms of Fe uptake and transport by plants, because Fe is an essential element in energy transformations that are fundamental for various plant cell processes. Iron is essential for photosynthesis and is a major constituent of chlorophyll. Inadequate uptake of Fe by plants causes chlorosis, which shows as a yellowing of foliage. Manganese deficiency and iron deficiency have

	V6	NIST 1575a
Target	0.08	0.005
Mean	0.08	0.0044
Std. Dev.	0.005	0.0006
RSD %	6	14

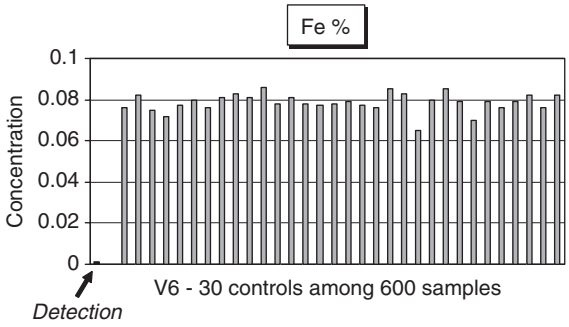


Fig. 9-25. Iron – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

similar results and so yellowing can be a good geobotanical indicator of Fe or Mn deficiency that the field geologist should note. Generally, a soil with high pH and high levels of carbonate will be more prone to cause iron deficiency in plants than soils with lower pH and carbonate levels. Excess Fe can cause toxicity in some wetland plant species by forming ochreous root precipitates. This results in the creation of an effective Fe-exclusion mechanism that manifests itself as an Fe-deficiency chlorosis. A detailed summary of Fe cycling in plants is given by Megonigal et al. (2005).

With regard to mineral exploration, elevated Fe concentrations in plants can be indicative of underlying ultramafic to mafic rocks, and can on occasion be used to assist in lithogeochemical mapping of concealed bedrock.

A correlation analysis of a biogeochemical dataset frequently defines an Fe-associated suite of elements. More detailed data analysis using principal components reveals this as the dominant factor in explaining the variability in many datasets. Multi-element associations with Fe constitute an 'iron factor' (Dunn, 1995a), of which a primary suite includes some or all of Al, Fe, Hf, Hg, Na, REE, Sc, Ti, and sometimes Co, Cs, Ni, Pb, Th and U depending on the chemistry of the substrate. Locally, in Au-rich areas elements that are associated with this Fe factor include Au, As, Sb and Cr. Examination of plant tissues under the scanning electron microscope reveals discrete accumulations of Fe as sulphides, oxides, and associations with other elements to provide inorganic phases that are not always recognized as rock-forming minerals – i.e., elemental combinations that are unique to plants.

Lanthanum (La), the rare earth elements (REE) and yttrium (Y) (Fig. 9-26)

Precision by ICP-MS for most of the REE is generally good, and for the more abundant elements (La, Ce) it is very good with RSDs of better than 10% when concentrations are greater than 0.5 ppm La or Ce. Similarly, precision by INAA is excellent. Twigs and bark from field survey samples commonly have La concentrations at or above 0.2 ppm, and so the data can be plotted with confidence that values

	V6	NIST 1575a
Target	0.9	(0.05)
Mean	0.9	0.03
Std. Dev.	0.04	0.01
RSD %	5	22

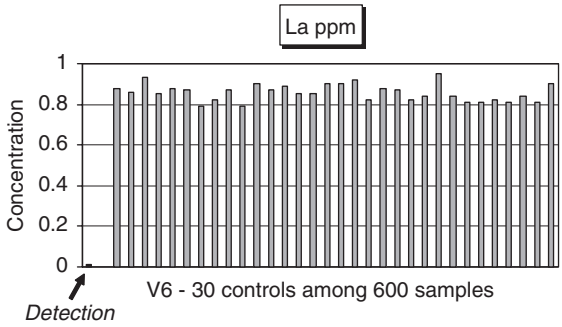


Fig. 9-26. Lanthanum – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

relate to true variations among the samples and not to any lack of analytical precision. In foliage, however, REE concentrations are lower (e.g., commonly <0.1 ppm La) and therefore the precision for REE in a dataset derived from foliage is not as good as that from twigs or bark. The pine needles comprising NIST 1575a contain about 0.05 ppm La at which level the RSD is 22%. Lanthanum in foliage samples collected from the Eden Project was below 0.1 ppm La in all but six samples, and the only species to show an appreciable concentration was fig (*Ficus carica*) with 2.8 ppm La. Similarly, Ce and Y were enriched only in this same sample. No other REE were determined.

A correlation matrix or factor analysis of a multi-element dataset frequently shows a strong relationship of REE with Fe. Further discussion of this association is given under the section on Fe.

Plants are capable of accumulating high levels of REE. Six REE determined by INAA in common species of the boreal forests growing near outcrop of REE-rich allanite yielded 8–10 ppm La in dry twigs and leaves of alder (*Alnus crispa*), with lesser concentrations in birch, black spruce, jack pine and Labrador tea (Dunn and Hoffman, 1986). There were similar concentrations of Ce and substantially less Sm, Eu, Yb and Lu in general accord with usual crustal REE abundances.

It seems that the more primitive the plant, the greater its ability to absorb REE. Many species of fern, a plant that has survived 350 Ma of evolution, are enriched in REE. Swordfern (*Polystichum munitum*) from various locations in south western British Columbia and deer fern (*Blechnum spicant*) from the same and other locations have been collected on several occasions over the past 20 years. Some elevated levels of REE have been noted in samples from sites with background concentrations in soils and rocks, e.g., swordfern from Stanley Park in Vancouver has yielded 6.5 ppm La, 8 ppm Ce and 3 ppm Nd.

Wyttenbach et al. (1998) determined concentrations of eight REE in foliage, including fern fronds, from six species growing in an experimental forest located at an elevation of 800 m in Switzerland. They found that the REE distribution patterns

of fir and spruce were almost identical, but were substantially different from those of maple, ivy, blackberry and wood fern. The fern exhibited strong REE fractionation with substantial relative enrichment of the light REE (LREE).

The results for wood fern obtained by Wyttenbach are in accord with a study in the Endako area of British Columbia where REE signatures of several common plant species were compared with those of the bedrock (Dunn, 1998b). In that region the chondrite-normalized REE patterns derived from rock analysis have been used to assist in distinguishing among various granitic phases, of which some are more 'fertile' with respect to Mo mineralization. The area has a widespread veneer of glacial till and the test was to assess if patterns of REE in overlying vegetation would assist in mapping the signatures of the concealed bedrock. Results showed (Fig. 9-27) that the fern had a marked enrichment of the LREE compared to the signature from the granite, and exhibited a REE pattern that was substantially different from the other species examined – outer bark of lodgepole pine, and twigs and leaves of the Sitka alder (*Alnus sitchensis*). The fern and both tissues from the alder had a slightly negative Ce signature that did not correspond with that of the granite. The pine bark had the signature that most closely corresponded to the REE pattern derived from analysis of the granite. REE signatures were different over other phases of the granite suggesting that this approach may be viable for differentiating the mineralized from the non-mineralized phases of the concealed granites. The limited scope of the study was insufficient to clearly establish its value to exploration and more studies of this sort would be needed.

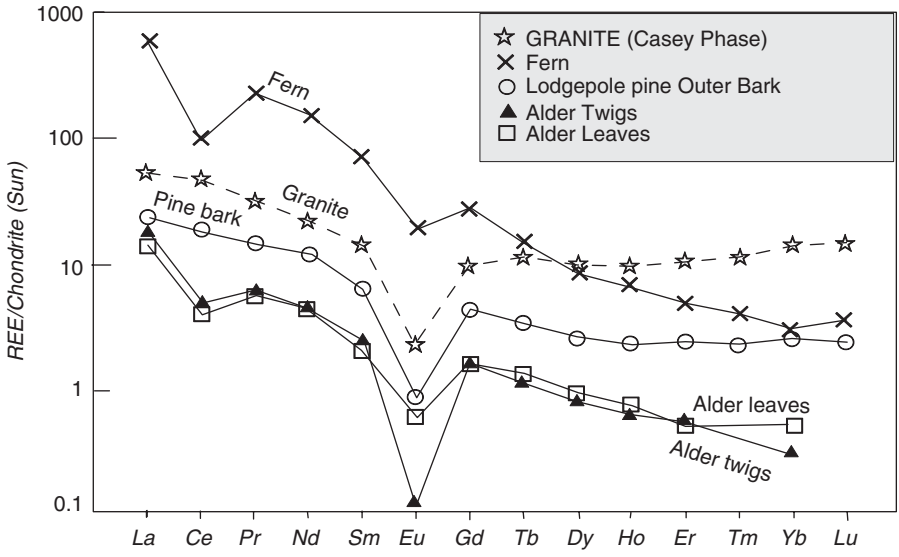


Fig. 9-27. Chondrite normalized REE patterns of granitic phase and overlying vegetation rooted in thin till cover. Endako area of central British Columbia.

Studies in China have found that the fern *Dicranopteris dichotoma* also shows this propensity to accumulate LREE with the highest concentrations reported for Nd with up to 1670 ppm in dry tissue (Shan et al., 2005). In accord with the chondrite-normalized pattern for REE shown in Fig. 9-8, *Dicranopteris* does not accumulate the heavy REE. Studies in Japan have added other fern species to the list of those that can take up unusually high concentrations of REE (Ozaki and Enomoto, 2001).

In another attempt to find a REE signature that might be diagnostic of mineralization, Gale (2002) analysed alder twigs collected along a traverse over a conductive body at the Reed property in Manitoba. Rock geochemical studies of drill core indicated that the area has the potential to contain massive sulphide deposits. He found that over the primary target alder twigs yielded REE signatures with a strong positive Eu anomaly. Subsequent work over other deposits has re-affirmed this observation of positive Eu anomalies over mineralization (George Gale, pers. comm., April 2005).

Another REE curiosity is the observation that, on occasion, there is a spatial relationship of positive Nd values flanking Au anomalies, while the other REEs exhibit a more subdued anomalous pattern. Prior to any development of the Seabee gold mine in northern Saskatchewan, a survey was conducted in 1986 in the vicinity of mineralization that was being investigated. Among the species collected was the outer bark of black spruce. Samples were ashed and the ash analysed by INAA. Included in the multi-element package were data for seven of the REE – La, Ce, Nd, Sm, Eu, Yb and Lu. Figure 9-28 shows that La and Ce exhibit similar profiles, yet Nd (bold line) has a markedly different profile, with highest values occurring to the west of the large Au peak (dotted line). The remaining REEs that were determined had similar profiles to La and Ce. This pattern of Nd enrichment marginal to Au anomalies has been noted at a few additional sites in the La Ronge Gold Belt of northern Saskatchewan (e.g., south of Puswawao Lake). Neodymium-Au phases have been

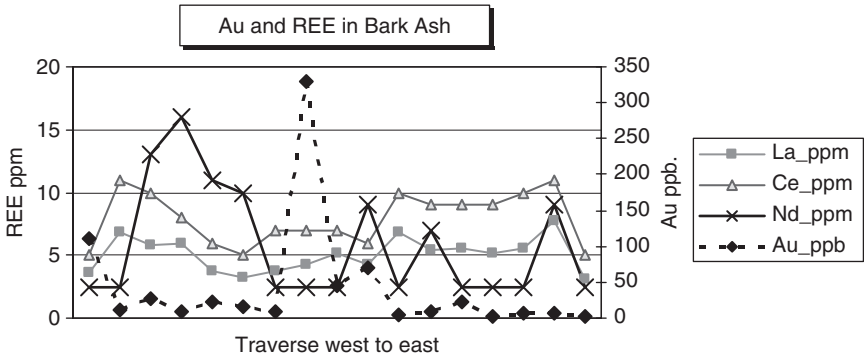


Fig. 9-28. Gold and REE in the ash of black spruce bark. Seabee Gold Mine (prior to development), Laonil Lake, Saskatchewan. Neodymium signature is significantly different from La and Ce, and flanks the Au anomaly. Analysis of ash by INAA.

studied experimentally, but none are known to occur at standard pressures and temperatures (Saccone et al., 1999).

For comprehensive accounts on the biogeochemical cycles of the REE detailed reviews are given by Yliruokanen (1975), Chiarenzelli et al. (2000), Tyler (2004) and Shan et al. (2005).

Lead (Pb) (Fig. 9-29)

Precision for Pb is very good at concentrations greater than 1 ppm, and analyses of biogeochemical samples appropriate for mineral exploration surveys commonly return concentrations in excess of 1 ppm Pb. Spikes in the data that might be related to analytical artefacts are very rare, and consequently field survey data can be plotted with confidence that values are the true composition of the samples. However, anomalous Pb levels should be treated with caution, because leaded gasoline leaves a lingering signature on samples from close to roadsides (Fig. 4-13). Friedland (1990) estimated that Pb derived from a point source of contamination has a soil retention time of 150–5000 years.

Despite the known toxic effects of Pb it occurs naturally in all plants, and it has been suggested that in small traces (2–6 ppb) Pb may possibly be an essential element (Broyer et al., 1972), although this has yet to be proved. Lead is taken up mainly via root hairs and stored as a pyrophosphate in cell walls.

There are several small plants reported to concentrate Pb (e.g., *Lychnis alpina* and various species of *Alyssum, Silene, Thlaspi* and *Minuartia*) but because of their small size and irregular distribution these are rarely practical to collect and are not of relevance to biogeochemical exploration.

Kovalevsky (1987) provides a list of 305 'biosamples' (as he referred to the individual types of tissue) from plant species (mostly trees and large shrubs) collected in eastern Siberia. They are arranged in order of their abilities to accumulate Pb, and at the top of the list are larch and pine, each of which is reported to concentrate

	V6	NIST 1575a
Target	18	0.17
Mean	18	0.21
Std. Dev.	0.5	0.05
RSD %	3	25

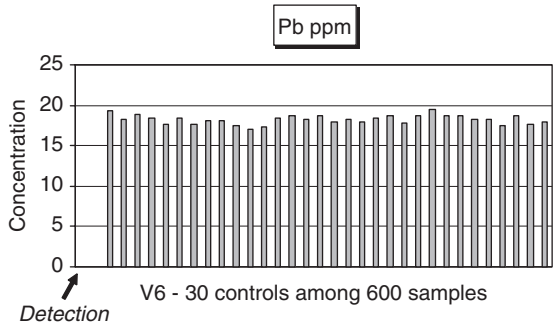


Fig. 9-29. Lead – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Pb by factors of 300 or more above the background level of 1 ppm in ash. Of the tissues recommended, the outer bark is the most practical to collect. In a detailed study to determine the source of Pb in pine tissues the highest correlation was found between Pb in bark and Pb in the soil C-horizon. Further investigation found that the zone supplying Pb to the soils and plants was at a depth of 1–3 m (Kovalevsky, 1987).

Considerable research on Pb in plants has been undertaken over the past 20 years, and detailed accounts of the complex transport pathways and mechanisms are summarized in Sahi and Sharma (2005). From an exploration perspective it is interesting to note that Kovalevsky's empirical observations that pine roots and needles are among the best accumulators of Pb are in accord with experimental results using Monterey pine (*Pinus radiata*). From ultra-thin sections of roots it could be determined that Pb grains were exclusively distributed in the outermost layer of the root cell wall. However, when Pb was introduced in an EDTA (ethylenediaminetetraacetic acid)-chelated form high concentrations were translocated to the needles (Jarvis and Leung, 2002). Various studies have demonstrated that the absorption of Pb through roots into aerial parts of several plants is greatly enhanced in the presence of chelating agents. In the natural environment, humic acids, citric acid and especially chlorophyll are common chelating agents.

A multi-species biogeochemical study was conducted of a 35 sq. km area that encompassed the past-producing Sullivan Pb–Zn mine in southern British Columbia (Dunn, 2000). At a site 5 km up-wind from the mine the highest Pb concentrations were found in the outer bark of lodgepole pine, western larch and Engelmann spruce (each approximately 7 ppm Pb in dry tissue). Concentrations in these media were close to 25 ppm Pb near mineralization reaching a maximum of over 400 ppm Pb in pine bark from sites near old mine workings, and around 100 ppm Pb at sites adjacent to the large open pit that was in production at the time. Twigs of Sitka alder (*Alnus sitchensis*) also proved to be capable of accumulating up to 400 ppm Pb (dry weight) while exhibiting no obvious sign of stress.

Data on seasonal variations in Pb content indicate that dead tissue, especially outer bark scales of conifers, is the optimum sample medium and sampling of growing tissues (foliage or twigs) should be conducted within a period of a few weeks. A sample programme involving growing tissues that extends over several months requires that corrections need to be made for seasonal fluctuations in Pb concentrations. Furthermore, a consistent number of years of growth should be collected. A study comparing elements in young versus older growth of twigs from Douglas-fir tops found that Pb was enriched three-fold in the 5–7 year growth compared to just the most recent 2–4 years of growth. A similar degree of enrichment occurred for REE and Tl, and lesser but consistent enrichments were present for Ba, Ca, Cd, Fe, Hg and Sr. Conversely, the younger growth was significantly richer in As, Cs, K, Mo, Ni, P, Rb and S. This stresses the importance of being consistent in the amount of growth that is collected during a biogeochemical sampling programme.

Lithium (Li) (Fig. 9-30)

Precision for Li by ICP-MS is fair. Field and analytical duplicates generally give more reproducible data than V6, suggesting some inhomogeneity intrinsic to V6. However, data should be treated with caution, and plotted using broad percentile intervals to obtain meaningful distribution patterns of relative concentrations. On occasion irregular blocks of data occur in large datasets. Figure 9-31 shows an apparent periodicity in the data obtained from analysis of more than 550 samples of dry Douglas-fir twigs. The two black arrows point to blocks of data that upon investigation could be traced to an analytical problem pertaining to specific racks of test tubes. This demonstrates the importance of carefully analysing sets of analytical data before conclusions are drawn as to their significance with respect to the natural environment.

	V6	NIST 1575a
Target	0.4	No data
Mean	0.4	0.15
Std. Dev.	0.05	0.02
RSD %	14	12

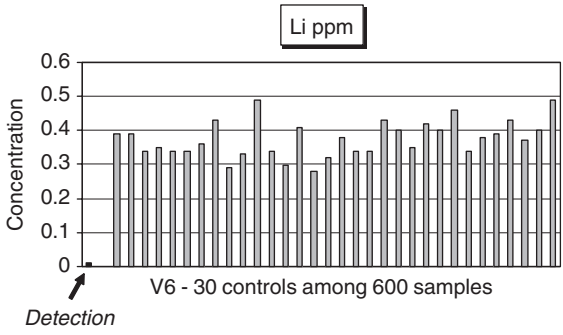


Fig. 9-30. Lithium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

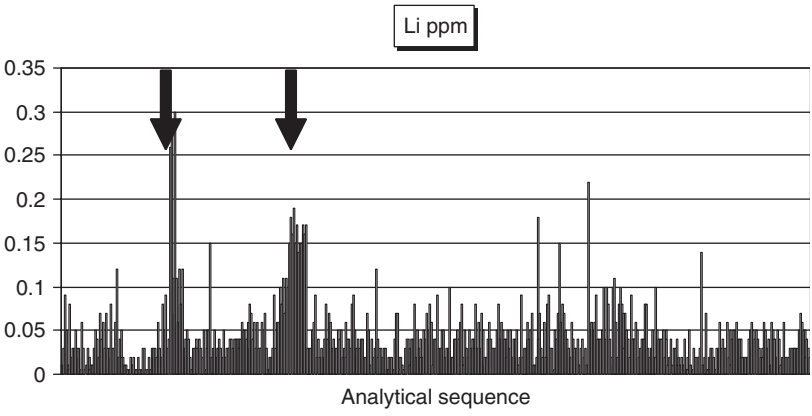


Fig. 9-31. Analytical sequence of Li data exhibiting suspect blocks of enrichments that subsequently proved related to specific blocks of test tubes containing the dissolved vegetation samples.

Gough et al. (1979) considered that the Li content of plant tissues should be a good guide to the Li content of soil, because of its high solubility. However, there are considerable differences in the ability of plants to accumulate Li, and so it remains important to ensure that plant species are not mixed. Members of the rose family are reported to have the greatest propensity to accumulate Li (Borovik-Romanova and Bielova, 1974).

Foliage samples from the Eden Project have concentrations of Li that are two orders of magnitude higher than common background levels of 0.2 ppm Li. This is probably because the material in which the plants are growing is derived from the china clay pit that houses the biomes. The Cornish granites from which the china clay is derived are enriched in Li. Twelve soils from the biomes yielded from 44 to 94 ppm Li that accounts for the high levels of Li in the plants. These data indicate that in the tropics, the leaves of banana plants (*Musa spp.*) might prove to be a suitable sample medium for Li. Calcium inhibits Li uptake, therefore the presence of carbonate substrates should be taken into account when interpreting Li distribution patterns from vegetation samples.

The rare metal pegmatites at the Tanco Mine (Bernic Lake, Manitoba, Canada) contain large reserves of Ta, Li and Cs with strong enrichments of Rb, Nb and Be. Near the mine, Li concentrations in dry jack pine (*Pinus banksiana*) tissues reach maxima of 107 ppm Li in outer bark, 52 ppm Li in twigs and 0.6 ppm Li in trunk wood. In dry twigs poplar (*Populus tremuloides*) had 48 ppm Li, mountain alder had 14 ppm Li and paper birch had only 5 ppm Li. Needles of black spruce (*Picea mariana*) contained similar levels to the pine bark.

Lutetium (Lu)

See Lanthanum and the Rare Earth Elements.

This is the heaviest of the REE. It is rarely present at concentrations above the detection limit of 0.02 ppm Lu in dry tissue and has no known significance to biogeochemical exploration for minerals. By reducing control V6 to ash it was possible to determine that the dry weight-equivalent concentration of Lu was 0.002 ppm (2 ppb Lu). Since V6 contains more REE than the average plant tissue, it is estimated that plants in general contain <1 ppb Lu.

Magnesium (Mg) (Fig. 9-32)

Magnesium values are far in excess of the detection limit, with the result that the precision is excellent. Analytical artefacts are unlikely to occur.

As a component of chlorophyll and an activator of enzymes, Mg is an essential element for plants, and like many essential macro-elements, it is usually of limited use in biogeochemical exploration. Over ultramafic rocks there is commonly an increase

	V6	NIST 1575a
Target	0.128	0.11
Mean	0.13	0.11
Std. Dev.	0.01	0.006
RSD %	7	5

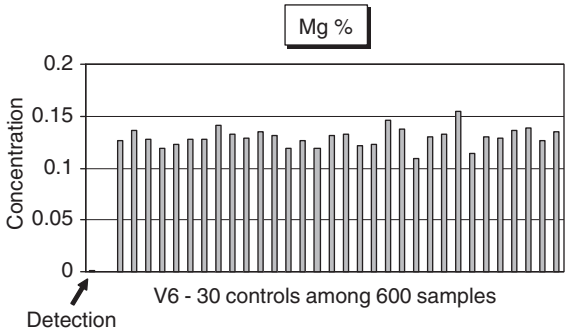


Fig. 9-32. Magnesium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

in Mg uptake by trees and shrubs (Fig. 1-1) and so it has the potential for assisting in lithological mapping. However, there are usually other indications of ultramafic rocks such as sparsity of vegetation and the presence of 'serpentine' flora – i.e., species that are characteristic of ultramafic rocks (Brooks, 1987). It is common for Mg data to exhibit a strong correlation with Ca, Ba and Sr (e.g., in foliage of ferns and vines from the western Amazon), or with P in some trees (e.g., Douglas-fir twigs). In bark of black spruce (*Picea mariana*) from Alaska Mg is associated with B, K, Mn and S, whereas in white spruce (*Picea glauca*) from the same area the association is with B, Mn, S and Zn. These differences attest to the different chemical compositions of these two spruce species.

Manganese (Mn) (Fig. 9-33)

Concentrations are invariably well in excess of the detection limit, and precision is very good. Manganese is easily absorbed by plants and it is an essential element that plays a significant role in photosynthesis. Once taken up and incorporated into plant tissue Mn is relatively immobile in a plant, and it is not readily relocated from old to young tissue. A comparison of 5-year top twig growth of mature Douglas-fir with 3-year growth has indicated that there is 30% more Mn in the older tissue, confirming this reluctance of Mn to relocate, and reaffirming a basic tenet of biogeochemical exploration that twigs of different ages should not be mixed. Consistency in sampling is of paramount importance for meaningful interpretation of analytical data.

Under conditions of low pH, such as a bog, Mn is readily absorbed creating strongly anomalous levels in vegetation samples collected from water-saturated acidic environments. The large amounts of Mn available to plants in acidic soils and bogs cause a Mn–Fe antagonism. This complex interaction is important to understand in helping to interpret patterns of both Fe and Mn distributions. In summary, Fe plays an essential role in plant growth, especially photosynthesis and nitrogen

	V6	NIST 1575a
Target	46	488
Mean	45	519
Std. Dev.	1.6	23
RSD %	4	5

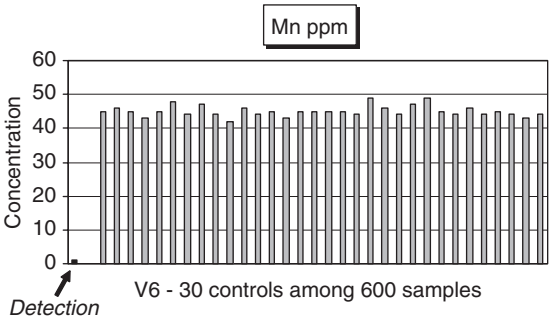


Fig. 9-33. Manganese – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

fixation (Kabata-Pendias and Pendias, 1992). It is not physiologically active in the ferric state, although it is often absorbed in this state and rapidly reduced to ferrous Fe within plant cells in order to commence its role in the synthesis of chlorophyll. The rate at which Fe is reduced in living cells is influenced by the quantity of Mn in the cells, since Mn acts as an oxidising agent (Meyer et al., 1973). Consequently, an excess of Mn may encourage the retention of Fe in its physiologically inactive ferric state. The result is mild stress, which can be manifested as chlorosis or delayed 'green-up' of vegetation in the spring. Such observations made during sample collection (e.g., yellowing of foliage or relative degree of saturation of the ground) are of value in interpreting the relationship of the vegetation to the underlying substrate. Detailed accounts of the complex interactions between Mn and Fe are summarized in Megonigal et al. (2005).

Various studies have noted the wide concentrations of Mn among plant species grown in the same soil (Loneragan, 1975), and this is confirmed by samples from the Eden Project. Whereas the soils have on average about 250 ppm Mn, the foliage of the plants growing in these soils ranges from 18 to 4196 ppm Mn.

Elements that are commonly statistically associated with Mn in plants are mostly other essential elements, such as Mg, Zn, Fe, B and sometimes K, Co and Cu. In tree tissues from the vicinity of the Sullivan Pb/Zn mine (with which Mn is associated) several species have more than 2% Mn in ash. This corresponds to 500–700 ppm Mn in dry tissue. A single lodgepole pine rooted in a zone of base-metal sulphide mineralization with tourmalinite yielded concentrations in dry tissue of 195 ppm Mn in top stems, 278 ppm in roots, 486 ppm Mn in lower twigs, and only 85 ppm Mn in outer bark scales.

Mercury (Hg) (Fig. 9-34)

In dry plant tissue Hg is rarely present at concentrations below the detection limit of 1–2 ppb Hg that some commercial laboratories report for ICP-MS.

	V6	NIST 1575a
Target	41	40
Mean	40	37
Std. Dev.	3.6	5
RSD %	9	14

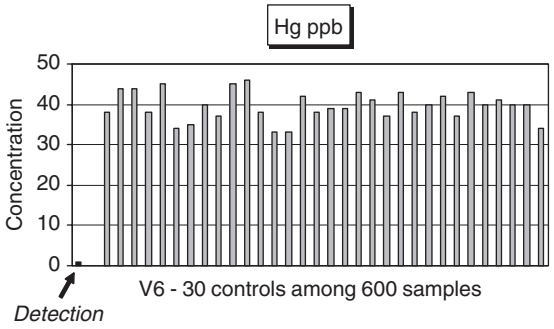


Fig. 9-34. Mercury – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Concentrations are commonly in the 10s of ppb and above 10 ppb analytical precision is excellent. Small spikes in control data are rare, and the very good reproducibility of Hg data from field and laboratory duplicates attests to homogeneity of Hg in typical survey samples.

Much has been written on the fate of Hg in the environment, with a considerable volume of literature on Hg in plants, some of which is quite controversial with regard to what portion has emanated from a natural source and what is from human activities. From the perspective of the exploration biogeochemist, suffice it to say that once potential sources of airborne contamination have been taken into account, the data can be quite enlightening. Trends in relative Hg concentrations may be related to bedrock structure such as dilation zones in antiforms, faults, fractures or breccias that have not become annealed. These structurally weak and porous zones act as conduits through which Hg emanations can migrate. Over zones of concealed Au mineralization Hg enrichment can occur; hence, because of its volatility, Hg acts as a pathfinder element for Au and other metals.

Rasmussen (1995) reported significant temporal increases of Hg in needles of balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) from two control sites remote from known mineralization. This observation is of relevance to biogeochemical exploration programmes conducted over a long period of time, because it demonstrates that there will be a temporal drift in the data. She noted that the Hg content of new foliage more than doubled with each growing season, and was 5–10 ppb higher in the 1990 growing season than in the previous year. This reinforces the recommendation made earlier that field surveys should be completed within a period of two to three weeks. However, by collecting several years of growth (e.g., five years) this variability can be reduced, because it is within the current year's growth that the most substantial variations take place. Also, this temporal drift can be monitored by returning to a particular location on each sampling session to take a repeat sample of a shrub or tree. From the data on changes in Hg concentrations (and other elements) accumulated over a period of time a regression curve can be calculated that would permit normalization of data to a common base.

With respect to Hg contamination from natural sources an interesting modern study of geological processes was reported from the 1980 eruption of Mount St. Helens in Washington State. One year after the eruption Siegel and Siegel (1982) collected soils and young (less than sixth-month old) horsetails (*Equisetum arvense*) that grew at distances of 30–140 km around the volcano. Prior to the eruption, background levels of Hg in this species collected between 1969 and 1975 at various locations in western USA and Canada yielded approximately 2 ppb Hg. In Portland, upwind from Mount St. Helens, horsetails collected in 1981 yielded the same regional level of 2 ppb Hg, whereas at Yakima some 140 km downwind, horsetails yielded 23 ppb Hg. The horsetails were capable of 'biomagnifying' the subtle increases of Hg in soil derived from the volcanic eruption. This study presented a clear indication of the capability of vegetation to accumulate Hg from natural sources.

Markert's (1994) estimate of 100 ppb Hg as the norm for his 'reference plant' seems high in light of an abundance of data accumulated over the last 15–20 years. A meticulous study involving the analysis of over 400 vegetation samples from the Precambrian Shield in Ontario achieved a detection limit of 1 ppb Hg by performing the analyses in a clean, mercury-free laboratory. It was shown that background levels in twigs and needles of balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) are less than 10 ppb Hg (Rasmussen et al., 1991). Elsewhere, analyses of tens of thousands of samples from around the world commonly return values in the range of 20–40 ppb Hg for foliage, although many species have lower concentrations. In conifer bark, however, several hundred ppb Hg is not uncommon. In western Canada, the outer bark of lodgepole pine (*Pinus contorta*) and western larch (*Larix occidentalis*) have a similar appearance and texture, yet compared to the larch the pine typically has double the concentration of Hg.

On the topic of seasonal variation, but in an environment with high Hg levels (hydrothermal mineralization with cinnabar), Znamirovskii (1966) found that in the summer and spring rings of xylem there was more Hg than in the autumn and winter rings. Highest concentrations occurred at the interface between the bark and xylem tissues. This intriguing account describes the discovery of metallic mercury in the stump of a 120-year-old pine tree from Siberia. The tree was felled in winter some 5–6 years prior to scientific observations, during which time curious locals had cut the stump several times when they noticed metallic globules. When first investigated by the scientific team Hg could not be seen, but on thawing during the spring draw of sap small drops of Hg (up to 3 mm diameter) appeared on the outside of some cracks. The same stump, sawn again 37 days later in laboratory conditions, revealed drops of Hg 0.1–0.2 mm in size in the tissue of the summer–spring rings. Metallic Hg was found in the surrounding soil over an area of 400 m^2, and drops of Hg around 0.3 mm in size could be seen in soil within a radius of 10 m from this stump.

This continual draw of sap after a tree has been felled is a normal phenomenon. Its 'life blood' continues to pump. What is unusual is that there was sufficient metal in the ground to actually form metallic globules on the sawn surface. Although rare, it is not, however, a unique occurrence. Alexander Kovalevsky found high levels of

Ag on stump surfaces from over a silver deposit in Siberia and suggested that stump surfaces might represent an appropriate biogeochemical medium for Ag exploration (pers. comm., ca. 1990).

With regard to normal environmental conditions, foliage tends to have higher concentrations of Hg than twigs. Figure 9-35 shows this relationship from a plot of Hg concentrations in tissues from the tops of Douglas-fir, southern British Columbia. There is usually a very high correlation between Hg in twigs and Hg in foliage that becomes evident when concentrations are in the tens of ppb Hg or higher. In the example shown the relationship is moderately good, but the profile for Hg in twigs is subdued because levels are close to detection.

In acidic aquatic environments bryophytes have been shown to accumulate HgS (metacinnabar) in cell walls (Satake et al., 1990). Two liverworts (*Jungermannia vulcanicola* and *Scapania undulata*) occurring in Hg-contaminated acid waters were investigated by several high-powered microscopic techniques and coagulated crystalline phases of HgS were identified attesting to the ability of the plants to absorb Hg in stream waters and nucleate crystals within their cell structures.

A study of mercury in 1208 dry plant samples (11 species) showed that all samples from the mercury-bearing Pinchi Fault (central British Columbia) contained 200 to 1600 ppb Hg (Warren et al., 1983). This contrasted with background concentrations from non-mineralized ground that rarely yielded in excess of 150 ppb Hg. A later study found 560 to 43,000 ppb Hg in the moss *Pleurozium schreberi* from the Pinchi mine site, and along the same structure 125 km to the north at the former Bralorne Takla mine the same species yielded 420–8560 ppb Hg (Plouffe et al., 2004). At the same time as the study by Plouffe et al., samples of vascular plants and mosses were collected from these areas, and scanning electron microscope investigation of the same species revealed the presence of small irregular grains of cinnabar (approximately 2 μm) that undoubtedly accounted for much, if not all, of the elevated levels in the moss. Outer bark of lodgepole pine and Douglas-fir yielded up to 5000 ppb Hg,

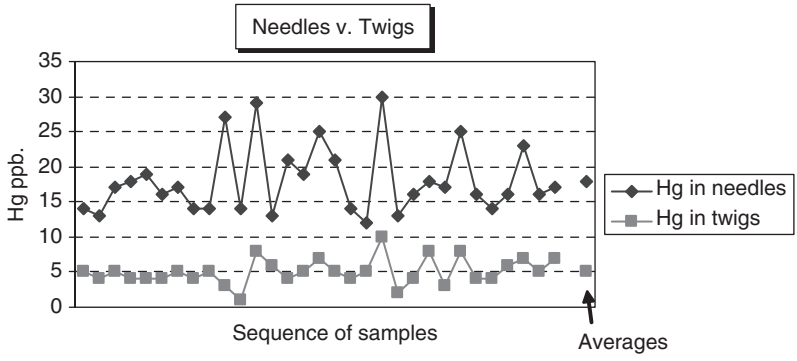


Fig. 9-35. Mercury in dry twigs (5 years growth) and needles from the tops of Douglas-fir (*Pseudotsuga menziesii*) from near the Shuswap River in southern British Columbia.

and various tissues from deciduous species at the mine site had from 40 to 1600 ppb Hg. These data are a sobering reminder that biogeochemical anomalies from immediately around a mine site may be related to airborne particulates of ore minerals derived from mining operations.

Russian studies have suggested that vegetation ash from samples over ore zones contain a non-volatile chemical species of mercury (perhaps mercury carbide) that bears a distinct relationship to the extent of the underlying ore. Kovalevsky (1986) reports a phenomenal 300,000 ppb Hg in ash from plants overlying mercury ore and up to 4300 ppb Hg in plant ash from samples over pyrite-polymetallic ore. The most sensitive plant tissues were found to be bark of birch (*Betula platyphylla*) and larch (*Larix dahurica*). The question here arises, are these truly Hg concentrations derived from Hg absorbed by the plants? Or could they be related to Hg associated with airborne particulates adhering to plant surface? From a personal database that includes many tens of thousands of analyses of ashed plant tissues, the only anomalous Hg in ash samples have been the following:

1. In a reconnaissance survey of western Nova Scotia a single sample of red spruce bark ash yielded a high and analytically repeatable level of 4000 ppb Hg. Upon investigation it was found that this sample also contained 640 ppm As, 2600 ppm Sb and 6% Pb – vastly more than the typical levels for the area of <5 ppm As and Sb and <200 ppm Pb in ash. The conclusion arrived at was that this tree must have got in the way of a hunter with a rifle, and that fortuitously the bark sample included a splattering of Pb shot!
2. In a reconnaissance level biogeochemical survey of a 200 km^2 area of northern Newfoundland, twigs and outer bark of black spruce were the principal sample media. All samples were reduced to ash by ignition at 470°C, and analysed by INAA and ICP-ES for a wide range of elements including Hg. At the time of the survey, mine tailings were exposed at an abandoned copper/gold deposit (Consolidated Rambler). Around this site, and for a distance of 3–4 km to the southeast, both bark and twigs of black spruce tissue yielded anomalous concentrations of Hg in ash with maxima of 255 ppb Hg in outer bark and 150 ppb Hg twigs. High values were coincident with enrichments of Au, As and Sb.

In the latter example, the Hg values, although repeatable, seemed suspicious because Hg in vegetation usually volatilizes completely at between 150 and 200°C. It is tempting to assign these coincident anomalies to new and undiscovered zones of gold mineralization, especially in light of the observations by Kovalevsky (1986), and also because the area is scattered with small Au deposits. However, in the case of the Newfoundland study a more probable explanation is that the metals are associated with fine particles of wind-blown sulphide-rich dust, derived from the large open area of mine tailings, which have become lodged in the tree tissues. This explanation is supported from anecdotal evidence that foresters in the area complained that their chain saws became dulled rapidly when cutting trees from the area down-wind from the mine site, suggesting that there was a relatively high abundance of wind-blown

dust particulates lodged in the bark. Mercury is associated with the ore, and is mostly associated with pyrite and pyrrhotite. Analysis of the mine tailings showed that most of the Hg, Au and As is present in very fine particles (<–230 mesh) that become readily airborne by winds (Table 9-IV). During the ashing process, not all the mercury volatilized because it was tightly locked within the crystal lattices of the sulphide grains. Despite careful washing of vegetation samples, it is not always possible to remove all dust because some becomes firmly embedded and with time tissue may grow around and over some grains, such that they become incorporated within the plant structure. This factor should always be considered as a possibility when interpreting biogeochemical data from samples collected close to mine sites.

In South America, studies in the Amazon have found high levels of Hg around the garimpo artisanal workings where the garimpeiros use Hg to recover the Au. The shrub species Vassoura de Botão, which is a pioneer species capable of surviving and invading disrupted environments, has been found to contain up to 4600 ppb Hg in dry tissue (Dunn and Angelicá, 2000). Of the 23 plants common to many areas of the Amazon it was found that the highest Hg concentrations were in foliage. Imbauba (*Cecropia spp.*), the most common species of the survey area, does not accumulate high levels of Hg (nor the other elements determined) but it reveals *relative* Hg enrichments around the garimpos for a distance of 200–300 m. Other species indicate that there is airborne contamination around the garimpos for a distance of at least 500 m.

Three thousand kilometres to the west, in the headwaters of the Amazon, lies the Chinapintza district in the Cordillera del Cóndor, located on the border between Peru and Ecuador. The epithermal Au deposits of this area have associated with them a strong Hg anomaly in the tree ferns (*Cyathea spp.*). The median Hg concentration for fern fronds throughout an area of approximately 120 km^2 is 76 ppb Hg. At Chinapintza concentrations reach almost 4000 ppb Hg with concentrations greater than 300 ppb Hg extending over an area of at least 1 km^2 and trending for

TABLE 9-IV

Metal concentrations in various size fractions of tailings from the former consolidated Rambler Mine, Baie Verte, Newfoundland, Canada

ASTM 'Tyler' sieve size	Hg (ppb)	Au (ppb)	As (ppm)	Mo (ppm)	Se (ppm)	Sb (ppm)	Cr (ppm)
Coarse (>–30 mesh [600 μm])	110	200	33	2	8	18	170
–30 to –80	175	300	70	8	15	31	160
–80 to –150	195	700	390	19	87	28	95
–150 to –230	485	750	600	26	120	19	45
Fine (<–230 mesh [63 μm])	1170	1700	670	41	120	37	190

several kilometres to provide a target for more detailed exploration. Elsewhere in the survey area other anomalous Hg trends are evident. Whereas consideration should be given to the possibility that the highest Hg concentrations may be derived from airborne particulates, regional trends serve as targets on which to focus exploration activities.

In light of the results from many recent studies on the use of Hg data to assist in mineral exploration, much of the advice given by Kovalevsky (1986) remains sound. In particular, that Hg data can be used to elucidate concealed structures (folds, faults, fractures, zones of brecciation), and it is advisable to use Hg biogeochemical data in prospecting mainly for non-mercury deposits. As shown above, the presence of cinnabar in outcrop or open pit is likely to generate false Hg anomalies from airborne particulates. Mercury haloes may emanate from deep-seated ore-bodies that have Hg associated with them. Kovalevsky (1986) suggests that because of the volatility of Hg, ore-bodies at depths of 200–2000 m can generate Hg haloes that can be reflected in plants. Foliage and conifer bark are the preferred sample media.

Molybdenum (Mo) (Figs. 9-36 and 9-37)

Reproducibility of Mo in analytical controls and duplicates is excellent at the levels of Mo typically found in vegetation. However, the data for NIST 1575a indicate poorer precision at the much lower levels present in that control material.

Molybdenum is an essential micronutrient in plant enzymes. Its most important function is the reduction of soil nitrate, but only small traces are required. Roots are commonly relatively enriched in Mo (especially nodulated roots such as those found in alder), and N-fixing micro-organisms have especially large requirements for Mo.

In plants, Mo is one of the most mobile elements, but the plant tissues in which there is relative enrichment of Mo vary by species. In general, the twigs of conifers

	V6	NIST 1575a
Target	0.28	(0.01)
Mean	0.27	0.02
Std. Dev.	0.02	0.01
RSD %	8	37

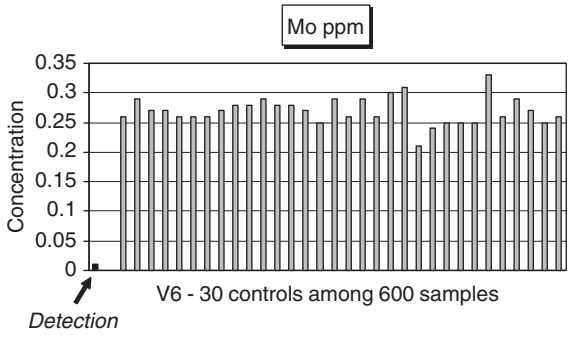


Fig. 9-36. Molybdenum – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

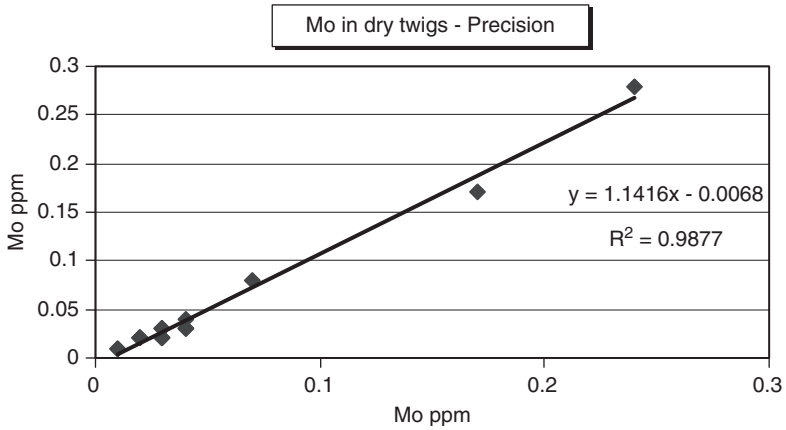


Fig. 9-37. Precision (analytical duplicates) of Mo in dry Douglas-fir twigs. Nineteen repeat analyses within batch of 555 samples, of which 10 analytical pairs returned identical values.

have higher Mo concentrations than the foliage. In deciduous species values are sometimes higher in the leaves and sometimes in the twigs, but the pattern is consistent for a particular species. Differences are not great, with one tissue type rarely having more than double the Mo concentration of another, although an exception appears to be plants that tend to accumulate Mo, such as alder. Molybdenum in alder twigs can be more than four times enriched than alder leaves, and especially enriched in the latest year of twig growth. Conversely, the genus *Combretum* (bush-willow), widespread throughout much of sub-Sahara Africa, has more Mo in leaves than twigs. Acacia from the North Mara district of Tanzania and also from Western Australia contains slightly more Mo in the twigs than the foliage.

Molybdenum is a significant pathfinder for Cu–Mo, Cu–Au–Mo, W–Mo and other Mo-bearing deposits. Copper–Mo porphyries are usually better defined from the Mo content of the vegetation than by that of Cu. Kovalevsky (1987) investigated the exploration characteristics with respect to Mo of 506 species and parts of plants and found most to be highly informative. From their Mo content suberized (woody) pine cones from the floor of Siberian forests were found to be particularly effective for focussing on exploration targets. However, because there are so many plant tissues that do not establish significant barriers to Mo uptake, most parts of most species are suitable for biogeochemical surveys with the exception of inner bark (bast). Inner bark is rarely an informative or practical medium to collect for any element.

Data from the Eden Project demonstrate the greatly varying capabilities of different plant species to accumulate Mo, with plants showing a range from 0.03 ppm Mo in *Protea* to 10.8 ppm Mo in *Acacia* grown in similar soils under a controlled environment.

Molybdenum is one of the few elements for which studies claim to be successful in quantifying the depth through overburden to ore. Kovalevsky (1987) reports that

> if the minimum … measure of the anomalies is their threefold excess over local background, then the maximum [overburden] depth of detecting lithogeochemical haloes having a relative intensity of 500 compared with the local background (i.e., ore = 1000 ppm and background = 2 ppm), is 22 m for the lower parts of the stems of Dahurian rhododendron [*Rhododendron dauricum*], 17 m for the upper parts, and 16 m for shoots and leaves.

This bold prediction of depth to mineralization, estimated from sampling different tissues in a 'non-barrier' plant, he defined as 'biogeochemical logging'. This quantification of depth to mineralization is an exciting concept, but it does not appear to have been tested outside of Siberia.

A survey over a Mo porphyry in the western Amazon showed that the Mo anomalies derived from four different plant parts (foliage from bamboo, a vine, treefern, and bark from the fern) were similar and spatially related to mineralization. However, concentrations were substantially higher in the fern (foliage and bark) than in the other two species (Fig. 4-3).

The ready availability of Mo causes high levels of Mo uptake by plants in contaminated sites. As a consequence, there are commonly large biogeochemical Mo haloes around Mo mining operations. Windblown Mo-bearing dust can settle on the ground and be dissolved by rain and leach into the soil. It is then absorbed by plant roots to give a substantial enrichment in plant tissues. This process probably accounts in large part for the extensive Mo halo that surrounds the Endako mining operations in central British Columbia, where reconnaissance level surveys using the outer bark of lodgepole pine (*Pinus contorta*) have outlined the regional extent of molybdenum enrichment. This survey involved reducing bark to ash which, in lodgepole pine throughout much of central British Columbia, has background levels of 5–10 ppm Mo (0.1–0.2 ppm Mo dry weight [DW] equivalent). The median value for a dataset of 217 samples was 78 ppm (1.5 ppm Mo DW), and values in excess of the median extended over an area of approximately 5000 km^2. Values greater than the 95th percentile value of 717 ppm Mo (34 ppm Mo DW) extended over an area of 500 km^2 attaining a maximum of 1.5% Mo in ash (300 ppm Mo DW) from trees close to the mine (Fig. 9-38).

Although dust from the mining operations contributes to the high levels encountered, historic records indicate similar levels in plants collected from the mine site around 1950 prior to any mining operations (Warren et al., 1953). At that time values of 65 ppm Mo in dry alpine fir needles were reported (equivalent to 2000 ppm Mo in ash) from an undisclosed site which from discussions with Professor Warren in the 1990s proved to be Endako. Clearing of the mine site for production did not begin until 1964. Analyses published in 1965 (Warren and Delavault, 1965) of a variety of species reported maxima in ash of 9600 ppm Mo in willow leaves and 17,000 ppm Mo in the annual plant 'fireweed' (*Epilobium angustifolium*). Samples of fireweed collected more than 30 years later from the mine site yielded 2950 ppm Mo in ash or 376 ppm Mo in dry tissue (Dunn, 1998b).

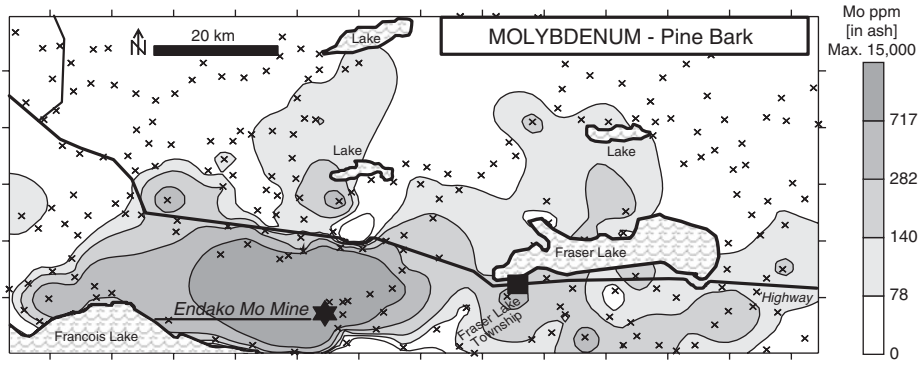


Fig. 9-38. Molybdenum in the ash of lodgepole pine bark – reconnaissance survey in the vicinity of the Endako Mo mine. Analysis by INA.

At the Lucky Ship prospect located 100 km farther west, outside of the large Endako anomaly, concentrations of more than 1000 ppm Mo in the ash of second-year growth of alpine fir (*Abies lasiocarpa*) needles were reported by Hornbrook (1969). In oven-dry needles of lodgepole pine and alpine fir concentrations exceeded 20 ppm Mo over the deposit, and anomalous values extended for 500 by 750 m. Subsequently, various companies held the property and undertook a limited amount of prospecting. In 2005, Cantech Ventures Inc. commenced a drilling programme and grades of $>0.089\%$ Mo were encountered within its first two holes. Drilling in 2006 'encountered Mo mineralization over its entire length with the best grade between 6.0 and 266.0 metres which averaged 0.084% Mo (including the first 36 metres which averaged 0.163% Mo)' (www.newcantech.com, August 9, 2006, press release).

Molybdenum serves as a good example of an element that can be very useful as a biogeochemical indicator of mineralization – both Mo deposits and Mo-bearing deposits, such as Cu–Mo–Au porphyries. Analytical data are precise and accurate, and because it is highly mobile Mo can be readily taken up by plants. However, because of its mobility, caution must be exercised to carefully evaluate the environment within which samples are collected (especially noting any nearby mine workings); and there should be vigilance maintained when interpreting results, because an anthropogenic 'footprint' can obscure the natural signature derived directly from concealed mineralization.

Neodymium (Nd)

See Lanthanum and the Rare Earth Elements.

Neodymium has no known biological role. In V6 it exhibits good precision with a mean of 0.7 ppm Nd. Databases are limited but sufficient to establish that Nd is usually concentrated in plants at levels just above its detection limit by ICP-MS of 0.02 ppm Nd in dry tissue. In South Africa, foliage of *Acacia mellifera* (Black thorn)

has yielded up to 2.7 ppm Nd adjacent to a kimberlite. Stems from this species had concentrations 50–70% lower. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada yielded an average concentration of 0.03 ppm Nd, with a maximum of 0.18 ppm Nd. The sagebrush *Artemisia* collected from near the Bayan Obo REE mine in northern China yielded a phenomenal 600 ppm Nd in dry tissue (3300 ppm Nd in ash). However, from the unusually high ash yield of this sample (18%) it is strongly suspected that a considerable proportion of the Nd was derived from airborne particulates.

On occasion, Nd enrichment has been observed in samples spatially related to zones of Au mineralization. Further details are given in the section on 'Lanthanum'.

Nickel (Ni) (Fig. 9-39)

The analytical precision for Ni by ICP-MS is very good and concentrations in plant tissues are commonly well in excess of the detection limit of 0.1 ppm Ni.

For some plants, Ni is an essential element present in the enzyme urease, and it probably plays a role in the translocation of nitrogen. It can also occur as a citrate, and it is in this form that some extraordinarily high concentrations of Ni in plants have been reported. The blue sap reported on the surface of bark from the serpentine-endemic tree 'Sève bleue' (*Sebertia acuminata*) from New Caledonia obtains its colour from Ni. Analyses have yielded 25.7% Ni in dried sap and 11.2% Ni in fresh sap (Jaffré et al., 1976). The Ni contents of other dried plant tissues from this tree were 1.17% Ni in leaves, 2.45% Ni in bark from the trunk, 1.12% Ni in bark from the twigs, 0.20% Ni in fruits and 0.17% Ni in trunk wood.

Lee et al. (1977, 1978) established that the Ni content of the sap in Sève bleue, and in the leaves of other New Caledonian and Zimbabwean species, was a citrate–nickel complex. In other species Ni is present as a malate complex. There are more plants that hyperaccumulate Ni than any other metal and Brooks (1998) lists 256 species noting that another 48 are known from Cuba. Subsequently, more have been

	V6	NIST 1575a
Target	3.3	1.5
Mean	3.2	1.6
Std. Dev.	0.25	0.16
RSD %	8	10

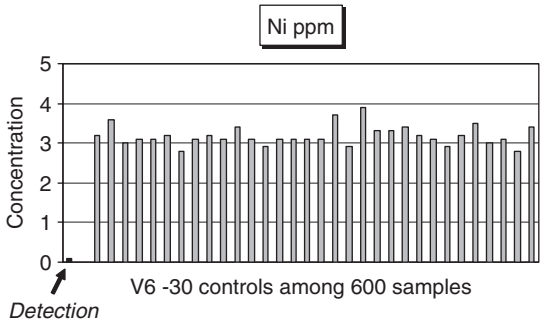


Fig. 9-39. Nickel – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

identified bringing the number now to 318. The dominant plant families that have species capable of hyperaccumulating Ni are the Buxaceae, Euphorbiaceae, Flacourtiaceae and (notably) the Brassicaceae. Examples are as follows:

- Buxaceae (e.g., boxwood) from Cuba. Up to 2.5% Ni in *Buxus vacciniodes*.
- Euphorbiaceae (flowering shrubs) – especially *Leucocroton* from Cuba (up to 6% Ni) and *Phyllanthus* from New Caledonia (up to 3.8% Ni).
- Flacourtiaceae (flowering trees and shrubs) from New Caledonia. Especially *Homalium* and *Xylosoma* with up to 1.5% Ni.

The Brassicaceae (herbs and forbs) are mainly from southern Europe. Most notable are *Alyssum* (48 species – up to 2.9% Ni – especially *Alyssum bertonii*) and *Thlaspi* (Pennycress – 23 species with up to 3.1% Ni). The species *Berkheya coddii* has been identified as a prime candidate for phytomining because of its high biomass (2 m tall) and its ability to concentrate $>2\%$ Ni.

With respect to mineral exploration surveys the above examples of Ni-hyperaccumulation are somewhat academic. Most of the Ni-hyperaccumulator plants are curiosities and not sufficiently abundant to conduct an exploration biogeochemistry survey. In the vast boreal and temperate forests of the world, no common plant is known to be a Ni-hyperaccumulating species. Foliage of plants from the Eden Project show more typical levels, with a range from 0.2 ppm Ni in pine needles to 5.2 ppm Ni in bamboo leaves.

In a survey using scrapings of outer bark from 455 Douglas-fir trees (*Pseudotsuga menziesii*) in the vicinity of the Old Nick showings in southern British Columbia it was found that the median concentration was 1 ppm Ni with a maximum of 4.6 ppm Ni. Mineralization is associated with pyrrhotite and pentlandite disseminated within serpentinite units and metasediments. Mineralization is fairly uniform with an average range of 0.15–0.2% Ni. The bark signature is, therefore, quite subtle although linear trends related to mineralization and bedrock are apparent from contoured bark data of values greater than 1.5 ppm Ni (British Columbia Dept. of Mines 2004 assessment report 27,579). By comparison, a large dataset of lodgepole pine outer bark samples from central British Columbia where no Ni deposits are reported yielded concentrations lower than those in Douglas-fir at Old Nick, with a median value of 0.3 ppm Ni and a maximum of 0.85 ppm Ni.

Nickel has a tendency to accumulate at the top of conifer trees, so values are higher than in bark. A set of 35 Douglas-fir top samples from a background area of south-central British Columbia (Shuswap River) yielded up to 4.4 ppm Ni in dry needles and 5.6 ppm Ni in five years growth of twigs. A broader survey of the area involving 562 tree tops had a maximum of 9.6 ppm Ni in twigs.

In northern Saskatchewan, the small ultramafic Rottenstone deposit that was mined out in the 1960s has some strong Ni anomalies within an area of more than 100 km^2 that are not related to mining activities and have yet to be explored. At the old mine site several common species of the northern forests are enriched in Ni and the

TABLE 9-V

Rottenstone ultramafic deposit (Ni–Cu–PGEs–Au), Saskatchewan. Nickel (ppm in dry tissue) in common species from close to the abandoned mine site

Species	Latin binomial	Tissue	Ni (ppm)
Black spruce	*Picea mariana*	Top twigs	35
Black spruce	*Picea mariana*	Twigs	32
Black spruce	*Picea mariana*	Needles	14
Labrador tea	*Ledum groenlandicum*	Twigs	18
Labrador tea	*Ledum groenlandicum*	Needles	21
Mountain alder	*Alnus crispa*	Twigs	25
Mountain alder	*Alnus crispa*	Leaves	60

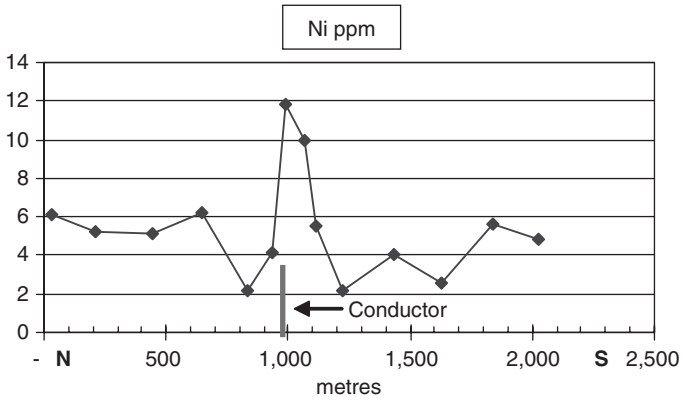


Fig. 9-40. Nickel in top twigs of black spruce (analysis by ICP-MS of 3 years growth of dry tissue). Thompson Nickel Belt, Manitoba, Canada. Data courtesy of Anglo American Exploration Ltd.

data serve to evaluate their relative uptake of Ni (Table 9-V). The high concentration in alder leaves attests to the sensitivity of this species to the presence of Ni. Leaves were collected from 5-year-old shrubs almost 40 years after mining operations had finished.

In Manitoba, Canada, the Thompson Nickel Belt yields an abundance of geophysical conductors, but geophysics alone cannot confidently differentiate between those that represent barren sulphides and those that are nickeliferous. Black spruce (*Picea mariana*) top twigs were collected from over several of these conductors and analysed for 53 elements. Nickel concentrations were elevated, reaching a maximum of 24 ppm Ni in three years growth of twigs over several conductors, and subsequent drilling has found them to be Ni-bearing. There was very little difference in the Ni content of each year of growth of either the twigs or needles. However, the twigs contained more than double the Ni content of the needles or cones. Figure 9-40

shows a typical profile of Ni in top twigs from a helicopter-borne flight over a known geophysical conductor.

Niobium (Nb) (Fig. 9-41)

Precision for Nb is poor at the concentrations usually present in vegetation. Occasional spikes in controls and fair to poor replication of field and laboratory duplicates considerably compromise the value of the Nb data. Interpretation of Nb results should be treated with caution, since the controls indicate that false anomalies may arise.

Niobium has low mobility in the natural environment. Kabata-Pendias (2001) reports that Nb may be relatively mobile under humid conditions, but notes that there is no evidence offered to support this contention. Kovalevsky (1987) stated that the low mobility of Nb over Nb-bearing ores results in low availability to plants except over 'cryogenic taiga-type [waterlogged] soils', which is interpreted to mean permafrost or discontinuous permafrost, and is likely to refer to studies in the arctic conditions of the Kola Peninsula where significant Nb ores occur.

Determinations of Nb can assist in the exploration for Nb/Ta deposits. Near Nb mineralization on the Kola Peninsula concentrations of up to 10 ppm in dry tissues of several species (notably the arctic raspberry *Rubus arcticus*) have been reported (Tiutina et al., 1959). At the Bernic Lake rare metal deposit in Manitoba various tissues of jack pine, black spruce, alder, poplar (*Populus tremuloides*) and paper birch (*Betula papyrifera*) were collected at eight sites near the mine. Niobium was determined by ICP-MS at the Geological Survey of Canada laboratories. The pine bark had, on average, the highest concentrations with an average of 4.3 ppm Nb. The spruce bark had 2.2 ppm Nb, the spruce twigs 1.5 ppm Nb and the pine twigs 1.2 ppm Nb. All of the remaining samples yielded <0.2 ppm Nb. A few comparisons of needles from spruce and pine indicated that the pine had twice as much Nb as the spruce, but both were substantially lower than in the twigs or bark.

	V17	NIST 1575a
Target	0.02	No data
Mean	0.02	0.004
Std. Dev.	*0.01*	0.003
RSD %	*53*	65

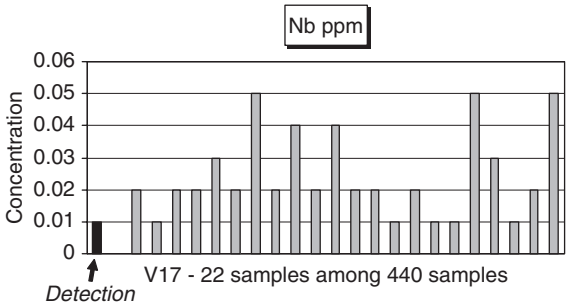


Fig. 9-41. Niobium – Precision and accuracy on dry tissues – V17 and NIST 1575a (see Fig. 9-1).

At Bernic Lake the Nb concentrations were probably somewhat enhanced by Nb-bearing dust from the mine workings, but the data serve to demonstrate that the conifer bark and twigs would be the preferred sample media for conducting a biogeochemical survey for Nb. These findings are in accord with those of Kovalevsky (1987) who considered that the external layers of bark or suberized cones from the forest floor are the most informative indicators of Nb.

Kimberlites commonly have elevated Nb values, and some enrichment of Nb at the margins of kimberlites has occasionally been found. The volcanic-facies kimberlite at Sturgeon Lake, Saskatchewan, yielded weakly anomalous levels of Nb in twigs of trembling aspen (*Populus tremuloides*) using an ICP-MS method developed to determine ultra-trace levels of selected elements (Hall et al., 1990b). Background concentrations in ash were 0.2 ppm Nb (0.006 ppm Nb dry weight), with a maximum adjacent to the kimberlite outcrop of 1.3 ppm Nb (0.02 ppm dry weight) (Dunn, 1993).

Osmium (Os)

See 'Platinum and the Platinum Group Elements'.

Palladium (Pd)

See 'Platinum and the Platinum Group Elements'.

Phosphorus (P) (Fig. 9-42)

Since P is an essential element for plants, concentrations are always well above the detection limit. Precision is excellent. There is a considerable volume of literature on

	V6	NIST 1575a
Target	0.046	0.11
Mean	0.045	0.13
Std. Dev.	0.003	0.006
RSD %	7	4

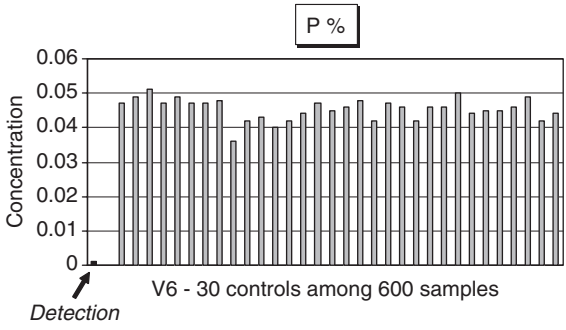


Fig. 9-42. Phosphorus – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

the role of P in plants; it is a component of various proteins, sugars and starches, and it is critical for energy transfer. The general ratio of C:N:S:P in terrestrial plants is 790:7.6:3.1:1 (Bolin et al., 1983). The P content of Markert's (1994) 'reference plant' is 0.2%, which is close to the average of 0.24% in foliage samples from the Eden Project. Twigs contain less P (generally about 0.05–0.15% P) and in conifer bark concentrations are lower still at 0.01–0.03% P.

With respect to exploration, the P content of plants occasionally assists in defining the location of mineralization. Phosphorus is sometimes enriched with U deposits, in which case anomalous P concentrations can occur in plants. However, U is a mobile element and U is a better indicator of U than P.

Some kimberlites have elevated levels of P. In these situations plants growing over the kimberlites can generate positive P anomalies. In central Alberta, a white spruce sampling programme found enrichments of several elements in the ash of the top stems, including a good response from P over most of the known kimberlites of which only one had an outcrop and the remainder were concealed beneath several metres of overburden (Fig. 9-43).

Over kimberlites from elsewhere P responses in vegetation are usually more subtle or absent. Several kimberlites from South Africa yielded no positive P response in the overlying vegetation, whereas others had a modest response. Similarly, there was a

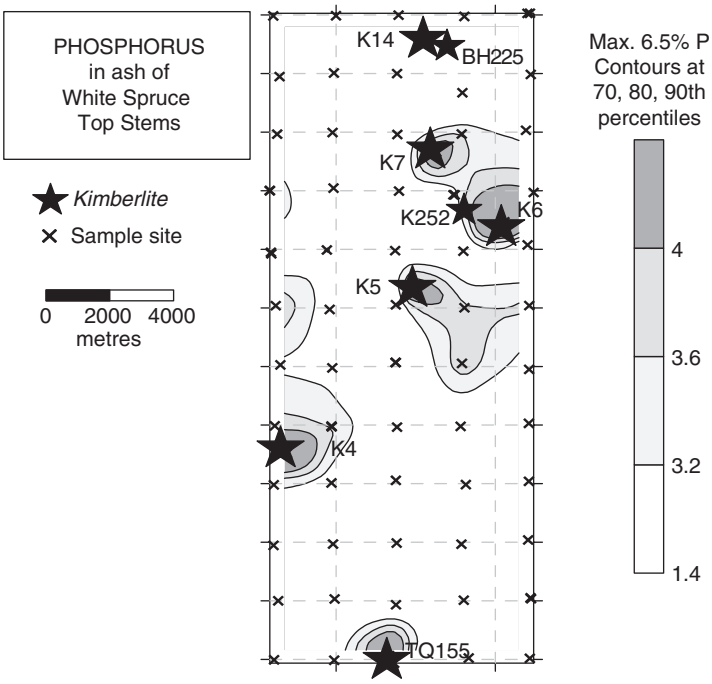


Fig. 9-43. Phosphorus in the ash of top stems from white spruce (*Picea glauca*). Buffalo Head Hills, Alberta, Canada.

positive response in leaves of dwarf birch from over some of the kimberlites in the Ekati diamond fields of Canada's Northwest Territories, and a subtle response in twigs of poplar from adjacent to the volcanic facies Sturgeon Lake kimberlite in Saskatchewan (Dunn, 1993). Commonly, positive signatures of P in plants growing over kimberlites are complementary to stronger signatures of other elements.

Platinum (Pt) and the platinum group elements (PGEs) – palladium (Pd), iridium (Ir), osmium (Os), rhodium (Rh) and ruthenium (Ru) (Figs. 9-44 and 9-45)

These are among the rarest of the elements and, although instrumental detection limits are now in the low-ppb range, the PGEs are infrequently detected in dry plant tissue from areas remote from cities. Of these elements only Pd and Pt are detected by ICP-MS without analysis by high-resolution instrumentation or the introduction of methods to pre-concentrate the PGEs (e.g., NiS fire assay). If traces of Pd or Pt are reported in dry tissue, results should be verified by re-analysis and careful consideration of the environment from which the samples were collected, always bearing in mind that modern vehicles have catalytic converters than emit Pt and Pd. Samples from close to highways are likely to be contaminated with small amounts of Pt and/or Pd. For example, Dongarrá et al. (2003) recorded up to 102 ppb Pt and 45 ppb Pd

	V7 (ash)	NIST 1575a
Target	~ 80 ppb	No data
Mean	84	
Std. Dev.	*16*	
RSD %	*19*	

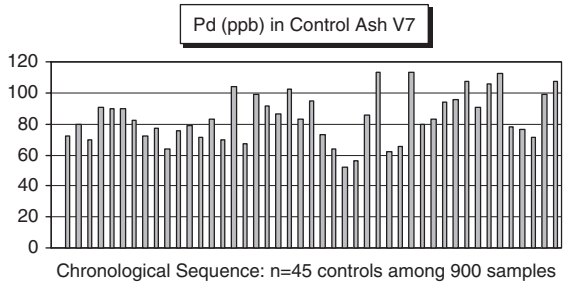


Fig. 9-44. Palladium – Precision and accuracy on ashed tissues – V7 (see Fig. 9-1).

	V7 (ash)	NIST 1575a
Target	~ 50	No data
Mean	59	
Std. Dev.	*51*	
RSD %	*86*	

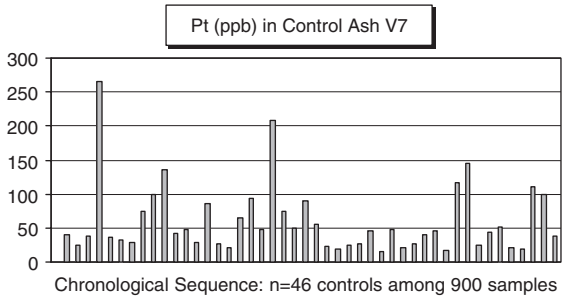


Fig. 9-45. Platinum – Precision and accuracy on ashed tissues – V7 (see Fig. 9-1).

in pine needles from the urban area of Palermo, Italy. From SEM studies they found Pd bound to micron-sized silica particles, probably in the form of a halogenated compound.

For PGEs other than Pt and Pd, HR-ICP-MS, INAA, GFAAS or special techniques are required for determining the very small traces present in plant tissues. Iridium is included in most INAA packages, with detection as low as 0.1 ppb Ir in dry tissue, but even so concentrations are nearly always below detection. Any positive values reported should be queried with the laboratory to ensure that appropriate corrections have been made. For all practical exploration purposes, at the present time only Pd and Pt need to be determined in plant tissues and any requirement for the other PGEs should be discussed with the analyst.

At this time there is no commercially available PGE standard for dry vegetation. The quality control charts for Pd and Pt shown above are data from the analysis of an unusually PGE-rich ash (designated V7) and they demonstrate precision that is quite good for Pd but poor for Pt. In field samples it is rare to obtain Pd values as high as these and precision at lower ppb Pd levels is likely to be considerably inferior. V7 has yielded extreme variability for Pt with a RSD of 86%. This level of variability does, of course, cast doubt on the validity of the data from field samples. However, if analytical duplicates are reasonably repeatable and the patterns of Pt distribution are similar to those of Pd and other typical pathfinder elements (e.g., Te, Se), there is good reason to more fully investigate their significance with regard to the potential presence of PGE-rich deposits.

Palladium determinations by ICP-MS are prone to a variety of complex inter-element interferences requiring many corrections by the analyst. Platinum gives a much cleaner signal, but like Au it exhibits the classic 'nugget' effect such that it appears to be highly heterogeneously distributed within plants. In conducting a biogeochemical survey for PGEs, the reduction of tissues to ash by controlled ignition is an effective way to preconcentrate them so that concentrations are appreciably above the instrumental detection limits. There are, however, some potential problems with respect to Pd, because the usual ashing temperature is about 475°C and at this temperature studies have indicated that Pd may create a poorly soluble oxide bond that does not breakdown until much higher temperatures are attained (Table 9-VI). Excellent agreement was shown between the ICP-MS method and lead fire assay (FA)/ICP-MS for twenty samples collected in bulk in the vicinity of the Rottenstone Deposit, Saskatchewan (Hall et al., 1990a).

Table 9-VI shows that the total Pd content of the samples was not obtained until samples were heated to about 850°C. However, an appreciable amount of the Pd could be recovered from ash obtained from controlled ignition at 470°C demonstrating that, for exploration purposes, meaningful inter-site distribution patterns could be obtained provided all samples were ashed at the same controlled temperature. It could be argued that in PGE exploration all samples should be ashed to at least 850°C. However, this would involve an extra cost and at that temperature other elements will have partially volatilized and others may have formed complex bonds

TABLE 9-VI

Effect of ashing temperature on the recovery of Pd (ppb) in black spruce using ICP-MS. Values normalized to ash weight at 470°C (Hall et al., 1990)

Tissue		Ignition temperature (°C)					
		470	700	800	850	870	900
Black spruce twigs	Pd (ppb)	810	890	958	1390	1428	1410
Black spruce needles	Pd (ppb)	91	117	170	220	230	228

creating new problems in interpreting a multi-element data set. Whereas it is desirable to release all of an element into solution, for exploration purposes this is not a requirement, because it is the spatial relationships of element concentrations that provide focus for exploration activities. Controlled conditions are the critical factors to be maintained.

In dry tissues, background levels of Pd, the most abundant of the PGEs, are in the sub-ppb range, and the other five PGEs (Pt, Rh, Ir, Os and Ru) occur as only a few parts per trillion (Dunn et al., 1989). Palladium in plants of the western United States has been studied in some detail by Kothny (1979, 1987, 1992), who found that plants growing in soils containing 40 ppb Pd had from 15 to 110 ppb Pd in ash. The highest levels were in California white oak (*Quercus lobata*), red pine (*Pinus resinosa*) and bearberry (*Arctostaphylos nummularia*). Seasonal variations determined mostly from samples of black walnut (*Juglans hindsii*) were similar to those reported elsewhere for gold, with highest concentrations occurring in the early summer (Kothny, 1992).

Under most natural conditions Pd is more mobile than the other PGEs, behaving in a similar manner to base metals. Studies show that Pd can migrate either as a true solute or in colloidal form (Pogrebnyak et al., 1986) whereas this has not been demonstrated for Pt. It can be expected, therefore, that a biogeochemical halo around PGE mineralization will be larger for Pd than Pt. Of all the PGEs, Pd is that which is likely to provide the best biogeochemical signature of underlying PGE mineralization. In sulphide-hosted PGE mineralization, Pd is commonly the most abundant of the PGEs. In oxide-hosted PGE mineralization, Ir may be relatively abundant and can be determined as part of a routine multi-element INA analysis. The low detection obtained by INAA (2 ppb Ir in ash and 0.1 ppb Ir in dry tissue) provides a simple first appraisal of the presence of PGEs in vegetation and, by inference, in the underlying substrate.

From a biogeochemical exploration perspective it is important to consider the anticipated type of mineralization that may be hosting PGEs. The biogeochemical response to PGE mineralization is strongly dependant on whether the host minerals are sulphides or oxides. Sulphide-rich mineralization (Ni and Cu-rich) may generate a good biogeochemical response, whereas PGEs locked tightly in crystal lattices, such

as chromite, are not readily released and are therefore absorbed only weakly by plants to give a subtle biogeochemical response.

In the Arctic tundra, gossans rich in Pt and Pd occur near Ferguson Lake within a suite of metamorphic rocks dominated by hornblende-rich gneiss, amphibolite and granitic gneiss. They are volcanic and sedimentary rocks of the Archaean Kaminak Group that were metamorphosed during the Hudsonian orogeny. Copper–nickel sulphides form narrow, massive lenses and disseminations, and the metallic minerals collectively contain 1.2–3.3% combined copper and nickel. Grab samples have yielded up to 590 ppb Pt and 2500 ppb Pd (Coker et al., 1991). A study of several arctic shrubs and lichens reported less than 50 ppb Pt, but up to 913 ppb Pd in ash of *Diapensia lapponica* from a gossan (Lee, 1987). This circumpolar alpine-arctic species is a small cushion-forming evergreen perennial shrub, up to 15 cm in height. A second study in the same area examined the PGE content of dwarf birch (*Betula glandulosa*), and Labrador tea (*Ledum palustre*) and reported maxima of 1350 ppb Pt, 3071 ppb Pd and 124 ppb Rh in ash from stems (Coker et al., 1991). The dry tissue equivalent concentrations of these values are approximately 25 ppb Pt, 55 ppb Pd and 2.4 ppb Rh. The ashed stems of the birch and Labrador tea had substantially more Pt, Pd and Rh than the leaves, with Rh showing the greatest stem to leaf contrast of more than 15:1 in ash.

The Stillwater Complex of Montana contains North America's largest known reserves of PGEs. Platinum, Pd and Ni sulphides of the braggite-vysotskite solid solution series are hosted by layered mafic to ultramafic rocks. Fuchs and Rose (1974) collected two samples of limber pine (*Pinus flexilis*) which yielded maxima of 56 ppb Pt and 285 ppb Pd in twig ash. Ten years later, prior to the commencement of mining operations, Riese and Arp (1986) collected twigs and needles of Douglas-fir (*Pseudotsuga menziesii*) from 65 sites along several traverses over the Howland Reef part of the complex. Samples were reduced to ash at 550°C and analysed by ICP-ES after a proprietary double MIBK-based extraction method. Twigs (5–7 years growth) were reported to contain remarkably high maxima of 3000 ppb Pt and 15,000 ppb Pd in ash. Concentrations were approximately one order of magnitude higher in the Douglas-fir twigs than in the soils, and biogeochemical anomalies were displaced a few tens of metres down slope from a known zone of mineralization. Platinum had the lowest signal-to-noise ratio, and it was concluded that Pt in vegetation was the preferred geochemical exploration method for this area.

The small ultramafic body comprising the Hall deposit at Rottenstone Lake in Saskatchewan was mined from 1965 to 1968 to extract PGE-rich violarite (Ni) and chalcopyrite (Cu). The deposit yielded 4779 ppb Pt and 3920 ppb Pd that was contained mostly in sperrylite (PtAs2), moncheite (PtTe2) and kotulskite (PdTe). Subsequently, PGE-rich phases of several mineralized boulders have yielded up to 38,000 ppb Pt, 13,000 ppb Pd, 270 ppb Rh, 180 ppb Ir, 200 ppb Os and 140 ppb Ru (Hulbert and Slimmon, 2000). Although the ore body was only 50 m by 50 m by 10 m, it was richer in PGEs than any other nickel–copper deposit in Canada.

The Rottenstone area is located in pristine boreal forest, far from any road or all-weather trail and as such comprises a 'natural biogeochemical laboratory' from

which collections have been made on numerous occasions between 1983 and 2006. Samples have been analysed for all the noble metals, and they show that twigs and outer bark of black spruce accumulate PGEs more than the other tissues that were examined (Table 4-VII). Labrador tea is also sensitive to the presence of most of these elements. During that time span plants were naturally seeded after cessation of mining operations, yet species have maintained a similar PGE composition – the precise locations visited in 1983 to collect samples were re-sampled in 2006.

Platinum group metals within the Alaskan-type Tulameen Complex of southern British Columbia occur mostly in chromite-rich pods within serpentinized dunite. Platinum and Ir are the dominant PGEs, with no detectable Pd in the chromites, but weak Pd enrichment in the clinopyroxenite, hornblendite and the rare sulphide phases. Platinum-iron alloys, sperrylite (PtAs2), and irarsite (IrAsS) are the most abundant PGE-bearing minerals, and all other PGE minerals reported are Pt-bearing. They occur either as euhedral to subhedral grains in chromite, or as anhedral grains interstitial to chromite. The recovery of platinum has been solely from placer operations. In view of the nature of the mineralization and the resistance of chromite to weathering, the PGEs tend to remain tightly bound in crystal lattices. Consequently, analyses of various trees have yielded only low concentrations of PGEs. However, most rock types comprising the complex do contain total PGE concentrations of at least a few tens of ppb demonstrating a regional enrichment of PGEs.

At Tulameen, plant cover is sparse on the steep slopes and on top of the serpentinized body that rises steeply from the moist wooded valley of the Tulameen River. Samples of trees and shrubs from a 6 km transect along the Tulameen valley (down-slope from known chromite pods on Grasshopper Mountain) yielded only a few ppb PGEs. Iridium was detected in only two of 70 ash samples: 15 ppb Ir in Douglas-fir (*Pseudotsuga menziesii*) twigs, and 5 ppb Ir in twigs from a yew (*Taxus brevifolia*). None of the 34 samples from sites close to chromite pods on the mountainside yielded over 2 ppb Ir or Rh in ash, but they contained <2 – 29 ppb Pt and <2 – 21 ppb Pd. Ash of Douglas-fir twigs had 17 ppb Pt and 7 ppb Pd, and bark had 7 ppb Pt and 18 ppb Pd. Highest concentrations in ash of the samples tested were 29 ppb Pt in outer bark of lodgepole pine (*Pinus contorta*), and 21 ppb Pd in bark of Whitebark pine (*Pinus albicaulis*) (Dunn, 1992, 1995a; Fletcher et al., 1995). Earlier unpublished work by Prof. H.V. Warren (pers. comm., 1989) noted some preferential uptake of Pt by the small 'umbrella plant' (*Eriogonum ovalifolium*), and analysis of a sample collected later confirmed his observation, yielding a relatively high level of 21 ppb Pt in ash. Several species of this plant occur on mountain slopes of western North America, especially on ultramafic rocks and, despite its irregular distribution, it could be used to provide an indication of the PGE potential of the substrate.

A biogeochemical component was included in a range of geochemical surveys conducted by the Minnesota Geological Survey over a 1000 km^2 area that encompassed the Duluth Gabbro (Buchheit et al., 1989; summarized in Dunn, 1995a). Among the objectives of the programme was to explore for PGEs by determining distribution patterns of Pt and Pd using the five most common species of tree and

shrub. In ash, maxima of 40 ppb Pt in white spruce twigs, and 52 ppb Pd in balsam fir twigs were recorded. Maps that accompany the report show that the few samples with detectable levels of Pt did not relate to any particular rock type or structure. The Pd data, however, showed weak clustering of higher values at several locations.

The Coryell alkalic intrusion near Grand Forks, in south-central British Columbia, has associated Cu mineralization with Au, Ag and PGEs that was worked almost a hundred years ago. Ore shipped out in 1915 had an average grade of 9.6% Cu, 230 ppm Ag, 0.68 ppm Au and 8.9 ppm PGEs. Fifty tissue samples from common trees, shrubs and herbs collected mostly from a site near the base of the mountain over shonkinite pyroxenite failed to yield an Ir analysis above the detection limit of 2 ppb in ash, and Pt and Pd were present in amounts similar to those found at Tulameen (Dunn, 1992).

In Siberia, over many years Kovalevsky (2001) analysed more than 25,000 plant samples, mostly rotted pine stumps, for PGEs resulting in the discovery of several new types of PGM mineralization in weathered bedrock. Maximum concentrations were reported to be 5000 ppb Pt in ash, with data obtained by a 'sensitive emission spectrographic method'. An initial survey involving extremely dense sampling intervals revealed twelve biogeochemical anomalies, 1–10 m wide, with concentrations of 50–500 ppb Pt in ash. Subsequent trenching disclosed seven types of PGM mineralization.

It is to be concluded that the value of biogeochemical methods to explore for PGE mineralization is low for most PGE-bearing rocks except sulphide-rich systems where a strong response can occur. For PGEs hosted by other rock types the biogeochemical signature is likely to be very subtle unless there has been considerable degradation of host minerals (e.g., chromite) through weathering processes.

Praseodymium (Pr)

See Lanthanum and the Rare Earth Elements.

Praseodymium is a REE that has no known biological role. In V6 it exhibits good precision with a mean of 0.19 ppm Pr. Databases are very limited, but Pr is usually concentrated in plants at levels close to its detection limit by ICP-MS of 0.02 ppm Pr. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 2.7 ppm Pr adjacent to a kimberlite. Stems from this species had concentrations 50–70% lower. Dwarf birch leaves from over kimberlites in the Ekati area of the Northwest Territories of Canada yielded concentrations consistently below detection.

Promethium (Pm)

This rare earth element has no stable isotopes. It is radioactive and there are no known measurements of Pm in plants.

	V6	NIST 1575a
Target	0.09	0.42
Mean	0.09	0.44
Std. Dev.	0.01	0.022
RSD %	7	5

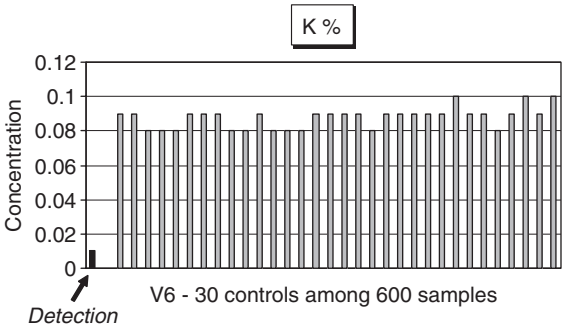


Fig. 9-46. Potassium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Potassium (K) (Fig. 9-46)

Concentrations of this essential macronutrient element for plants are typically well in excess of the detection limit. Precision is excellent and concentrations in field samples can be plotted with confidence that they are not analytical artefacts. Trends in K enrichment can sometimes be attributed to bedrock alteration, particularly where K feldspar has been degraded due to weathering, or there has been some K metasomatism. In the latter situation plants may be useful indicators of metalliferous porphyry environments. In a survey over a Mo–Cu porphyry in the Amazon, fronds from tree ferns had high K levels (up to 2.86%) that corresponded to the locations of highest Mo concentrations. In this situation, whereas the Mo data were the more useful for defining areas of mineralization, the K data were supplementary in that they helped support the interpretation that Mo was associated with K-rich rocks.

Potassium data from foliage collected in the Eden Project show concentrations ranging from 0.38% K in bamboo to 5.09% in wild coffee.

Radium (Ra)

Most of the work on Ra in plants that has been directed towards mineral exploration has been undertaken in the former Soviet Union (Kovalevsky, 1972, 1973). Kovalevsky (1987) reported that of 139 plant tissues tested 100% showed no barrier to Ra uptake, and there is no other element that exhibits this non-barrier property. He noted that 'samples with anomalous alpha activity are subjected to radiochemical analysis of radium and thorium (via radon and thoron), to determine the nature of their radioactivity'. He later concluded that mosses and lichens are recommended for prospecting for ores whose indicator element is Ra (i.e., U).

Essentially all naturally occurring radium is present as radium-226. The concentration of Ra in plants is typically about 3% of that in soil. It seems that the only

recorded unusual enrichment of Ra in plants is by Turner et al. (1958) who reported that Brazil nuts growing in areas of high natural Ra have orders of magnitude higher concentration plant-to-soil ratios than those typically found.

Rhenium (Re) (Fig. 9-47)

Rhenium was the last naturally occurring element to be discovered (1925). In rocks, it occurs primarily with minerals containing Pt, Nb, REE and Mo. Rhenium is one of the rarest elements, and there was scant information on its biogeochemical characteristics until the advent of ICP-MS with its high sensitivity to Re (detection of 1 ppb). Large databases now demonstrate that Re is commonly present in dry tissue at levels of less than 1 ppb. No chart is presented to show accuracy and precision in dry tissue because there are no known controls with concentrations above the detection limit of 1 ppb Re. Consequently, the histogram illustrating Re precision is based upon analysis of internal control V7 comprised of plant ash from a bulk sample collected at a PGE deposit. This ash has returned excellent precision on concentrations of only 6 ppb Re, with an RSD of 8%. On a dry-weight basis this corresponds to just 0.1 ppb Re, which is probably a reasonable assessment of background levels of Re in plants. Tests on the volatility of Re indicate that little or none is lost during reduction of plant tissue to ash at 475°C.

A comparison of *Acacia* bark, roots, wood and foliage has revealed that only the foliage contained Re concentrations above detection, with values up to 5 ppb DW. This is in general accord with observations for other species. Consequently, for any surveys requiring Re analyses the foliage would be the preferred sample medium.

Commonly, if Re levels are elevated there is a coincident increase in Mo and/or PGEs because of their geochemical affinity. Figure 9-48 shows two parallel sample traverses 100 m apart that both show a strong Re response in needles of sub-alpine fir near the eastern end of each traverse. These lines were at Mount Polley in central British Columbia where Cu–Mo–Au porphyry mineralization is present. In this case

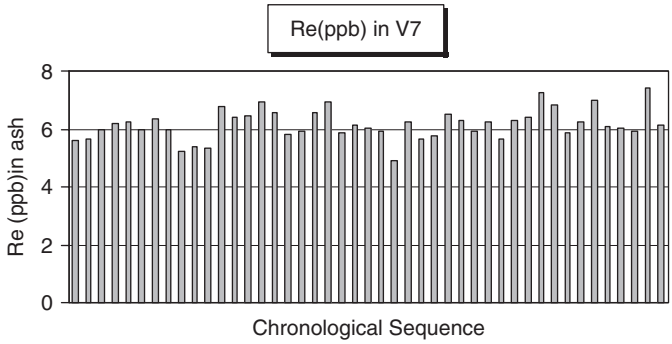


Fig. 9-47. Rhenium – Precision and accuracy on ashed tissues – V7 (see Fig. 9-1).

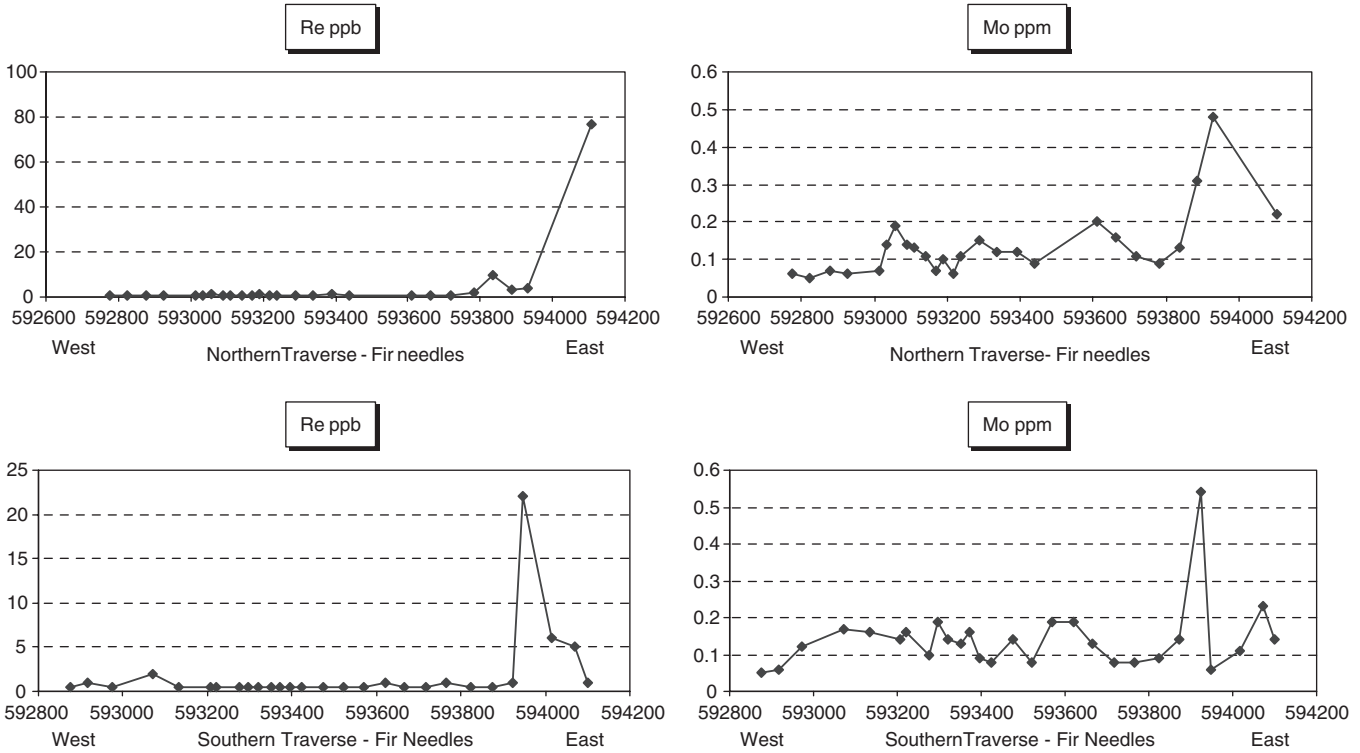


Fig. 9-48. Rhenium and Mo in dry sub-alpine fir foliage from undisturbed forest near the Mount Polley Cu–Au–Mo porphyry.

the Re is clearly in association with Mo. Of note is that the soils from the same sample stations also yielded Re anomalies, but concentrations were an order of magnitude lower. The trends of Re and Mo enrichment indicate that the strike of metal enrichment is towards the northeast.

Kimberlites sometimes yield anomalous levels of Re. The Welgevonden kimberlite in South Africa has up to 10 ppb Re in dry foliage of the thorny common plant 'Hak en Steek' growing over and marginal to the concealed kimberlite.

A survey involving approximately 800 black spruce tree tops from a 120 km^2 area centred on the small Rottenstone PGE–Ni–Cu deposit in Saskatchewan yielded a maximum of 40 ppb Re in dry top twigs. Of note was the spatial relationship of Re enrichments to the location of known mineralization, since it followed a structural trend adjacent to the zone of PGE enrichment (Fig. 9-49).

Another environment in which elevated levels of Re in plants may occur (invariably associated with Mo) is over black shales, or reduced muds such as ancient lake sediments which, upon oxidation, can release Re for uptake by plants.

Samples of foliage from the Eden Project have unusually high levels of Re, especially in samples from the Hot Tropical Biome where concentrations reach 149 ppb Re. Such concentrations are most unusual and in this environment they are probably attributable to high levels of organic matter added to the soils.

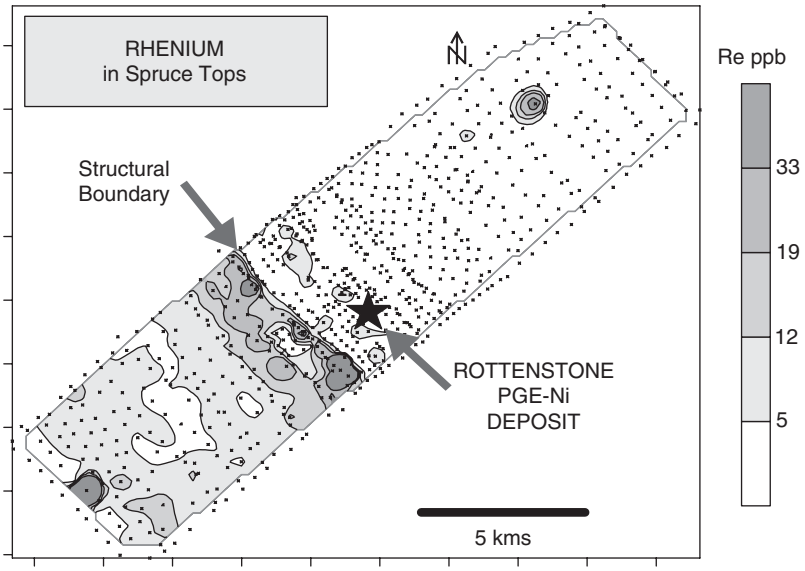


Fig. 9-49. Relationship of Re enrichment in black spruce tops to known PGE-Ni deposit. Shaded contours represent isolines of concentrations greater than the median of 1.5 ppb Re in dry tissue. Rottenstone, Saskatchewan. Analysis by ICP-MS on ash (normalized to dry weight).

Rhodium (Rh)

See 'Platinum and the Platinum Group Elements'.

Rubidium (Rb) (Fig. 9-50)

Rubidium is the sixteenth most abundant element in the earth's crust. Consequently, the levels present in plants are correspondingly high and well above the detection limit by ICP-MS of 0.1 ppm Rb. Precision for Rb is consistently excellent, with controls showing no spurious 'spikes' in the data.

Rubidium has no known biological role although there are indications in the literature that it may be an essential micronutrient. For example, in a study of root function and overlap Hawkes and Casper (2002) used Rb as a nutrient analogue. Rubidium has a slight stimulatory effect on plant metabolism, probably because of its geochemical affinity to the large and essential monovalent cation potassium. The two elements are found together in minerals, soils and plants although K is much more abundant than Rb and their paths diverge within plant structures. Consequently, in plants there is not always a positive correlation between Rb and K, nor does Rb always exhibit a strong relationship with the other large monovalent cation Cs, although the association occurs more frequently than with K.

Plants readily absorb Rb and most plant tissues have concentrations considerably higher than the level of 1.05 ppm Rb that is highly reproducible in V6. The pine needles comprising NIST 1575a have 17 ppm Rb, such that the precision is even better with a RSD of 3%. Figure 9-51 shows the near perfect reproducibility of Rb in needles of balsam fir (*Abies balsamea*) from two splits of 26 samples (blind duplicates) interspersed within a series of over 500 samples.

Samples from the Eden Project demonstrate the wide variations in Rb uptake. Concentrations range from 3 ppm Rb in leaves of avocado and protea to 216 ppm Rb

	V6	NIST 1575a
Target	1.05	17
Mean	1.05	17
Std. Dev.	0.06	0.6
RSD %	5.4	3

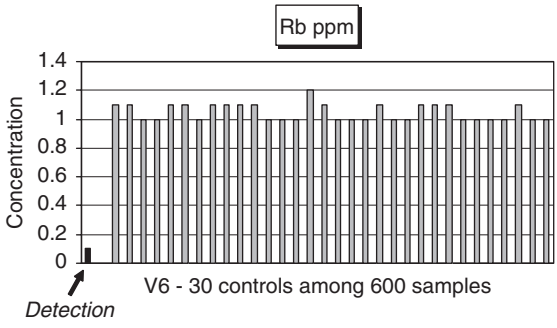


Fig. 9-50. Rubidium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

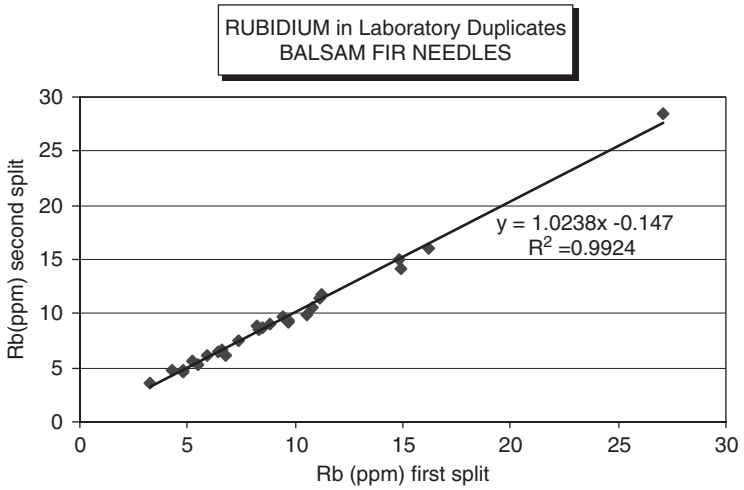


Fig. 9-51. Near perfect precision obtained for Rb in 26 analytical pairs of balsam fir needles.

in torch ginger. Rubidium is usually more concentrated in conifer twigs than needles, but in *Acacia* and deciduous species in general it is higher in leaves than twigs. It is slightly lower in outer bark than in either twigs or foliage.

Several surveys have found Rb to be concentrated, usually with Sr, in vegetation samples from over and around some kimberlites (Dunn, 1993). Rubidium forms chemical compounds that can be extremely soluble, especially as carbonates. It is commonly enriched in phlogopite contained within kimberlite where it is likely to occur at inter-lattice sites, substituting for K, and held between the lattice layers by weak van de Waals forces. Carbonatization occurs with many kimberlites, and during weathering, weak carbonic acids may strip Rb from the phlogopite and transport it in solution until it is absorbed by plants in the acidic environment around their roots.

Ruthenium (Ru)

See 'Platinum and the Platinum Group Elements'.

Samarium (Sm)

See Lanthanum and the Rare Earth Elements.

Samarium in V6 exhibits very good precision with a mean of 0.14 ppm Sm. Samarium is usually present in plants at levels below its detection limit by ICP-MS of 0.02 ppm Sm. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded

up to 0.56 ppm Sm adjacent to a kimberlite, with stems containing lower concentrations. Dwarf birch leaves from the Ekati area of the Northwest Territories of Canada yielded concentrations below detection. Near the REE-bearing allanite at Hoidas Lake in northern Saskatchewan alder foliage was the vegetation sample medium that contained the highest Sm concentration with 0.8 ppm.

Scandium (Sc) (Fig. 9-52)

Precision for Sc is fair to poor largely because Sc concentrations in plants are usually close to the detection limit. Many types of plant tissue have concentrations below or close to the detection limit of 0.1 ppm Sc in dry tissue, although concentrations up to 0.5 ppm Sc are not uncommon. The majority of the plants from the Eden Project that were tested yielded between 0.2 and 0.4 ppm Sc in dry leaf tissue. Ferns seem capable of accumulating a wide range of elements, and the tree fern *Cyathea* from the western Amazon has returned concentrations of up to 5.2 ppm Sc in dry fronds compared to a background level of 0.2 ppm Sc. A factor analysis of the dataset ($n = 354$) demonstrated that Sc is most strongly associated with Al, with positive loadings for Ba, Ga, REE, S and Se. Leaves of a tropical vine from the same area had lower Sc concentrations, but also exhibited an association among Sc, Al and REE. The significance of this association is not known to be of importance to mineral exploration and probably simply reflects the geochemical affinities of these elements.

In general, Sc concentrations are highest in roots, substantially lower in stems and least concentrated in leaves (Horovitz, 2000), but Shtangeeva (2005) notes that the biogeochemistry of Sc is still poorly understood.

In the event that a biogeochemical survey sample shows an anomalous level of Sc, the Fe and Al data should be checked to see if there is a corresponding increase, because in most species it appears that Sc is closely associated with Fe or Al in plant tissues. To date Sc distribution patterns have not contributed any obvious assistance in the exploration for concealed mineralization.

	V6	NIST 1575a
Target	0.27	0.01
Mean	0.2	0.18
Std. Dev.	0.05	0.08
RSD %	27	47

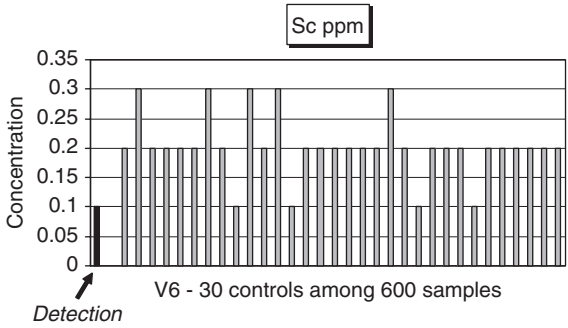


Fig. 9-52. Scandium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Selenium (Se) (Fig. 9-53)

Control V6 has close to the detection limit of 0.1 ppm Se, and exhibits precision at the usual +/–100% at this level. Values obtained for NIST 1575a indicate poor accuracy compared to published values. Selenium is an element for which some laboratories declare that low levels cannot be accurately measured by ICP-MS, whereas others claim to have circumvented this problem and provide consistently precise data. Interpretations of Se data by ICP-MS should, therefore, take these uncertainties into account.

From a biogeochemical exploration perspective Se is an element that has received considerable attention for more than fifty years. 'Selenium floras' have been identified in the western United States, Canada, Columbia and Queensland (Brooks, 1983). The presence of Se floras indicates the presence of Se enrichment in the substrate, either because they can tolerate high concentrations or they have a specific requirement for Se. Perhaps best known of these plants are the poison (or milk) vetch (*Astragalus*) and the locoweed (*Oxytropis*). Some species are geobotanical indicators (i.e., they are present only when Se is enriched in the ground), whereas others are Se accumulators that have been found to take up more than 1% Se dry weight. Concentrations can be sufficiently strong to detect in the field the garlic and horseradish-like odour emitted from these plants that is characteristic of volatile Se compounds. On the Colorado Plateau, Cannon (1957) used various species of *Astragalus* for indirect prospecting for U, because carnotite (potassium uranium vanadate) is commonly associated with zones of Se enrichment in sedimentary U roll-front deposits. As Brooks (1983) noted

> Her classic work represents one of the most successful known applications of the geobotanical method.

Recent work by Pickering et al. (2000, 2003) on Se in *Astragalus* using the synchrotron has added significantly to an understanding of the location and speciation of Se during various growth stages (Fig. 6-6). Among the revelations of this study is

	V6	NIST 1575a
Target	0.2	0.1
Mean	0.18	0.226
Std. Dev.	0.06	0.07
RSD %	31	29

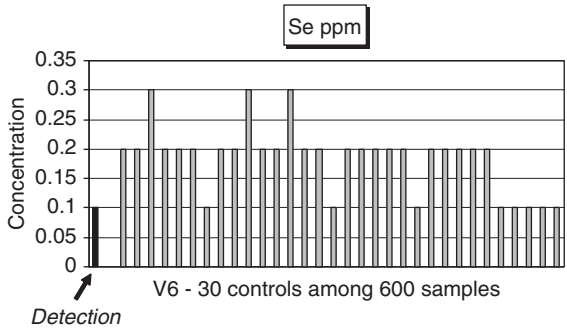


Fig. 9-53. Selenium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

that selenate is concentrated in leaf tissue, with very little in twigs. This finding is in accord with observations on the relative concentrations of Se in coniferous species of North America and in stems and foliage of a number of deciduous species. In the widespread genus *Combretum* (bushwillow) found throughout much of sub-Sahara Africa and tropical regions of South America, concentrations in foliage and stems are similar. In general, for surveys requiring measurements of Se the foliage would be the preferred tissue to collect.

There are no notable concentrations of Se in the foliage samples analysed from the Eden Project, although it is noticeable that slightly elevated concentrations occur in members of the Rubiaceae family (bedstraw or coffee family).

Geochemically, Se follows S and relatively high concentrations in plants can be a good indication of sulphide-bearing minerals. Selenium can also be enriched in vegetation from over PGE-bearing mineralization, and S/Se ratios can be used to assist in focusing in on drill targets. However, it should be borne in mind when evaluating distribution patterns that the precision of the data is at best only fair for both Se and S. Also, it should be noted that reduction of plant tissues to ash results in partial volatilization of both elements, and so unless these losses can be firmly quantified caution should be exercised when attempting to interpret S/Se ratio distribution patterns from the analysis of ashed tissues.

In a survey that encompassed the Fox River Sill in eastern Manitoba, black spruce treetop twig samples were reduced to ash and analysed by ICP-MS after digestion in a nitric acid/hydrogen peroxide mixture. This method provides more precise, accurate and stable data than digestion by aqua regia. The distribution pattern of subtle Se enrichments formed a striking linearity that closely followed stratigraphic trends. Highest concentrations were located close to but spatially displaced from sites on the lower part of the Fox River Sill that yielded elevated levels of Pt and Pd in the treetops. About 7 km to the west, a linear trend of similar Se intensity lay parallel to a zone of elevated Pt and Pd, and was coincident with the projected extent of a volcanic unit covered by glacial deposits.

Silver (Ag) (Fig. 9-54)

In dry plant tissue Ag determined by ICP-MS is rarely below the detection limit of 1 or 2 ppb Ag obtained by commercial laboratories (Fig. 9-54). Values are commonly in the low to tens of ppb at which levels precision is usually very good. At higher levels the precision is excellent. V17 has a higher concentration of Ag and has shown remarkable precision from 336 repeat analyses inserted as blind controls within a sequence of more than 7000 field samples over a two-year period (Fig. 9-55). The mean value for this control is 74 ppb Ag with a standard deviation of 2.1 and an RSD of 2.9%.

On occasion within a sequence of field survey samples, there are very rare spikes in the Ag data. They are rarely repeatable and after extensive checks for contamination

	V6	NIST 1575a
Target	18	(12)
Mean	17.6	12
Std. Dev.	1.7	1.5
RSD %	10	12

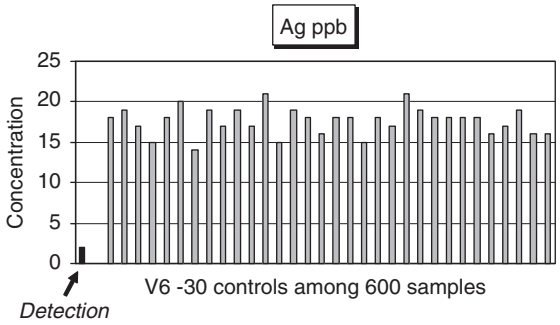


Fig. 9-54. Silver – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

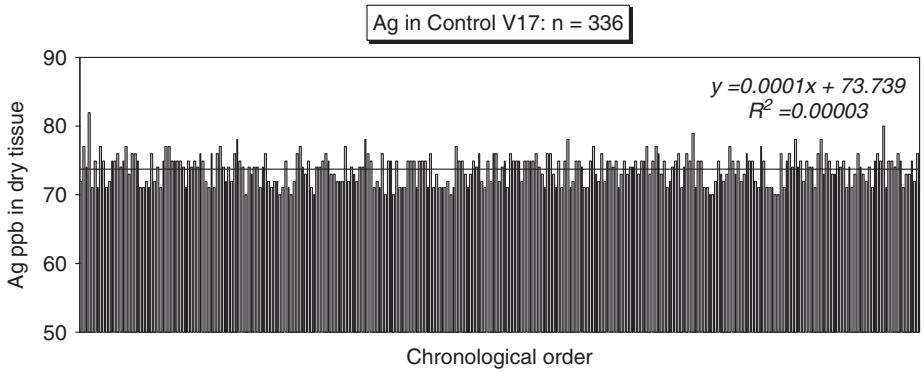


Fig. 9-55. Silver in control V17 inserted as a blind control among 7000 samples over a two-year period.

their source has not been identified. Consequently, it is as well to be suspicious of single point Ag 'spikes' in the field data. Unless they make geological sense they should either be ignored or, preferably, the analysis should be repeated.

Data on foliage from the Eden Project demonstrate the wide range in uptake of Ag among the samples tested, but data should be viewed in context of the Ag content of the soils, which is substantially different in the two biomes. In the warm temperate biome (WTB) the soils average 162 ppb Ag, whereas in the hot tropical biome (HTB) concentrations average two orders of magnitude higher than this with almost 17,000 ppb Ag, presumably because of the higher levels of organic matter mixed in with the HTB soils. Consequently, the WTB samples (listings shown in black font in the table of foliage analyses on the CD) should be viewed as a separate population from the HTB samples (listings shown in red font). Concentrations in the WTB samples range from 14 to 185 ppb Ag, except for the African Boxwood (*Myrsine africana*) that yielded 754 ppb Ag; whereas in the HTB concentrations ranged from 38 ppb to 9670 ppb Ag in rattan (*Calamus spp.*). Although there is considerable variability in the Ag content of soils in different environments from around the

world, soils in general contain Ag concentrations much closer to those in the WTB than those in the HTB, and so plant tissues usually have a range of Ag concentrations similar to those encountered in the WTB.

Conifer trunk wood has a propensity to accumulate Ag. Concentrations in ashed wood are commonly 10–15 ppm Ag which at first sight appear to be extraordinarily high until data are normalized to a dry-weight basis. These values correspond to 50–75 ppb Ag in dry tissue, because the ash yield of conifer wood is about 0.5% – i.e., a 200-fold concentration.

Several tissues were analysed from a single lodgepole pine rooted in tourmalinite with massive sulphides near the Sullivan Pb/Zn/Ag mine in southern British Columbia. The dry roots and outer bark scales each contained 270 ppb Ag; twigs had 50 ppb Ag and at the top of the tree the stem had 15 ppb Ag.

There is an inconsistency among species as to whether foliage or twigs have the higher concentrations of Ag. As a broad rule of thumb it appears that conifers have more Ag in twigs than needles, and deciduous plants have the opposite. Some examples show the patterns obtained from various locations.

Conifers

- Mountain hemlock from Mt. Washington on Vancouver Island, where there is undeveloped Au/Ag mineralization, has five times more Ag in twigs than in needles.
- Black spruce twigs from near the Rottenstone PGE/Au/Ni/Cu mineralization in Saskatchewan have twice the Ag concentrations of needles.
- Douglas-fir tops from southern British Columbia have slightly higher Ag concentrations in the twigs.

Deciduous species tend to have higher Ag concentrations in the foliage.

- At North Mara in Tanzania *Acacia* has considerably more Ag in foliage than stems. Elsewhere in Central Africa in other species the foliage has similar concentrations to the stems.
- In northern Saskatchewan Labrador Tea has similar concentrations in foliage and stems, yet alder foliage has double the Ag content of the stems.

In the design of a biogeochemical survey that focuses on the exploration for Ag deposits, these patterns of relative enrichments among plants serve as a guide for selecting a suitable sample medium. However, the low detection limits and high precision of the data obtained for Ag by ICP-MS mean that either foliage or stems would be equally informative, because there is a good correlation between the patterns obtained from each tissue type.

Over a number of years in the 1980s and 1990s, Dr. Alexander Kovalevsky and his wife Olyesa Kovalevskaya conducted some extensive studies relating the Ag content of rotted cones and stumps of pine (*Pinus sylvestris*) and stumps of larch (*Larix dahurica*) to concealed Ag mineralization in eastern Siberia. This work was

presented as posters and talks at a number of international conferences at which complex maps were displayed showing patterns of Ag distribution. The results were summarized in several extended abstracts (e.g., Kovalevsky and Kovalevskaya, 1990; Kovalevskaya and Kovalevsky, 2003). In the latter publication it is stated that

> the best case history for NBP (Non-barrier Biogeochemical Prospecting) with ore-grade prediction is for Ag in the Transbaikal South … Ag-bearing Gilbera Zone of Deep Faults (GZDF). Exploration of NBP for Ag in GZDF involved analysis for 47–70 elements in ~25,000 biogeochemical and 4,000 rock samples. This has resulted in delineation of > 250 'Supposed Ore Biogeochemical Anomalies' (SOBA) of Ag. Trenching of 29 SOBA has revealed 27 'Veined Silver Ore Bodies'.
>
> The suberized cones and rotted pine and larch stumps outlined
>
> > 250 SOBA of Ag up to 8 × 20–200 m with 70–3,000 ppm Ag in ash [approximately 0.4 to 15 ppm Ag in dry tissue]. These are the highest recorded concentrations of Ag in plants. Within an area of 10 km^2, 11 zones were outlined, ranging from 100 × 150 m to 250–400 m. A predictable relationship between Ag in rocks and Ag in plants was established … sample traverses 40–60 m apart and sample spacing of 1–3 m are recommended.

Sodium (Na) (Fig. 9-56)

Determinations on V6 and NIST1575a exhibit moderately good precision. Many plant samples have concentrations quite close to detection at which level precision is fair to poor. In trace amounts Na is classified as a beneficial element for many plants, and only essential for some species. It is usually found more concentrated in foliage than in twigs. Many samples of foliage from the Eden Project yielded much higher levels of Na than typically found in natural forest environments, even though the soils had levels similar to those found in boreal and temperate forests. Concentrations ranged from 0.004 to 1.6% Na in dry tissue.

	V6	NIST 1575a
Target	0.009	0.006
Mean	0.009	0.007
Std. Dev.	0.002	0.0008
RSD %	15	11

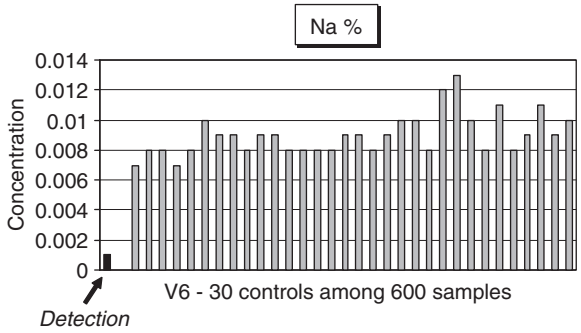


Fig. 9-56. Sodium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Plants that are enriched in Na include the halophytes that thrive in salty environments (e.g., the saltbush *Atriplex*) and seaweeds. The common rockweed or wrack (*Fucus*) has on average 23% Na in ash (Vinogradov, 1953), or about 6% Na dry weight. Brown, red and green seaweeds all have high levels of Na (Dunn, 1998a).

Sodium in vegetation is commonly associated with Fe and Fe-related elements. On occasion Na concentrations can be used to assist in lithogeochemically mapping Na-rich volcanic rocks, albitization and zones of Na metasomatism to provide focus to areas favourable for mineralization.

Caution should be exercised in interpreting Na enrichments in vegetation that is collected in coastal areas (because of salt spray), and in northern climates alongside roads that may have been salted in the winter months to melt the ice.

Strontium (Sr) (Fig. 9-57)

Concentrations in the control samples V6 and NIST 1575a are all well above the detection limit, and precision is excellent. Plant tissues commonly have concentrations in the tens to hundreds of ppm Sr, and so data can be plotted with confidence that they represent true variations among the field survey samples.

Strontium is an essential element for some plant species, but its general essentiality has not been confirmed. It performs a function similar to Ca in plants and may be incorporated into their structural components, but it seems that Sr cannot replace Ca in biochemical function (Kabata-Pendias, 2001).

Positive correlations between Sr and Ca occur sometimes in survey samples, but a strong relationship is not always present. However, in plants growing on carbonates there are frequently positive correlations among Ca, Sr and Ba such that, in the absence of outcrop, this association can give an indication of underlying lithology.

Highest concentrations of Sr are found in the wood of stems and tree-trunks. For many plants, the concentrations of Sr are similar in leaves and stems. A study in the western Amazon found that leaves of the vine *Clusia* yielded higher Sr concentrations (median of 61 ppm Sr in dry tissue, $n = 360$) than the fronds of tree-ferns *Cyathea*

	V6	NIST 1575a
Target	31	(6.8)
Mean	31	7.5
Std. Dev.	1.6	0.3
RSD %	5	4

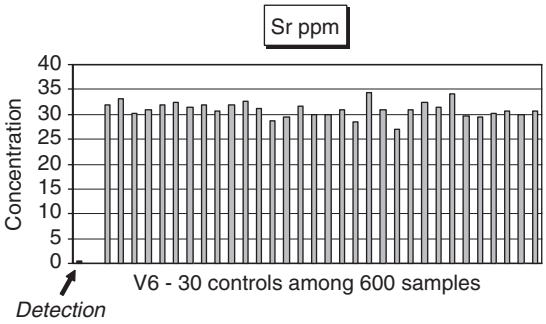


Fig. 9-57. Strontium– Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

collected at the same sites (median 18 ppm Sr). The foliage of plants from the Eden Project showed a wide range in composition, from a low of 1.4 ppm Sr to over 110 ppm in the fig (*Ficus*) and *Acacia*. *Acacia* at North Mara in northern Tanzania contained well over 200 ppm Sr in both stems and foliage. Similarly, *Acacia* from Western Australia typically has concentrations up to 200 ppm Sr in most components of its structure, with highest levels occurring in roots and thick stems.

With respect to conifers, in the temperate forests of British Columbia Douglas-fir have similar concentrations in needles and twigs with values commonly in the range of 10–30 ppm Sr except higher over carbonates. Black spruce from the boreal forests has higher concentrations in the twigs. Scales of outer bark from fir, spruce, pine and larch generally have 20–30 ppm Sr. These are broad generalizations, since concentrations up to 100 ppm Sr are not uncommon.

These data indicate that for most species there is some filtering of Sr from roots through trunk wood into twigs, but that there is little or no barrier mechanism to inhibit the passage of Sr from twigs into foliage.

Sulphur (S) (Fig. 9-58)

The analytical precision for S at levels below 0.2% is generally only fair, and most plant tissues contain concentrations below this. Consequently, the interpretation of plots of S distributions determined by ICP-MS should be treated with caution, since data should be considered semi-quantitative.

Sulphur is an element that is essential to all plants, although only in trace amounts as a component of proteins and enzymes, and as an agent to assist in resistance to cold conditions. Concentrations in twigs are commonly in the range of 500–1000 ppm S (0.05–0.1%) and the same to half this amount in foliage. Conifer outer bark has similar concentrations to twigs. There is usually a good correlation between S in twigs and foliage, with variations mostly attributable to imprecision in the analytical data or to inconsistency in sampling procedures. The latter is because concentrations of S vary with the age of stem/twig growth, and emphasizes the

	V17	NIST 1575a
Target	0.03	(0.1)
Mean	0.03	0.08
Std. Dev.	0.01	0.01
RSD %	23	14

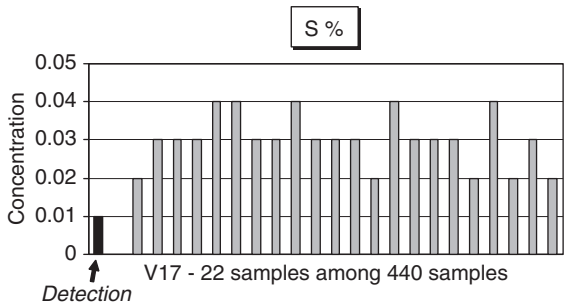


Fig. 9-58. Sulphur – Precision and accuracy on dry tissues – V17 and NIST 1575a (see Fig. 9-1).

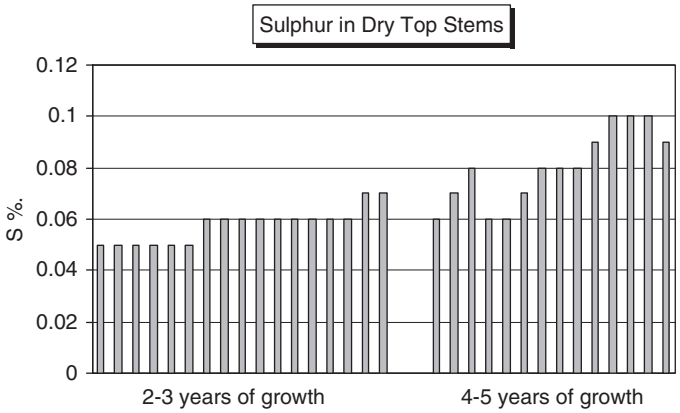


Fig. 9-59. Sulphur in Douglas-fir top stems from 35 sample sites in southern British Columbia, demonstrating the differences in composition with the amount of twig growth.

importance of maintaining a consistent number of years of growth to be collected at each sample station. Figure 9-59 shows this relationship with a histogram of S concentrations in Douglas-fir top stems.

Sulphur data from the Eden Project samples show concentrations in foliage from 0.11% in fig (Ficus) up to 1% in several species including sweet garlic. Garlic in general is known to accumulate both S and Se compounds that account for its distinctive smell.

Distribution patterns of S concentrations in a population of plants from a biogeochemical survey can be of relevance to mineral exploration. Whereas the many published studies on antagonistic relationships between and among elements can make the thought of arriving at meaningful interpretation quite daunting, patterns can make geological sense. Many of the antagonisms that have been observed are with respect to crops or seedling plantations. Once a forest or desert scrub vegetation has become firmly established, it seems that these antagonistic effects are subdued and may no longer be relevant. However, the astute geochemist should remain keenly aware of the possibilities that spatial patterns of element distributions *may* have resulted from deficiencies or toxic levels of element assemblages. As an example S has been shown to inhibit the transport of Pb from roots to shoots so that S deficiency can increase the Pb movement into the tops of plants (Jones et al., 1973). From a practical exploration point of view it is therefore as well to consider if a Pb anomaly might be related to S deficiency. By reviewing the data for both Pb and S a judgement can be made as to whether this is a possible explanation. The answer is usually that it is not the reason, and the more data that accumulate the clearer it becomes that by sampling well-established plants, the biogeochemical method is robust and, when possible sources of contamination have been taken into account, anomalies are related to variable concentrations in the substrate – whether it is the soil, groundwater or underlying bedrock.

As part of a survey conducted over an epithermal Au/Ag system at the 3Ts property in central British Columbia, needles from white spruce (*Picea glauca*) were collected along traverses over mineralized veins. The Ted vein on this property comprises quartz-calcite with finely disseminated sulphide minerals. Pyrite is dominant, but also present are chalcopyrite, sphalerite, galena, Ag-sulphides, tellurides and sulphosalts. Figure 9-60 shows the S signature in the dry needles, demonstrating a strong positive signature over the Ted vein and slightly elevated concentrations farther to the east towards some mineralized boulders. Just west of the Adrian Boulders an undisturbed zone of sulphide mineralization occurs beneath a cover of glacial till. This exemplifies the potential use of S to assist in delineating the locations of buried sulphides using foliage. In this case spruce needles were used, but fir and pine should be equally informative. Data listings for this occurrence are part of the 'Halogen Project' for which digital data listings and a full account are contained on the CD that accompanies this book.

Sulphur data, when used in conjunction with data for commodity metals and when due consideration is given to the S data quality, can provide good focus for more detailed exploration activities.

Tantalum (Ta) (Fig. 9-61)

At the very low levels of Ta usually present in dry vegetation, analytical precision by ICP-MS is poor. Samples from the Eden Project were all below the detection limit of 0.001 ppm Ta, except for three species from the Mediterranean region (maximum of 0.006 ppm Ta in Arar (*Tetraclinus*)), and one just above detection from the hot tropical biome. However, given the poor precision for Ta these data must remain suspect until they can be verified.

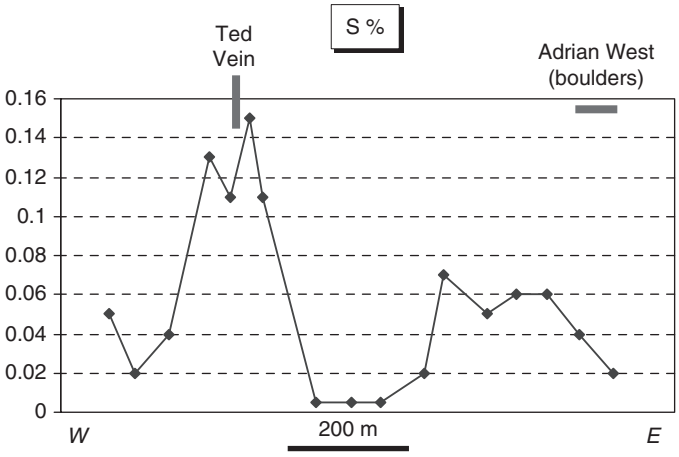


Fig. 9-60. Sulphur in dry needles of white spruce (*Picea glauca*). Ted vein (epithermal Au/Ag with base metal sulphides), 3Ts property in the Nechako Plateau area of central British Columbia.

	V6	NIST 1575a
Target	0.002	(0.002)
Mean	0.002	0.001
Std. Dev.	0.002	0.001
RSD %	73	99

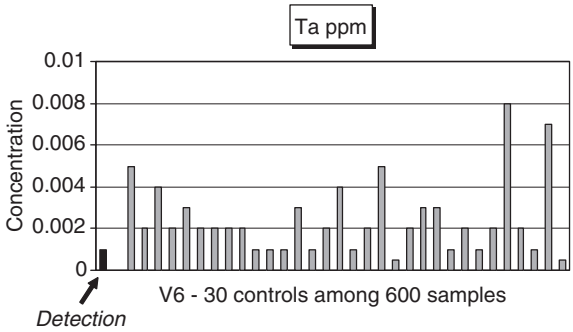


Fig. 9-61. Tantalum – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

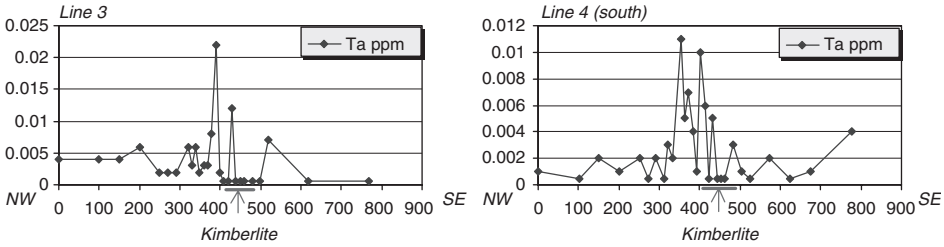


Fig. 9-62. Tantalum in foliage of South African thorny shrub 'Hak en Steek'. Two parallel profiles over concealed kimberlite. Concentrations in ppm of dry tissue. Distance in metres. Data courtesy of Dr. W.B. Coker, BHP Billiton Ltd.

Kovalevsky reported values of 10–60 ppm Ta in the ash of a few species using an emission spectrographic method with a detection limit of 10 ppm Ta. Determinations by both INAA and ICP-MS on samples from the vicinity of the Ta–Li–Cs Bernic Lake Mine in Manitoba showed that twigs and bark of jack pine (*Pinus banksiana*) had the highest concentrations with locally more than 1000 ppm Ta in ash. At eight sites, the average concentrations on a dry-weight basis were, pine bark 34 ppm Ta, spruce bark 22 ppm Ta, pine and spruce twigs both 13 ppm Ta. Other species and tissues contained mostly less than 1 ppm Ta. At the time of sample collection in 1990 the Bernic Lake mine was in operation and it was the largest Ta mine in North America. Whereas the high concentrations in the vegetation could be attributed partly to airborne contamination, the data indicate that in the exploration for Ta, in order to provide the best anomaly to background ratios, bark of pine or spruce would be the preferred sample medium, followed by twigs of either species.

Some kimberlites are enriched in Ta, and on occasion Ta anomalies have been recorded in vegetation growing over or, more usually, marginal to kimberlites. If Ta enrichment occurs, like many elements associated with kimberlites, anomalies tend to form an annulus of relative enrichment at the margins of the diatreme. Figure 9-62 shows two parallel profiles of Ta in dry foliage from a common South African thorny shrub known as 'Hak en Steek', collected over the Welgevonden kimberlite.

The translation from the Afrikaans is 'grab and stab' that gives a good idea of the nature of this plant, and signifies that good leather gloves are required for its collection. Background concentrations were close to the detection limit of the ICP-MS method, but at the margins of the kimberlite values were an order of magnitude higher (0.01 ppm Ta).

Tellurium (Te) (Fig. 9-63)

Tellurium is a rare element that is barely detectable in most plant tissues. Tellurium in V6 is at or close to the detection limit of 0.02 ppm Te. Occasional values for V6 of up to 0.05 ppm have been reported, indicating poor precision at low concentrations. There are no data published for NIST 1575a and all personal determinations obtained to date by ICP-MS have returned values below detection. Owing to this lack of control samples for Te in dry vegetation, the chart to demonstrate precision has used ash control V7, collected from a PGE-rich site. The data indicate some instrumental drift with time, and precision is only fair, given an RSD of 23%.

Dried tissue sometimes yields concentrations above detection. As with any element, single point anomalies should be viewed with suspicion since they may be analytical artefacts. In the event that a series of field survey samples show detectable Te values, repeat analyses would be advisable to confirm their validity, because Te can be a useful 'pathfinder' element for precious metal deposits. Situations have been found where a series of values above detection have not been repeated when re-analysed, and so immediately upon receipt of data it is necessary to scrutinize them for patterns of element concentrations that may be related to analytical artefacts such as instrument drift or loss of calibration.

There are reports in older literature of some suspiciously high concentrations of Te in plants. Schroeder et al. (1967) indicated that concentrations in onion and garlic can be as high as about 300 ppm Te, and the garlic smell of some plants is from the volatile compound dimethyl telluride. Analysis of leaves from plants at the Eden Project showed only one plant (rattan, 0.04 ppm Te) with a concentration above detection. In Schroeder et al. (1967), it was stated that Te is among the most abundant

	V7 (ash)	NIST 1575a
Target	~ 1	No data
Mean	1.1	
Std. Dev.	*0.2*	
RSD %	*23*	

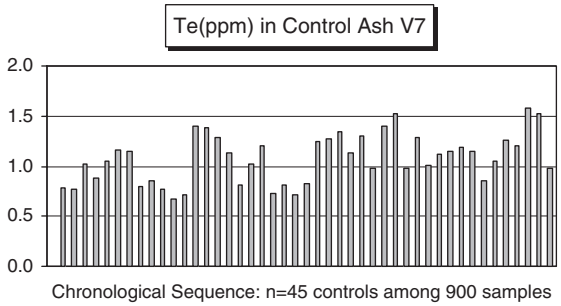


Fig. 9-63. Tellurium – Precision and accuracy on ashed tissues – V7 (see Fig. 9-1).

of trace elements in the human body, which, if true, would indicate an enormous biomagnification from rocks through the food chain to humans. It was claimed that the average human body contains 560 mg Te, which put it fourth in abundance after Fe, Zn and Rb (see also Cohen, 1984). The evidence seems to be that these workers reported erroneous data, because it has since been shown that instead of 560 mg Te in humans there is only 0.7 mg Te in an average body of 70 kg – almost three orders of magnitude less Te (Emsley, 1998). As a result of this and other findings, it would appear that the extraordinarily high levels of Te in biological systems reported by Schroeder et al. (1967) and Cohen (1984) were incorrect.

Probably a more realistic assessment of typical Te concentrations in plants comes from a study conducted twenty years later using a more refined analytical technique (Cowgill, 1988). This was a comprehensive study of Te in vegetation from the vicinity of precious metal–mercury–telluride mineralization in the Ely mining district of Nevada. It involved the collection and analysis of 480 samples of trees and shrubs, and 505 samples of flowering plants. An additional 105 samples were collected from various areas of western Colorado. All samples were analysed for Te, Se, Fe, S, Zn, Cu and Pb. On average, flowers were found to contain significantly more Te than other plant parts. In trees, the highest Te concentrations were in the foliage and the lowest in the branches. Seleniferous species of the vetch *Astragalus* contained larger amounts of Te than plants in the Te-rich Ely area, whereas non-seleniferous species of this genus contained much less. This pattern is in accord with the usual sympathetic correlation between Te and Se, because of their geochemical affinity in nature. No plants contained more than 1 ppm Te in dry tissue.

Limited data on dry samples from near PGE/Au mineralization have indicated that Te in Labrador tea is more concentrated in leaves than twigs, whereas black spruce twigs have more Te than in needles. To obtain information on the geochemical 'relief' of Te datasets, samples need first to be reduced to ash by controlled ignition at 475°C because of the very low concentrations of Te in dry tissues. It appears that little or no Te volatilizes from spruce at this temperature – samples of black spruce twigs from a PGE deposit have returned concentrations of 0.06 ppm Te in dry tissue and 0.05 ppm Te when ash of the same sample is normalized to a dry-weight basis. Preliminary data indicate that more Te volatilizes from foliage of deciduous species than conifers, but this needs further investigation. Figure 9-64 shows data from the determination of a set of dry tissue samples. The diamond symbols indicate data from the direct analysis of 1 g samples of dry tissue, and show that only two samples yielded values above the detection limit of 0.02 ppm Te (values below detection are shown at half the detection limit). Fifteen-gram samples of the same material were reduced to ash and Te determinations were made. The data were then normalized to a dry-weight basis by adjusting for the loss on ignition. This exercise permitted two important observations to be made.

- The only two samples of dry tissue that yielded concentrations above detection proved to be the samples with the highest (and very similar) concentrations

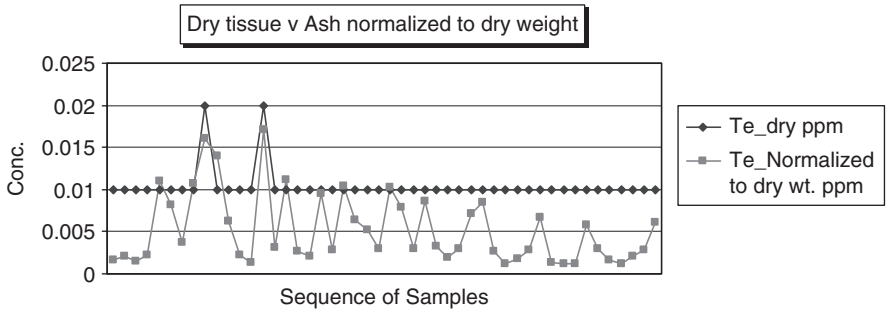


Fig. 9-64. Tellurium in dry *Acacia* tissues. Concentrations in dry material versus ash normalized to dry weight.

obtained from analysis of the ash. This indicated that Te values that are above detection in dry tissues may well be meaningful values.

- By analysing the ash, considerably more detail of the low-level geochemical variability of Te could be discerned.

Surveys conducted in Canada over the PGE deposits associated with the Fox River Sill in Manitoba and the Rottenstone deposit in Saskatchewan both involved the analysis of ashed twigs of black spruce. In both environments, close to mineralization the maximum concentrations were between 1.5 and 2 ppm Te in ash, which is the equivalent of 0.03–0.04 ppm (30–40 ppb) Te in dry tissue. Over kimberlites in the Buffalo Head Hills area of Alberta, white spruce top twigs yielded up to 0.12 ppm Te in ash with values at background sites of <0.02 ppm Te. Over Au deposits in various parts of the world the maximum values have been 0.5 ppm Te in dry foliage.

At the time of writing, it seems that unless high-resolution ICP-MS is available, for surveys requiring detection of subtle variations in Te distribution (e.g., many types of precious metal deposit) it would be advantageous to analyse ashed portions of the samples.

Terbium (Tb)

See Lanthanum and the Rare Earth Elements (La).

Terbium is one of the rarest rare earth elements. Its concentration in V6 is below the detection limit of 0.01 ppm Tb and it is rarely above this level in plant tissues. Ash of V6 contains 0.26 ppm Tb that equates to 0.006 ppm Tb in dry tissue. With the exception of ferns, most plants contain lower concentrations of REE than V6.

Thallium (Tl) (Fig. 9-65)

V6 has a Tl content of almost exactly the detection limit of 0.02 ppm Tl. V17 has 0.03 ppm Tl and consistently returns this value. Other data also indicate that precision for Tl by ICP-MS is excellent for values above 0.05 ppm. Figure 9-66

	V17	NIST 1575a
Target	0.03	No data
Mean	0.03	0.02
Std. Dev.	*0*	0
RSD %	*0*	0

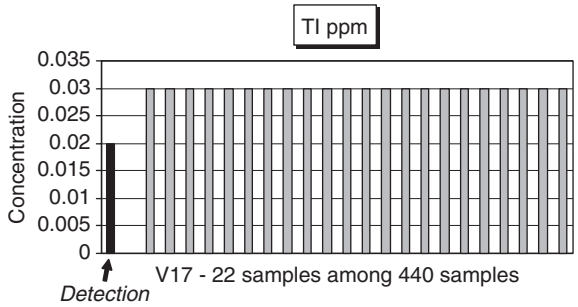


Fig. 9-65. Thallium – Precision and accuracy on dry tissues – V17 and NIST 1575a (see Fig. 9-1).

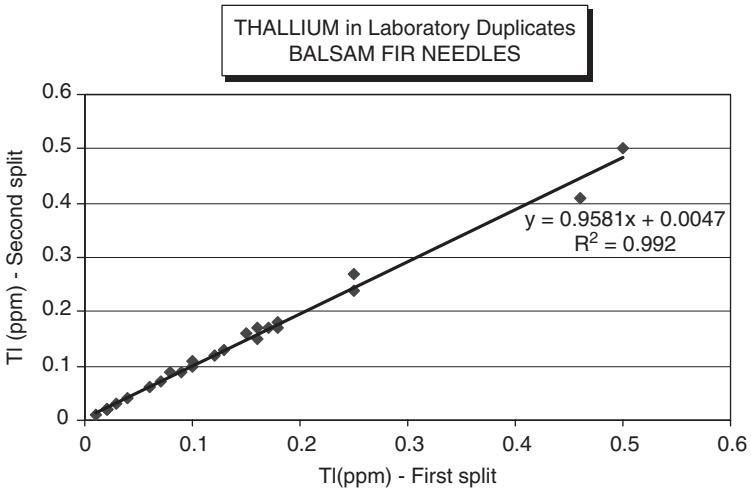


Fig. 9-66. Precision for thallium on replicate samples of balsam fir needles ($n = 19$).

shows the near perfect laboratory precision obtained for replicate samples of balsam fir needles. This level of precision is common for Tl and not exceptional for the dataset shown.

Thallium chemistry is similar to that of alkali salts. In rocks, it closely follows K and Rb and is, therefore, reflected in K metasomatism. Thallium minerals form during epithermal stages of hydrothermal processes generating a high Tl content to marcasite, sphalerite and galena. It is especially enriched in polymetallic deposits, including those with Au along with As, Sb and Ag. It is a highly mobile element that disperses during oxidation of sulphide ores, so that it can quite readily be made available to plant roots. Given these properties and the excellent precision obtained from the analysis of plant tissues, Tl can be a very useful pathfinder element for a number of different types of mineral deposit. Warren and Horsky (1986) noted a near perfect correlation between Tl and Au in several tree species from British Columbia and suggested that the Tl data could be used as a prospecting tool for Au, especially

since Tl data for vegetation are generally more precise than those for Au. The fact that potential sources of Tl contamination are few in areas distant from base metal smelters enhances its value as a pathfinder.

Whereas background levels of Tl are usually <0.02 ppm Tl, there are plants that can hyperaccumulate Tl. Leblanc et al. (1999) reported an extraordinary concentration of more than 500 ppm Tl in dry tissue of two brassicaceous plants from the south of France. Both *Biscutella laevigata* and *Iberis intermedia* are crucifers and, since they are classified as weeds and are therefore quite widespread, they would make suitable candidates to collect for a biogeochemical survey in certain parts of southern and central Europe. Perhaps more importantly, these species could be used for phytoremediation and possibly phytomining of Tl by planting and harvesting a crop over Tl-rich mine tailings. A synchrotron study of Tl in *Iberis intermedia*, for which a maximum of 3100 ppm Tl was recorded in the study by Leblanc et al. (1999), has demonstrated that Tl is present primarily as a water-soluble chemical species distributed throughout the vascular network. A direct relationship of vein size to Tl concentration was observed (Scheckel et al., 2004).

Plants from the Eden Project mostly yielded concentrations below the detection limit of 0.02 ppm Tl, with the most notable enrichments (0.8 and 0.5 ppm Tl) occurring in two species of fig tree, one growing in the hot tropical biome and the other in the warm temperate biome.

Thorium (Th) (Fig. 9-67)

Thorium concentrations in V6 are an order of magnitude above the detection limit, and precision is good. For NIST 1575a, all determinations were at or below the detection limit of 0.01 ppm Th by ICP-MS. Plant tissues commonly have values lower than those of V6, so interpretation of the data should be treated with caution, given the inferior level of precision that is obtained at low concentrations.

Thorium has a geochemical affinity for U, but the two elements usually exhibit distinctly different biogeochemical distribution patterns. Whereas there are many

	V6	NIST 1575a
Target	0.12	(0.02)
Mean	0.1	<0.01
Std. Dev.	0.01	
RSD %	12	

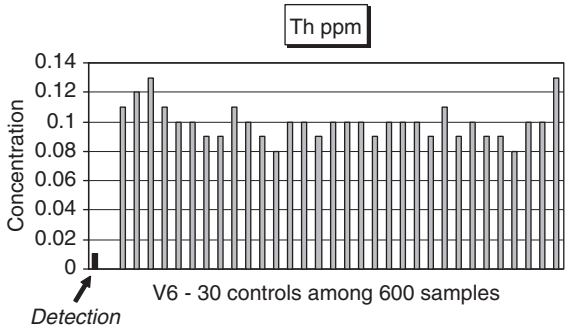


Fig. 9-67. Thorium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

records in the literature of extremely high concentrations of U in plant tissues, this is not the case for Th. A study of the composition of common boreal forest species growing in a remote area of northern Saskatchewan over Th-REE-rich allanite provides an example of the comparative uptake of Th by different tissues (Table 9-VII) in a pristine environment. Even in this Th-rich environment the maximum Th concentration in dry tissue was only 0.077 ppm Th (77 ppb) in dry alder leaves. However, these low concentrations must be partly a function of Th being structurally bound in the crystal lattices of the allanite, and not readily released in solution.

Only five plants from the Eden Project yielded concentrations above the detection limit of 0.01 ppm Th. The highest value, still only 0.05 ppm Th, occurred in the 'star cluster' (*Pentas lanceolata*).

TABLE 9-VII

Concentrations of Th in common plants growing over Th-REE-rich allanite at Hoidas Lake, Saskatchewan. Determinations on ash by INAA. Dry equivalent weights calculated from the ash yield (Dunn and Hoffman, 1986)

Tree or shrub	Botanical name	Tissue	Th_ash (ppm)	Th_dry (ppm)	Ash yield (%)
Birch	*Betula papyrifera*	Twig	2.0	0.036	1.82
Birch	*Betula papyrifera*	Leaf	1.1	0.044	3.96
Birch	*Betula papyrifera*	Outer bark	2.4	0.067	2.78
Birch	*Betula papyrifera*	Trunk wood	<0.5	<0.003	0.62
Labrador tea	*Ledum groenlandicum*	Root	4.4	0.035	0.8
Labrador tea	*Ledum groenlandicum*	Stem	2.2	0.028	1.25
Alder	*Alnus crispa*	Twig	<0.5	<0.012	2.43
Alder	*Alnus crispa*	Leaf	2.1	0.077	3.65
Spruce	*Picea mariana*	Twig	1.7	0.032	1.87
Spruce	*Picea mariana*	Twig-treetop	1.9	0.032	1.71
Spruce	*Picea mariana*	Outer bark	0.9	0.025	2.81
Spruce	*Picea mariana*	Trunk wood	0.8	0.003	0.32
Spruce	*Picea mariana*	Cones	2.6	0.013	0.5
Spruce	*Picea mariana*	Twig[1]	2.9	0.044	1.52
Jack pine	*Pinus banksiana*	Twig	0.8	0.013	1.68
Jack pine	*Pinus banksiana*	Needles	<0.5	<0.0116	2.92
Jack pine	*Pinus banksiana*	Trunk wood	<0.5	<0.002	0.41
Jack pine	*Pinus banksiana*	Outer bark	1.0	0.020	2.03
Jack pine	*Pinus banksiana*	Cones	2.5	0.007	0.26

[1]Small tree in trench.

On occasion, modest increases of Th in plant tissues have been noted over and around mineral deposits, attesting to the role of radioactive elements to assist in transporting and nucleating metals. Some examples are as follows:

- At the Rottenstone PGE deposit in Saskatchewan black spruce twigs yield 0.5 ppm Th.
- At the Old Nick Ni deposit in southern British Columbia Douglas-fir bark contains a maximum of 0.55 ppm Th. Several surveys over Au deposits in British Columbia and Alaska have recorded concentrations in black spruce bark of up to 0.2 and 0.3 ppm Th in dry tissue.
- At the Jasper Gold Mine, long before there was any disturbance of the ground, Th enrichment in Labrador tea was coincident with Au enrichment. The maximum value was only 3.6 ppm Th in ash (by INAA), equivalent to about 0.07 ppm Th dry weight, but this represented a concentration that was nine times the background value (Dunn et al., 1990).
- Leaves of *Acacia* from northern Tanzania (North Mara Au) yielded Th concentrations that were on average seven times higher than stems, with concentrations up to 0.14 ppm Th in dry tissue.

It appears that bark of conifers and deciduous species concentrates Th to a greater degree than other tissues. In deciduous species, Th is generally more concentrated in foliage than twigs. In black spruce, the most common conifer of the boreal forests, twigs have more Th than needles.

Thulium (Tm)

See Lanthanum and the REE.

Thulium is the rarest of the REE and is rarely present in vegetation at concentrations above its detection limit of 0.01 ppm Tm.

Tin (Sn) (Fig. 9-68)

For the most part, the precision of Sn data by ICP-MS is fair to poor. Overall precision improves at higher concentrations, but reproducibility remains somewhat erratic. It has recently been argued that Sn is beneficial, if not essential to plant metabolism (Nagy et al., 2000). Tin is not readily available to plants under most natural conditions resulting in generally low concentrations in most plant tissues. According to Romney et al. (1975), most of the Sn accessed by plants remains in the roots and is not readily translocated to aerial parts. However, data from *Acacia* in Western Australia contradict this observation, since higher concentrations of Sn were found in bark and phyllodes than in the roots.

	V6	NIST 1575a
Target	0.19	No data
Mean	0.19	0.04
Std. Dev.	0.05	0.02
RSD %	27	40

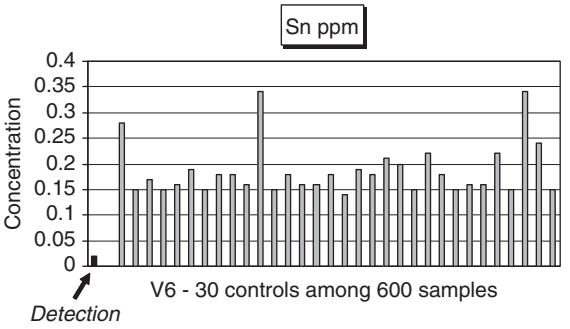


Fig. 9-68. Tin – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Foliage of plants from the Eden Project contained values from 0.03 to 0.52 Sn in dry tissue with the highest concentrations occurring in the She-Oak (*Casuarina*), which is a common tree in Australia.

In Siberia Sn enrichment was reported in several species of pine, spruce, birch, maple, raspberry and willow, with highest concentrations occurring in outer bark scales of the conifers (Kovalevsky, 1987).

In western Nova Scotia, Sn was mined from a fluorine-rich pegmatite phase of the South Mountain Batholith at East Kemptville. A reconnaissance biogeochemical survey of western Nova Scotia found high levels of Sn around the mine site. Although some of the Sn was probably derived partly from airborne dust, the propensity of different species to accumulate Sn could be determined from multi-species determinations. Red spruce (*Picea rubens*), bearing close similarities and properties to black spruce with which it probably hybridizes, contained a maximum of 81 ppm Sn in dry twigs, and bark of the same tree had 48 ppm Sn. Samples of larch (*Larix laricina*) bark from the same sample station returned a concentration of 47 ppm Sn, and twigs of balsam fir (*Abies balsamea*) had 16 ppm Sn.

The East Kemptville concentrations are exceptionally high for Sn in plant tissues. Anomalous Sn values from other surveys, where there is no known Sn mineralization are 1.1 ppm Sn in dry tree-fern fronds (*Cyathea*) from the western Amazon; 0.27 ppm Sn from leaves of the vine *Clusia* from the same 350 locations; 0.25 ppm Sn in *Acacia* bark and phyllodes from Western Australia; 0.26 ppm Sn in Douglas-fir top stems from an area in southern British Columbia containing potential Broken Hill-type Pb/Zn mineralization. Acacia twigs from central Africa have higher concentrations of Sn (median [$n = 37$] of 0.13 ppm Sn) than leaves (median of 0.09 ppm Sn).

For reasons that have yet to be explained there is relative enrichment of Sn over some kimberlites. At the Welgevonden kimberlite in South Africa concentrations of 0.1 ppm Sn were present in the thorny shrub known locally as Hak en Steek along four traverses where they passed over the kimberlites. This represented at least five times the background level of < 0.02 ppm Sn. Similarly, Sn enrichment up to 0.2 ppm

	V6	NIST 1575a
Target	24	No data
Mean	22	5
Std. Dev.	1.2	0.9
RSD %	5	19

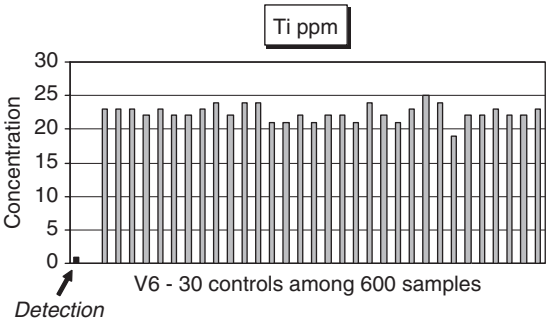


Fig. 9-69. Titanium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

Sn occurs over and peripheral to several of the kimberlites in the Ekati diamond fields of the Northwest Territories.

Titanium (Ti) (Fig. 9-69)

Ti values, although only in the tens of ppm, are usually well above the detection limit of 1 ppm, hence precision is very good. High levels of Ti could be an indication that samples are contaminated by airborne dust, although natural uptake should not be discounted because studies have suggested that a higher rate of growth, greater chlorophyll content and higher productivity may be attributed to the uptake of this element (Kelemen et al., 1993). Titanium levels are commonly in the range of 10–30 ppm Ti, with no consistent pattern as to whether the higher concentrations occur in leaves or twigs. The pattern seems to be species related, but with leaves usually having the higher concentrations (e.g., Douglas-fir, alder, Labrador tea and acacia). Bark tends to contain slightly higher concentrations.

Tungsten (W)

Tungsten is a rare element, which does not readily enter plant structures, and is below the level of detection (0.1 ppm W) in V6 and in NIST 1575a. There is no SRM that has W values in excess of detection limits. A few samples of the uraniferous twig SRM CLV-1 have returned values of 0.5 ppm W in ash, which is the equivalent of 10–20 ppb W in dry tissue. Any positive values of W should be carefully scrutinized to ensure that they have not been milled in equipment that contains any tungsten carbide components. This is especially true for hard tissues such as *Acacia* twigs. It is not uncommon for 5–10 ppm W to be recorded from samples that have been processed in grinding equipment containing W-carbide components.

About 75% of the foliage samples from the Eden Project yielded concentrations at or below the detection limit, but a few yielded approximately 1 ppm W, and

the 'star cluster' (*Pentas lanceolata*) returned a value of 7.1 ppm W which is extraordinarily high for plant material. The soil in which it is growing has a typical background level for soils of 2.5 ppm W.

The mineralization associated with the East Kemptville pegmatites in the South Mountain Batholith of Nova Scotia is primarily Sn and W. As noted under the discussion of Sn, although some of the metals were probably derived initially from airborne dust, the propensity of different species to accumulate them could be determined from multi-species determinations. Tungsten in trees from a single location near the mine site yielded concentrations in red spruce (*Picea rubens*) of 3.3 ppm W in dry outer bark scales and 3.1 ppm W in twigs. Balsam fir (*Abies balsamea*) twigs contained 0.6 ppm W. No larch was present at that site, but at other sites where the spruce and fir were collected, the larch had about 75% of the concentrations found in spruce.

Elsewhere in Canada, leaves of mountain alder (*Alnus crispa*) have been found to accumulate small amounts of W at sites where none is detected in other species.

Uranium (U) (Fig. 9-70)

V6 shows that the precision for U is very good. Similarly, the data for NIST 1575a are excellent, and to date the values obtained on the 19 one-gram samples submitted as blind controls have shown perfect precision.

Patterns of U distribution can be of considerable significance to mineral exploration, because many styles of mineralization have minor enrichment of U (i.e., a slight radioactive component). A subtle U enrichment could indicate the presence of concealed mineralization, especially since U is quite mobile.

Uranium was one of the first elements to receive detailed attention with respect to biogeochemical exploration. In the 1950s, the work of Helen Cannon and her co-workers at the United States Geological Survey was particularly important in that they recognized a number of plants that were both botanical and biogeochemical indicators of U mineralization. Work on the Colorado Plateau and the surrounding

	V6	NIST 1575a
Target	0.06	(0.009)
Mean	0.06	0.01
Std. Dev.	0.01	0
RSD %	11	0

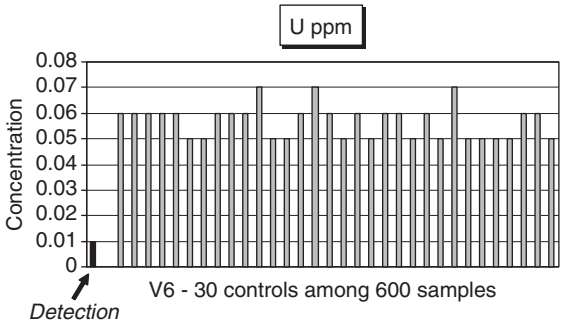


Fig. 9-70. Uranium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

area recognized Se as a pathfinder for U in the roll-front deposits that received much exploration attention at that time. Determinations were made of the U content of many plant species that grow in the arid to semi-arid environments of the mid-west and southern United States. It was found that the poison (or milk) vetch (*Astragalus spp.*) and juniper (*Juniperus monosperma*) were able to accumulate more U than most other species, although a single sample of greasewood (*Sarcobatus vermiculatis*) returned a phenomenally high 7400 ppm U in ash (Cannon, 1952). In subsequent studies, the uranium content of many more species was determined (Cannon, 1964). A full review of this and other work worldwide up to the mid-1980s was published as a Nuclear Energy Agency/International Atomic Energy Commission (NEA/IAEA) 'state-of-the-art' report with a comprehensive list and brief summary of 128 papers detailing U biogeochemistry in mineral exploration (Dunn et al., 1985).

Uranium concentrations in plant ash are commonly less than 1 ppm (<0.02 ppm U in dry tissue), but the high mobility of U in the natural environment has resulted in some remarkably high levels that may extend over large areas. In northern Saskatchewan, near the eastern edge of the Precambrian (Helikian) sandstones comprising the Athabasca Group, there are some of the world's largest and richest U deposits. Biogeochemical investigations in that area have shown that the most recent ten years growth of twigs from black spruce (*Picea mariana*) are enriched in U, and they provide a simple, practical and effective medium for outlining local and regional zones of U enrichment (Dunn 1981, 1983b,c). Details of this study are given as a case history in Chapter 11.

Over zones of U mineralization there is considerable heterogeneity of U in and among plant species. Whereas some studies report that roots contain more U than other tissues, other investigations have found more U in bark (Kovalevsky, 1973) or twigs (Dunn, 1981). Either bark or twigs can be effective, but roots are impractical to collect and it is difficult to remove inorganic particulates.

In the Athabasca area tissues from common plants were analysed to establish a hierarchy of their relative ability to accumulate U. Table 9-VIII shows that there are two orders of magnitude difference between concentrations of U in black spruce twigs and concentrations in conifer trunk wood, in horsetails and in water lilies. Near U mineralization, the ratio of U in twigs to that in needles of black spruce is 10:1, whereas in areas remote from mineralization this ratio is usually 2:1.

Plants from the Eden Project give further information on the relative uptake of U by different species. Concentrations in dry foliage range from below the detection limit of 0.01 ppm U to almost 1 ppm U in Arar (*Tetraclinis articulata*).

Bouda (1986) sampled heather, gorse, fern, grass, ash and several conifers growing on the Dartmoor granite, and found highest concentrations in heather (*Erica tetralix*) and gorse (*Ulex spp.*) with 0.14 and 0.13 ppm U, respectively. About 80% of the U in the Dartmoor granite of southwest England occurs as uraninite.

Concentrations similar to those in the samples from Dartmoor represent maximum concentrations encountered in tree ferns from the western Amazon and in conifer bark and twigs from western Canada in areas with no known U mineralization, but where

TABLE 9-VIII

Relative concentrations of U in common plants from the eastern edge of the Athabasca Group, northern Saskatchewan, Canada

Common name	Botanical name	Plant organ	Relative concentration (%)
Black spruce	*Picea mariana*	Twigs	100
Labrador tea	*Ledum groenlandicum*	Stems	50–70
Leather leaf	*Chamaedaphne calyculata*	Stems	50–70
Blueberry	*Vaccinium spp.*	Stems	50–70
Jack pine	*Pinus banksiana*	Twigs	50
Mountain alder	*Alnus crispa*	Twigs	50
Paper birch	*Betula papyrifera*	Twigs	50
Tamarack	*Larix laricina*	Twigs	50
Labrador tea	*Ledum groenlandicum*	Leaves	30
Leather leaf	*Chamaedaphne calyculata*	Leaves	30
Blueberry	*Vaccinium spp.*	Leaves	30
Jack pine	*Pinus banksiana*	Needles	10–30
Mountain alder	*Alnus crispa*	Leaves	10–30
Paper birch	*Betula papyrifera*	Leaves	10–30
Tamarack	*Larix laricina*	Needles	10–30
Black spruce	*Picea mariana*	Needles	10
Willow	*Salix spp.*	Twigs and leaves	10
Grass	–	All	10
Labrador tea	*Ledum groenlandicum*	Roots	5
Black spruce	*Picea mariana*	Trunk wood	1
Jack pine	*Pinus banksiana*	Trunk wood	1
Horsetail	*Equisetum spp.*	All	1
Water lily	*Nupa spp.*	All	1

there is a subtle U signature related to base or precious metal mineralization. In northern Canada and in Western Australia, U at similar concentrations occurs in twigs over PGE mineralization. In northern Tanzania, U in the leaves of *Acacia* from the vicinity of the North Mara Au deposit is considerably more concentrated than in stems. Concentrations are subtle with a maximum of 0.12 ppm U, although substantially higher than stems that all yielded U concentrations below the detection limit (0.01 ppm).

Biogeochemistry can be a powerful method in the exploration for U deposits. In addition, careful review of subtle U signatures can assist, also, in providing focus for exploration efforts for other minerals where mildly radioactive mineralizing fluids have assisted in the emplacement of mineral deposits.

Vanadium (V) (Fig. 9-71)

The precision for V data close to detection is only fair, because of interference on the ICP-MS by a major isotope of Cl (often present in vegetation in hundreds of ppm, and also in the aqua regia used to digest the samples). This explains why V has a higher detection limit (2 ppm) than most other trace elements and why some laboratories maintain that low ppm levels of V cannot be accurately measured by ICP-MS on an aqua regia digestion. However, other laboratories claim to have circumvented this problem and provide data with consistently adequate, if not good, precision.

Vanadium is an element that is essential in small traces to several classes of plants, in particular fungi and algae (e.g., seaweeds, and especially the green sea lettuce '*Ulva*' [Dunn, 1998a]). Studies have demonstrated that there is a biotransformation of V from vanadate (VO_3^-) to vanadyl (VO_2^+) during uptake by plants (Morrell et al., 1986), and it has been suggested that V may substitute for Mo in fixing nitrogen.

It is unusual for V to be present at concentrations >2 ppm V in dry tissue. To examine the geochemical relief of V in a set of vegetation samples, it is usually necessary to reduce tissues to ash and, after accounting for loss on ignition, results can be normalized to a dry-weight basis. From data obtained by this method it is apparent that concentrations for many types of tissue are similar to the 'reference plant' value of 0.5 ppm V (Markert, 1994).

Samples from several common boreal forest species collected over the ultramafic Bird River Sill in Manitoba were reduced to ash prior to analysis. Black spruce twigs and outer bark both yielded the highest average concentrations of 0.7 ppm V in dry weight equivalent. Average concentrations in dry tissues of other species were white spruce twigs, 0.5 ppm V; white spruce bark, 0.3 ppm V; balsam fir twigs, 0.4 ppm V; jack pine bark and twigs and birch twigs all had <0.2 ppm V. ICP-ES analysis of outer bark ash from 217 samples of lodgepole pine collected during a reconnaissance survey over the Nechako Plateau of central British Columbia yielded dry-equivalent

	V6	NIST 1575a
Target	<2	(<0.3)
Mean	2	<2
Std. Dev.		
RSD %		

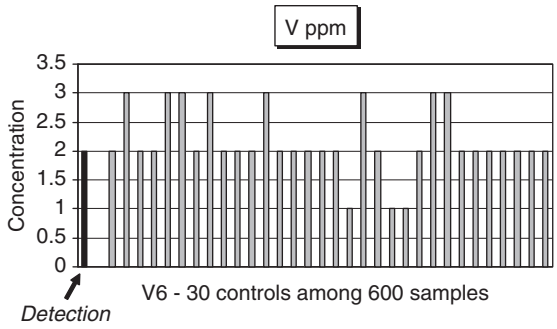


Fig. 9-71. Vanadium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

concentrations up to 2.3 ppm V with a mean of 0.6 ppm V (Dunn and Hastings, 1998).

Patterns of V distribution have not been of particular value in biogeochemical exploration for minerals. Vanadium is more concentrated in mafic than acidic rocks, and therefore enhanced values in vegetation may reflect underlying mafic lithologies. More importantly, V is enriched in some micas (fuchsite and roscoelite) that are sometimes associated with late-stage epithermal Au deposits.

In interpreting V patterns consideration should be given to potential environmental contamination if samples were collected near phosphate processing plants or from areas where phosphatic fertilizers have been applied, because of the geochemical affinity of V for P. Another source of contamination can be from burning of fuel oil that may contain V-bearing porphyrins.

Ytterbium (Yb)

See Lanthanum and the REE.

Ytterbium is a heavy REE that has no known biological role. In V6 it exhibits good precision with a mean of 0.04 ppm Yb. Databases are limited, and Yb is not usually present in plants at concentrations in plants that are above its detection limit by ICP-MS of 0.01 ppm Yb except near REE deposits. In South Africa, foliage of *Acacia mellifera* (Black thorn) has yielded up to 0.08 ppm Yb adjacent to a kimberlite, with lower concentrations in stems. Dwarf birch leaves from over kimberlites of the Ekati area of the Northwest Territories of Canada all yielded concentrations below detection. Near the Bayan Obo REE mine in northern China Yb concentrations in the ash of Russian thistle (*Salsola*) and desert apricot (*Amygdalus*) were below the detection limit of the INAA method employed (<0.25 ppm Yb in ash), whereas the same samples were highly enriched in the LREE. Early reports of 300 ppm Yb in ash of woody plants (Shacklette et al., 1978) are remarkably high and in light of subsequent studies by others such concentrations seem unlikely. At that time optical emission spectroscopy was used to determine REE concentrations.

Yttrium (Y) (Fig. 9-72)

Precision for Y is very good and data are highly reproducible. Concentrations in survey samples are typically well in excess of the detection limit of 1 ppb (0.001 ppm) Y. Yttrium closely follows the REE, so for discussions related to Y the section on La and the REE should be consulted. In the event that Y occurs with elevated levels of P and no corresponding increases in the REE, although a rare situation, the presence of the Y phosphate 'xenotime' (YPO_4) should be considered.

In areas remote from Y and REE mineralization, concentrations of Y in spruce twigs are generally from 0.005–0.05 ppm (5–50 ppb) Y in dry tissue. In a survey

	V6	NIST 1575a
Target	0.04	No data
Mean	0.04	0.023
Std. Dev.	0.001	*0.003*
RSD %	4	*12*

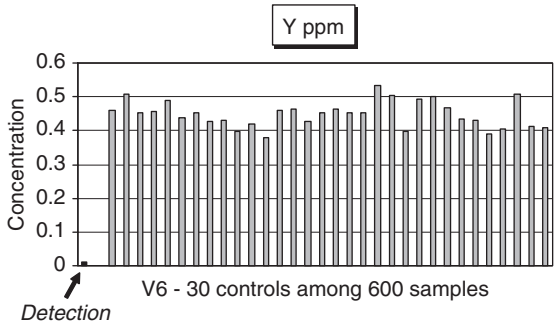


Fig. 9-72. Yttrium – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

involving 529 Douglas-fir top stems from southern British Columbia the median concentration was 0.012 ppm Y with a maximum of 0.083 ppm Y. In Africa, leaves of *Acacia* have an order of magnitude more Y than stems, with concentrations locally exceeding 2.5 ppm Y over lake sediments that once formed part of Lake Victoria in the North Mara area of Tanzania. Plants from the Eden Project yielded from a low of 0.007 ppm in leaves of the 'serendipity berry' (*Thaumatococcus*) that is native to the rain forests of West Africa, to more than 1 ppm Y in foliage from a fig tree (*Ficus*).

Zinc (Zn) (Fig. 9-73)

Zinc is an essential element for plants, hence it is present at concentrations well above its detection limit of 0.1 ppm Zn, commonly 100–1000 times greater, and analytical precision is extremely good. Some species can 'hyperaccumulate' Zn resulting in concentrations sometimes exceeding 1% Zn in dry tissue. However, these are small plants (e.g., several species of *Viola*, *Thlaspi* and *Minuartia*) and therefore unlikely to be of significant use in mineral exploration other than for the field geologist to recognize their presence as potential geobotanical indicators of Zn mineralization. These species are discussed in Brooks (1983) and Reeves and Brooks (1983). Similarly, the so-called Zn moss (the bryophyte *Pohlia wahlenbergii*) is a geobotanical indicator that stands out as an iridescent green covering of mineralized outcrops at Howard's Pass in the Yukon.

Zinc is essential for carbohydrate and protein metabolism, therefore some variability of Zn data obtained from a biogeochemical survey is related to the health of the tree rather than subtle changes in substrate chemistry. Consequently, in order to delineate areas of Zn mineralization it is necessary to look for quite substantial changes in Zn concentrations. Cadmium closely follows Zn in nature, but it is not known to be an essential element for plant metabolism. In plants it can, therefore, be a better pathfinder element for Zn mineralization than Zn itself. A considerable

	V6	NIST 1575a
Target	40	38
Mean	39	42
Std. Dev.	1.8	1.8
RSD %	5	4

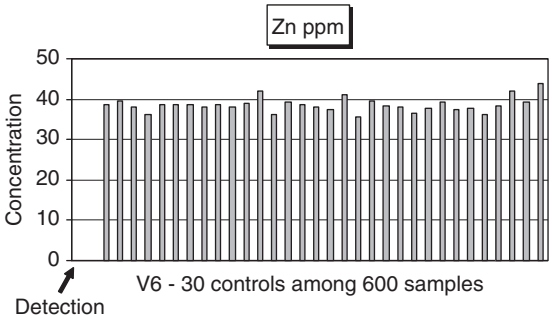


Fig. 9-73. Zinc – Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

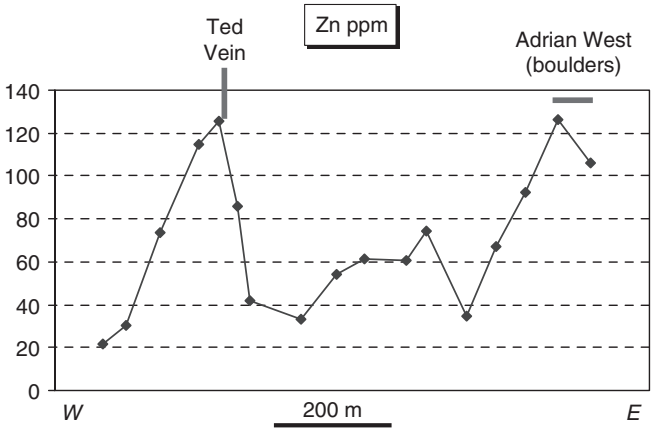


Fig. 9-74. Zinc in dry spruce needles (*Picea glauca*) along transect over the Ted epithermal Au/Ag/base metal vein. 3Ts property (Silver Quest Ltd.), central British Columbia (Dunn et al., 2006a,b). Additional details with 'halogen study' on the CD.

amount of research has been undertaken on the uptake of Zn by plants, and its movement within plants. A good review, dealing mostly with crops, is given in Kabata-Pendias (2001).

A survey over an epithermal Au/Ag system at the 3Ts property in central British Columbia included analysis of needles from white spruce (*Picea glauca*) collected along traverses over mineralized veins. One of these traverses crossed the Au-bearing Ted vein that comprises quartz-calcite with finely disseminated sulphide minerals. Pyrite is dominant, but also present are chalcopyrite, sphalerite, galena, Ag-sulphides, tellurides and sulphosalts. Figure 9-74 shows the Zn signature in the dry needles, demonstrating a strong positive signature over the Ted vein and the 'Adrian West' mineralized boulders. A similar profile was obtained from analysis of outer bark from lodgepole pine, but with lower concentrations and less geochemical relief than values from the spruce needles. Data listings for this occurrence are part of the 'Halogen

Project' for which digital data listings and a full account are contained on the CD that accompanies this book.

Trees that are renowned for accumulating high levels of Zn are birch and willow. Their bark, leaves and twigs commonly have background levels in excess of 100 ppm Zn in dry tissue. By contrast spruce, pine and Douglas-fir outer bark normally have 30–50 ppm Zn, with 100 ppm being an unusually high level. Outer bark from larch has lower concentrations with 15–25 ppm as a common range of background values. Twigs of all of these conifers have similar Zn concentrations to their respective outer bark. Needles of Douglas-fir contain lower Zn concentrations than twigs (typically 20–30 ppm Zn compared to 50 ppm for twigs). Foliage from subalpine fir typically has 50–60 ppm Zn, whereas western redcedar has only 10–15 ppm Zn.

Samples of foliage from the Eden Project returned concentrations of more than 100 ppm Zn in seven species, with a maximum of 455 ppm Zn in *Impatiens* demonstrating its capacity to scavenge Zn from the substrate. This enrichment is in accord with the findings of Tiagi and Aery (1982) who recognized *Impatiens balsamina* as a geobotanical indicator of Zn-enriched mine tailings in India.

The lowest concentration of Zn in the Eden Project samples was 13 ppm Zn in foliage of *Acacia*. This is in accord with foliage from field samples of various *Acacia* species from Africa and Australia. The *Acacia* branches, bark and roots that have been tested have all contained lower concentration than the foliage. However, *Acacia* seems to be sensitive to the presence of mineralization with concentrations in Zn-enriched areas substantially higher than the values of 5–20 ppm common at background sites.

In the vicinity of the former Sullivan Pb–Zn mine in British Columbia alder (*Alnus sinuata*) twigs provided the medium with the greatest range in concentration between background sites (approximately 40 ppm Zn in dry twigs) to sites over mineralization where there was over 1000 ppm Zn (Dunn, 2000).

Zirconium (Zr) (Fig. 9-75)

Precision for Zr is generally good and concentrations, although quite low, are usually above the detection limit of 0.01 ppm Zr. The availability of Zr to plants is low so concentrations in dry tissues are rarely above 1 ppm Zr and are usually below 0.1 ppm Zr. Any high values reported should be viewed with the suspicion that there may well have been some airborne dust contamination in the field, since contamination in the laboratory or analytical artefacts are unlikely unless a high Zr ceramic vessel was inadvertently used for grinding the tissues.

Eden Project foliage samples show a range from 0.02 to 0.7 ppm Zr. Approximately 70% of the samples yielded concentrations below 0.2 ppm Zr. Ferns appear to have a propensity to be able to accumulate small amounts of Zr.

A study that included the analysis of 148 samples of dwarf birch leaves from the tundra of Canada yielded a mean concentration of 0.03 ppm Zr with a maximum of

	V6	NIST 1575a
Target	0.05	No data
Mean	0.05	0.03
Std. Dev.	0.01	0.01
RSD %	13	24

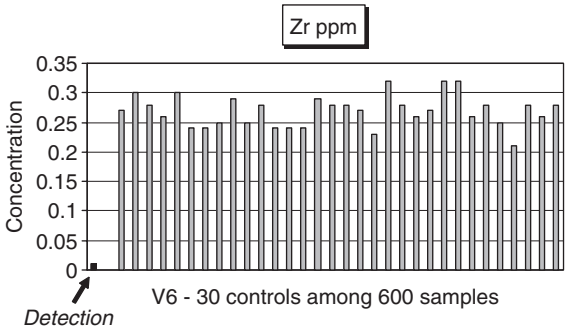


Fig. 9-75. Zirconium– Precision and accuracy on dry tissues – V6 and NIST 1575a (see Fig. 9-1).

0.08 ppm Zr. Acacia from southern and central Africa has more Zr in foliage than stems, with concentrations up to 4 ppm Zr in foliage and 0.6 ppm Zr in stems, although most stem samples yielded less than 0.1 ppm Zr. Zirconium seems to play no significant role in biogeochemical exploration for minerals other than to provide an indicator of possible airborne dust contamination. Black spruce from a remote area of northern Saskatchewan has demonstrated that twigs (up to 0.17 ppm Zr) have higher Zr concentrations that needles (0.03 ppm Zr), and that twigs and leaves of deciduous species (e.g., alder, *Alnus crispa*) have similar concentrations of 0.04 ppm Zr.

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Chapter 10

DATA HANDLING AND ANALYSIS

FIRST ESTIMATION OF THE DATA

On receipt of a spreadsheet of data in digital form, it is advisable to immediately make a 'working copy' and store the original in its exact format. This allows the user to return to the original in case subsequent manipulations of the data inadvertently corrupt the data (e.g., misalignment of cells, columns or rows during a sorting procedure). The 'working copy' needs to have cross-reference numbers and field parameters inserted that subsequently comprise the base document that can be sorted and manipulated in any manner that the user feels necessary.

After following through the rigours of collecting samples from a survey area and awaiting results from the analytical laboratory, as soon as the data are received there is a natural tendency to quickly scan the numbers to look for anomalously high concentrations of the commodity metals of particular interest. Two immediate scenarios may arise.

- There are no obvious anomalies and so there is a natural sense of disappointment and the data are given little further consideration.
- There are anomalous element concentrations, and it is very tempting to jump to conclusions as to their significance.

It is prudent to step back from the data and, before any conclusion is drawn, to systematically investigate the entire data set, starting with the controls. To reiterate what has been said earlier, no set of samples should be submitted without inserting controls – to establish both analytical precision (duplicate field and laboratory samples) and analytical accuracy (using vegetation controls of known composition). Whereas these controls should be inserted 'blind' so that a completely objective assessment of precision and accuracy can be made, many laboratories also provide the data from their own analytical duplicates and control samples. It is useful to interrogate all of these data in assessing the data quality.

Microsoft Excel is now a universally available and comprehensive software programme that can be used to meet all of the initial requirements of assessing quality control and sorting data. Most laboratories now report analytical data in Excel format or in an Excel-compatible format. Since it is recommended that 'blind' controls be inserted within a sequence of samples, the first step is to identify these controls and extract them into separate spreadsheets.

A visual appraisal of data structure is an important aspect of exploratory data analysis. By plotting data as histograms or on X–Y plots, the eye is quick to focus on associations, outliers and trends. An examination of the dataset in the format that it is received from the laboratory should constitute the first assessment. Construction of a histogram (bar chart) of each individual element is useful for identifying trends in the analytical data (perhaps attributable to analytical instrument drift) and any 'blockiness' that might be attributed to changes in instrument calibration (or contamination) after a block of samples is analyzed, such as an individual rack of test tubes. Figure 10-1 gives an example of set of samples that has a suspicious-looking blocky appearance. This proved to be an artefact of the analytical procedure, since it was later learned that each 'block' of 33 samples (notably that identified by the arrow) represented a single rack of test tubes, and that the laboratory checked instrument calibrations after each rack and made appropriate adjustments. Most of the single point peaks (~4 ppm Cr) represent a blind control sample. These could later be extracted and their precision and accuracy assessed separately. However, the control sample proved to have concentrations that were more than double the average values of the field samples, and by looking at only the controls the changes by blocks of samples would probably have been missed. In the case of Cr the analytical data obtained by ICP-MS on an aqua regia digestion require considerable correction (by the laboratory) for high inter-element interferences. Chromium data obtained by this method are, therefore, of lower quality than for many other elements, and due consideration should be given to this lack of quality during data interpretation.

Another feature of the data that should be looked for is any periodicity to the element concentrations. On occasion a trend has been noted such as that shown in Fig. 10-2.

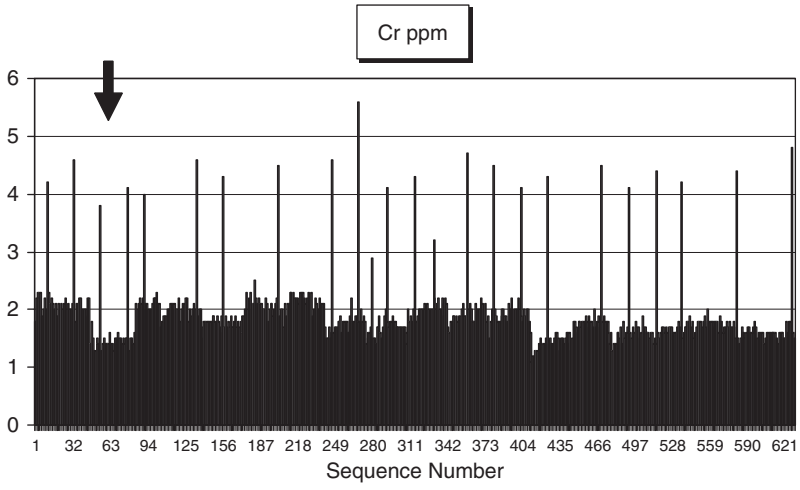

Fig. 10-1. Analysis of a set of 630 Douglas-fir twig samples showing a blocky appearance in the data for Cr. Individual peaks are mostly the blind control samples.

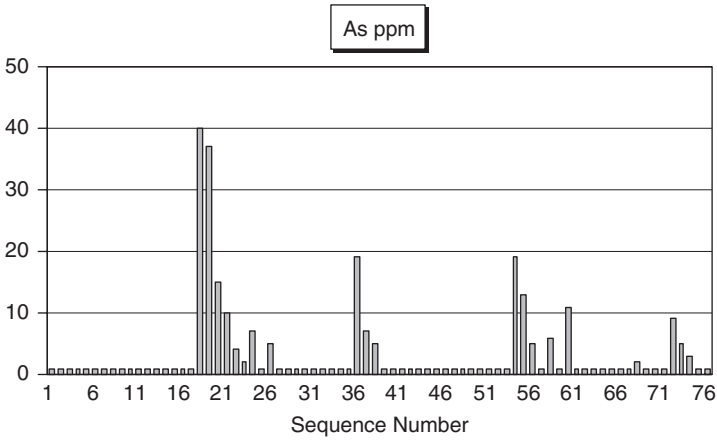

Fig. 10-2. Periodicity in data for As from a sequence of 74 samples of plant ash analysed by ICP-ES.

In the situation shown in Fig. 10-2 plant ash samples were submitted for analysis with standards inserted as blind controls after the 10th, 30th, 50th and 70th samples in the data sequence. However, the standard contained a similar As concentration (1 ppm) to most of the field samples and so on the first pass no analytical abnormalities were found. In this situation data were plotted without thorough preliminary investigation of data quality, and the locations of the anomalies made no geological sense. On re-examination of the data, as the histogram shown in Fig. 10-2, it soon became evident that the peaks in As enrichments occurred on a regular cycle of every 18th sample, and that values decreased over the following few samples. The laboratory had not reported their own quality control data, and so they were contacted to discuss these trends. It turned out that they had inserted their control at every 18th site and the control they used was material that originated from As-rich tailings! Consequently, because their analyst did not thoroughly purge the pipette used to extract the solution from each test tube after each analysis, there was some contamination transferred to the subsequent sample which, after a few samples, was finally flushed from the system. This particular data set was received a good many years ago, and nowadays any reputable laboratory is unlikely to follow such practices. However, although mistakes are few they can still be made and the geochemist receiving the data has to be alert to potential problems and errors. In the situation described, there were also some Cu enrichments and clusters of samples with elevated Cu and As could have led to some wasteful follow-up studies if the analytical data had been taken at face value.

The need for quality control has been emphasized in Chapter 6 with an extensive discussion of Standard Reference Materials. In a discussion of data quality, (Thompson, 1989) stated

> all analytical measurements are wrong; it is just a question of how large the errors are and whether they are acceptable

With this in mind the concept of 'Fit for Purpose' was introduced in Chapter 7, and in Chapter 9 discussion continues by demonstrating from the charts at the beginning of each element description, the realistic precision that can be expected from low-cost multi-element analysis by ICP-MS. For those looking for an in-depth evaluation of quality control in geochemical exploration, there are good accounts given by Thompson and Howarth (1978), Long (1999), Sketchley (1999), Smee (1998, 1999), Steger (1999), Taylor (1987) and Vallee and Sinclair (1999).

COMPUTER SOFTWARE TOOLS

There are many excellent competing software products for examining, evaluating and plotting geochemical data. It often comes down to personal preference or an employer's company policy as to which system to use. Excel is an excellent programme for the raw database platform, and many manipulations of the data can be undertaken within Excel. Programmes for more advanced statistical interpretations are, among others, SPSS, SAS, S-plus and Geosoft.

Data analysis systems are available that require a modest financial outlay for a stand-alone product, and/or perhaps an annual license fee. Among these products there is DataShed, provided by Maxwell Geoscience (Fremantle, Western Australia). This is a desktop software product, which is a data management system, designed to allow storage, management, analysis, reporting and integration of data in a functional and intuitive environment.

New software designed specifically for the geochemist is ioGAS (Geochemical Analysis System) by ioGlobal of Perth, Australia. The components of this software package have been designed by geochemists to make complex geochemical data analysis intuitive and easy to use for all levels of geoscientific knowledge. As such, it is not a generic statistics package, but focuses on the use of statistical tools that are of specific use for the geochemist and biogeochemist. Among its features are intuitive ways of rapidly plotting data by such tools as X–Y plots, dynamic histograms, probability plots and box and whisker plots. Menus permit the selection of different colours and symbols to represent differing classes of attributes, such as readily comparing the composition of leaves and stems and their spatial distributions with respect to underlying geological structures and lithology. There is a direct link with Google Earth upon which spatial data distribution plots can be draped to assess relationships to topography and cultural features. The website for this product (http://www.ioglobal.net/ioGas.aspx) includes coloured screenshots and a comprehensive range of free videos that explain how to navigate the software. As with many such programmes, there is a wide range of features that suffice to fill almost any aspect of data interpretation and analysis, but of relevance to the exploration geochemist is that the content is designed by geochemists with meaningful examples to which the geochemist can readily relate.

Geographical Information Systems (GIS) are used extensively in plotting the spatial relationships of data. MapInfo and the ERSI products (including ArcView, ArcGIS and ArcInfo) are some of the most widely recognised and widely used systems. Low-cost applications include Manifold System and Surfer (Golden Software, Colorado). The latter two systems are among the best for the individual with limited budget, because for a few hundred dollars they can be purchased outright, and require no annual licence fee. With a modest amount of effort in learning these latter systems, the non-computer specialist can generate clear and informative pictorial output, and the programmes allow the user to quickly interact with many parameters to obtain a perspective that is tailored to explore for a particular commodity. An up-to-date list of GIS options can be found at http://en.wikipedia.org/wiki/List_of_GIS_software.

DATA ANALYSIS

Biogeochemical data from samples that have been carefully and consistently collected, prepared and analyzed, require statistical evaluation by the same procedures that would be applied to any set of geochemical exploration data. Standard statistical treatment by univariate and bivariate methods should be applied, and it is usually advantageous to undertake further analysis by a multivariate method.

There are many textbooks on statistical analysis of data. Two old but good texts are by Moroney (1951), and for non-parametric statistics Siegel (1956). Among the more recent publications that provide accounts of statistics and computer applications with how and why to use a wide range of techniques, there are those by Rock (1988), Davis (2002), Grimm and Yarnold (1995, 2000), Helsel and Hirsch (2000), Taylor and Cihon (2004) and Tabachnick and Fiddell (2006). In Europe, Eurachem is a 'network of organisations … having the objective of establishing a system for the international traceability of chemical measurements and the promotion of good quality practices'. On their website (http://www.eurachem.ul.pt), they include a number of documents that can be downloaded, each addressing a particular aspect of quality control and data analysis.

A summary of statistical techniques used in biogeochemical data analysis is given by Brooks (1982, 1995). He provides succinct explanations, each within a few pages, of populations and distributions, the differences between parametric and non-parametric methods, tests of significance, bivariate analysis and multivariate analysis.

The following brief accounts of a number of statistical options outline the essence of methods commonly used in geochemical data analysis in general. There are many others that an individual worker may wish to invoke or experiment with, in order to understand the structure of a data set. The reader is referred to the publications listed above for a more in-depth understanding of statistical methods.

Univariate statistics

Univariate statistical analysis explores each variable in a data set by examining the range of values and the central tendency of the values. One of the simplest to start with is the histogram, which is very easily and quickly plotted by, for example, Excel. In order to comprehend the structure of a database, it is necessary to examine frequency distributions and cumulative frequencies, as well as some basic statistics that include calculation of maximum, minimum, mean, standard deviation and percentile values. Detailed accounts of cumulative frequencies and probability plots are provided by Sinclair (1976) and as a computer programme by Stanley (1988).

The median (50th percentile) of the data for each element provides a useful estimation of 'background' concentrations – i.e., the normal levels found in a particular area or on a particular geological substrate. Table 10-I shows a typical set of basic statistical parameters that were calculated from a suite of 455 samples of Douglas-fir outer bark during a survey in southern British Columbia. The software programme SPSS was used to make these calculations.

The example in Table 10-I shows a selection of percentile values, with the 98th percentile of the dataset as the highest percentile calculated. For the 455 samples in this dataset, that represents only 9 samples with concentrations higher than the 98th percentile (i.e., 2% of the 455 samples in the survey). For a dataset of this size the 98th percentile is an appropriate maximum percentile because of the small number of samples that it represents. For larger datasets (>1000) the 99th percentile can be of relevance. The software programme provides frequency distribution plots and permits the calculation of any number of percentiles, depending on the degree of interrogation of the dataset that is required and the precision of the analytical data. If there is poor precision, then the subdivision into narrow percentile ranges becomes meaningless. For data with good precision at low concentrations, it may sometimes be of use to interrogate the data structure of values below the median in order to set contour values for subsequent plotting of the data at, say, the 10th and 25th percentile values – or any preferred combination. It should be remembered that in geochemistry in general, negative anomalies might often be of use in understanding geochemical patterns, because zones of element depletion may point toward adjacent zones of enrichment derived from the depletion. This remains true for biogeochemical datasets.

An effective method of viewing data other than by absolute values is as a response ratio, which is the ratio of the concentration in a sample to the background value for each element. This is especially useful for comparing concentrations among different tissue types, different species or with other sample media. By this method all sets of results from different media are levelled to a common baseline for inter-comparison. For example, a response ratio of unity indicates a sample concentration at the median (50th percentile) of the dataset. A response ratio of three indicates a concentration of three times the median, regardless of the absolute value.

TABLE 10-I

Univariate statistics calculated for a set of 455 Douglas-fir outer bark samples. Concentrations in dry material determined by ICP-MS on a nitric acid/aqua regia digestion. Values below detection expressed as half the detection limit (see table 7-III)

	N	Mean	Standard Deviation	Minimum	Percentiles						Maximum
					50	70	80	90	95	98	
Ag (ppb)	455	7.5	3.3	2	7	9	9.8	11	13	16	43
Al (%)	455	0.035	0.014	0.005	0.03	0.04	0.05	0.05	0.06	0.07	0.1
As (ppm)	455	1.4	0.76	0.4	1.3	1.5	1.8	2.3	2.9	3.5	7.6
Au (ppb)	455	0.26	0.5	0.1	0.2	0.3	0.3	0.4	0.5	0.9	9.2
B (ppm)	455	6.2	2.3	2	6	7	8	9	10	13	19
Ba (ppm)	455	107	51	22	100	127	144	168	195	258	336
Bi (ppm)	455	0.01	0.004	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03
Ca (%)	455	0.77	0.23	0.28	0.74	0.88	0.96	1.08	1.192	1.298	1.7
Cd (ppm)	455	0.31	0.15	0.05	0.28	0.36	0.41	0.50	0.60	0.78	1.08
Co (ppm)	455	0.21	0.08	0.04	0.20	0.24	0.28	0.31	0.35	0.41	0.59
Cr (ppm)	455	2.97	0.8	1.70	2.90	3.20	3.50	4.00	4.40	4.99	8.4
Cs (ppm)	455	0.033	0.02	0.003	0.029	0.039	0.046	0.057	0.067	0.079	0.108
Cu (ppm)	455	8.0	1.4	4.2	7.9	8.7	9.0	9.8	10.4	11.2	13.0
Fe (%)	455	0.036	0.017	0.005	0.033	0.043	0.052	0.06	0.069	0.084	0.1
Ga (ppm)	455	0.1	0.04	0.05	0.1	0.1	0.1	0.1	0.2	0.2	0.3
Hg (ppb)	455	152	61	27	143	177	198	235	276	306	365
K (%)	455	0.093	0.03	0.03	0.09	0.1	0.11	0.13	0.15	0.179	0.25
La (ppm)	455	0.41	0.19	0.06	0.37	0.48	0.57	0.66	0.77	0.93	1.18
Mg (%)	455	0.028	0.008	0.011	0.027	0.031	0.034	0.038	0.042	0.049	0.074
Mn (ppm)	455	120	48	25	114	140	154	184	211	235	376
Mo (ppm)	455	0.10	0.04	0.02	0.09	0.11	0.12	0.14	0.16	0.19	0.29
Na (%)	455	0.003	0.002	0.001	0.003	0.004	0.005	0.006	0.007	0.008	0.021

Continued

TABLE 10-I *Continued*

	N	Mean	Standard Deviation	Minimum	Percentiles						Maximum
					50	70	80	90	95	98	
Ni (ppm)	455	1.05	0.7	0.05	1	1.3	1.5	1.9	2.4	2.7	4.6
P (%)	455	0.031	0.007	0.017	0.031	0.035	0.037	0.041	0.044	0.047	0.058
Pb (ppm)	455	5.8	2.7	0.7	5.4	6.7	8.1	9.5	10.9	12.5	18.2
S (%)	455	0.04	0.02	0.01	0.04	0.05	0.05	0.07	0.082	0.1	0.15
Sb (ppm)	455	0.07	0.04	0.01	0.06	0.08	0.1	0.12	0.14	0.16	0.21
Sc (ppm)	455	0.17	0.06	0.05	0.2	0.2	0.2	0.2	0.3	0.3	0.4
Se (ppm)	455	0.16	0.06	0.05	0.2	0.2	0.2	0.2	0.22	0.3	0.4
Sr (ppm)	455	47	18	13	46	55	62	72	81	89	113
Te (ppm)	455	0.01	0.004	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.04
Th (ppm)	455	0.05	0.04	0.01	0.05	0.06	0.07	0.09	0.1	0.12	0.55
Ti (ppm)	455	20	10	3	18	24	28	34	41	49	58
Tl (ppm)	455	0.01	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
U (ppm)	455	0.015	0.01	0.005	0.01	0.02	0.02	0.03	0.03	0.04	0.15
V (ppm)	455	1.5	0.6	1	1	2	2	2	3	3	3
W (ppm)	455	0.05	0	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1
Zn (ppm)	455	39	12	12	39	45	50	55	61	69	77

Bivariate statistics

Bivariate statistics are procedures used to describe the relationship between two variables, examining primarily the extent to which they co-vary. 'X–Y' plots ('scatterplots') provide a rapid and simple view of the relationship between two variables, and most software programmes (including Excel) permit rapid calculation and insertion of a trend line along with its regression equation. Scatterplots should be constructed when the relationship between two variables is of interest, because statistical summaries are not an adequate substitute for a visual appraisal of relationships.

Brooks (1982) shows how a regression line can be fitted to a series of points on a graph. He works through the mathematics of calculating a regression line for a plot of Ni concentrations in plant ash as a function of the Ni content in the soil, and the subsequent calculation of a reduced major axis. He shows, also, the calculations for the Pearson product moment correlation coefficient (r). This coefficient quantifies how good the linear relationship is at equating, say, the Ni content of a plant with respect to the Ni content of the underlying soil. Correlation coefficient matrices provide a quick evaluation of the relationships between each pair of elements obtained from a multi-element analytical package. However, to determine the multi-element relationship among all of the elements, a multivariate statistical technique needs to be applied.

Multivariate analysis

Multivariate statistics are procedures used to describe the relationship between three or more variables. In the case of geochemical data in general, the objective is to elucidate 'hidden' (i.e., subtle and not apparent) relationships in a dataset, which may assist in defining spatially related associations derived from concealed mineralization. In the case of biogeochemical datasets, a multivariate analysis can help to isolate those elements, or that portion of an element content that can be ascribed to plant nutrition from elements derived from a mineralized source. For example, Cu is an essential element, so some of the Cu content of plant tissues is directly related to the metabolic processes that result in Cu uptake and the health of the plant. However, plants growing in the vicinity of a Cu deposit may absorb greater quantities of Cu, and a part of that Cu concentration may be attributable to mineralization. A multivariate analysis may therefore indicate, for example, a Cu, Mo, B, Ca, K association (i.e., a nutritional factor) separate from a Cu, Mo, Au, As, U association that is derived from a concealed mineralized porphyry deposit. Factor analysis is a useful tool for separating out these associations.

At the outset, it must be realized that a basic assumption of multivariate methods is that the data are valid (i.e., the analytical precision is good). It should also be realized that multi-element analytical data have variable precision, and so the application of any multivariate statistical procedure should be entered into with caution.

These methods do not use the same logic of statistical inference that dependence methods do, and there are no robust measures that can overcome problems in the data. So, multivariate methods are only as good as the input. When using statistical software packages on a dataset, it is as well to first test the default settings, and preferably undertake a data analysis in two or more ways to see if the results are consistent. If they are, there is a strong underlying structure in the data and the patterns are robust. If not, it is advisable to discuss the methods with someone knowledgeable in these techniques, because it is tempting to believe the output and rely on what may be false assumptions.

Heydorn (2005) provides an up-to-date overview of exploratory multivariate analysis of soil and plant multi-element data. He endorses the caveat in the previous paragraph by stating that it is

> absolutely necessary that proper mathematical and statistical techniques be applied to scientific multivariate data to extract reliable information and to avoid drawing unjustified conclusions.

In many respects plant systems are extraordinarily complex, and with an ever-increasing number of elements derived from the systematic chemical analysis of plant tissues, it becomes a formidable task to establish the multi-element relationships that occur within and among plants. Multivariate analysis is, therefore, becoming an increasingly important tool in helping to sort out these interactions and their significance. From the perspective of biogeochemical exploration, the ultimate objective is to isolate those factors that are simply a reflection of intra-plant processes from those that are directly related to mineral deposits. Elements required for the 'organic' (plant) processes need isolating from the inorganic components that the geologist seeks in the quest for defining the location and extent of concealed mineralization.

There are several basic multivariate methods that are used in data analysis:

- cluster analysis,
- discriminant analysis (also referred to as Linear discriminant analysis),
- factor analysis, and
- principal components analysis (PCA – analogous to principal components analysis is metric multidimensional scaling).

The first two techniques seek groups of variables that can be extracted as separate and significant entities from a seemingly homogeneous dataset. Factor analysis and PCA are closely related, with factor analysis being the more complex and sometimes the more difficult to interpret, although both PCA and factor analysis methods commonly give similar results.

Most commercially available statistical analysis software programmes can perform all these multivariate analyses, with adjustments that can be made for methods to extract the desired output. The user can select the preferred technique. Rather than

elaborate on each technique, only factor analysis is described here, because in many situations it provides the most useful and relevant information for biogeochemical datasets.

The starting point for factor analysis is a spreadsheet of data (e.g., Excel) with the data all in a numeric (rather than text) format. The spreadsheet is imported into the statistical analysis software programme and 'factor analysis' is selected from the drop-down menu. The range of elements (with any quantifiable field parameters) to be incorporated into the factor analysis is selected and the command 'extract' is given. There is then a choice of methods that can be made, of which the default is usually 'Principal Components' and the default matrix for analysis is the correlation matrix. In factor analysis, vector 'eigenvalues' are calculated and for most purposes a value of one is an appropriate cut-off level. The programme needs to know how many iterations of calculations are required to generate the factor matrix. The default value of 25 is usually adequate, but for large datasets a higher value may be required to bring convergence to the dataset.

Factor analysis is used to explain variability among observed random variables in terms of fewer unobserved random variables called factors. The observed variables are linear combinations of the factors, plus error terms, and help to provide insight to the structure of large amounts of data. The process of factor analysis can be visualized as examining a dataset in n-dimensional space and inserting axes (typically orthogonal) into the data matrix to optimize the fit and thereby explain the variance in the data. This generates coordinates (from -1 to $+1$) with loadings according to the strength of correlation. The first iteration extracts the factor that explains the greatest proportion of the data variability, expressed as a percentage and represents a list of coordinates in factor (or component) 1. Once this variability has been extracted from the dataset, a new calculation is automatically generated to extract the combination of elements that accounts for the next largest percentage of the data variability. Commonly, about 7–10 factors will be extracted by this technique and these usually account for 70–90% of the data variability. The sort of information gleaned from this process may be isolation of elements related to plant structure (e.g. Ca, K, Zn, Cu) from those related to plant nutrition (e.g., B, Mo, P), and perhaps data related to mineralization (e.g., Au, As, Sb, Bi, Ag, Hg, U), a carbonate-rich substrate (Ca, Sr, Ba, Mg), or mafic bedrock (Ni, Co, Cr, Mg). There is commonly, too, a significant factor involving Fe and associated elements such as REE, Al, Hf, Sc, Ti and Hg.

It should be appreciated that results from factor analysis should not be considered as a definitive solution. The data that are entered are invariably of mixed quality with respect to analytical precision, because the data for some elements are considerably more precise than data for others. The uncertainty surrounding results from a factor analysis becomes evident if the user generates several rotated factor solutions using first a complete array of elements, then extracting some of those elements and re-calculating the factors for the remaining matrix. Commonly, there may be some

changes in element associations, although some pervasive associations will reflect the underlying robust components of the data structure.

Factor analysis provides another perspective on a dataset that can contribute toward an understanding of data structure and gives indications of associations that may be relevant to a mineral exploration programme. Interpretations are subjective and become based upon the intimate knowledge of a field area with respect to the natural environment, geology and mineralogical associations.

MAP PLOTS OF DATA DISTRIBUTIONS

A geologist needs maps – topographic, geological, geophysical and geochemical. Data from a biogeochemical survey can be presented in many formats, which are generic to the plotting of geochemical data from other exploration sampling media. Proportional dots of different colours can be used to represent either different species or different concentration levels of elements from a single species. Larger datasets can be contoured and sample sites superimposed as symbols with or without the data values (so-called 'Post' maps). The GIS packages discussed above are ideal for undertaking these map plots. For the small operator who does not have ready access to large and expensive GIS software programmes, a useful option is the software package 'Surfer'. A concise overview of the capabilities of this programme can be viewed on website http://www.goldensoftware.com/products/surfer/surfer.shtml.

Surfer is a contouring and 3D surface mapping programme that runs with Microsoft Windows. It converts data into contour, surface, wire-frame, vector, image, shaded relief and post maps, and has a wide array of options for customizing output. It is a rectangular grid-based contouring programme that requires the generation of a grid file for many plotting operations. Gridding methods use weighted average interpolation algorithms and various gridding methods can be applied. In summary, the principal features of the more common gridding methods are as follows.

- *Kriging* generates the best overall interpretation of most biogeochemical data sets. The user should be alert to the fact that maps generated from kriged data will extrapolate contours into areas with no data control. These areas should be disregarded, since they invariably provide a false impression of the extrapolated element concentrations.
- *Nearest Neighbour, Natural Neighbour, Minimum Curvature* and *Radial Basis Functions* all provide similar smooth contour patterns to kriging.
- *Inverse Distance* and *Shepard's Method* tend to generate 'bull's eye' patterns.

It is a useful exercise to experiment with each of the various gridding options to be satisfied that the one selected is optimal for generating the output that the user finds most meaningful for representing and interpreting the dataset.

UNUSUAL CONCENTRATIONS OF SELECTED ELEMENTS

The data received from a set of biogeochemical samples may sometimes yield some anomalous results that can be attributed to either analytical errors or certain environmental situations. The following examples are not exhaustive, but they represent problems that have been encountered from time to time, and are presented to alert the user to possible explanations for unusual enrichments or depletions of elements. There are many potential sources of these anomalies. A few to look out for are the following.

Analytical artefacts

All elements high. Could be a weighing error.
All elements low. Could be a weighing error.
Extremely low levels of all elements in an isolated sample. Automated pipette failed to enter the solution in the test tube (or the test tube was empty) with the result that no material was aspirated into the analytical instrument.
Poor precision. Can result from the sample weight being too low. Typically, 1 g of dry tissue or 0.25 g of ash would be a suitable amount. Lower sample weights commonly result in a marked decrease in analytical precision.
Precision of Au determined by INAA (Repeat analysis).
a) If very fine particles of Au (sub-micron) are distributed evenly throughout the sample, excellent precision can be expected.
b) If larger particles of Au are present, during the first count a Au particle may be in one position with respect to the detector and in another position for the second count resulting in either a lower or higher value being recorded.
INAA – All data out by a factor of 5. Incorrect placement of the samples with respect to the detector.
INAA – Reanalysis of material that has been irradiated previously: Isotopes of certain elements (e.g., Co, Zn, Sc, etc.) created during the first irradiation have not fully decayed, resulting in elevated concentrations of these elements (whether subsequent analysis by INAA or any other analytical technique).
ICP-ES – Data for an individual sample high by a factor of 2. The laboratory may have taken a smaller portion of a sample for analysis and failed to adjust for the smaller sample weight. This situation is only likely to occur if the laboratory has been given insufficient material for them to be able to analyze samples of consistent weights. As a result, they may take only half the usual weight (i.e., 0.5 g of dry tissue), and it has been known that a technician has forgotten to adjust the data for the smaller sample weight.
Cobalt. Small amounts of Co contamination can come from the preparation of tissues in mills with tungsten carbide surfaces, because the W particles are embedded in a Co-based binding matrix.

Mercury. Background concentrations in dry vegetation are usually 10–20 ppb. If samples have been dried at a temperature over 100 °C, invariably some Hg will have volatilized (Table 6-VI). Studies in the former Soviet Union have claimed that some Hg remains in plant ash (as a carbide) but such claims have not been substantiated by studies elsewhere, unless the Hg is structurally bound in a crystal lattice of a rock-forming mineral (e.g., pyrite).
Nickel. On occasion, a miniscule fragment of the cutting blade used to mill the samples becomes incorporated into a sample, resulting in a spurious spike in Ni data, along with a slight increase in Fe, although, because of the relatively high concentration of Fe in the plant tissue, Fe contamination is less evident.
Tungsten. Hard tissues that are milled in equipment with tungsten carbide components may contain several ppm W in dry tissue.
Zirconium. Substantial enrichments in plant tissues will be recorded if samples are ground in a ZrO mortar and pestle.

Sampling and sample preparation artefacts

Very high levels of gold, arsenic and antimony in ash: Samples may have been ashed in equipment used for fire assay. In one case, an analysis of over 200,000 ppb Au was returned from a sample that was reduced to ash in a fire-assay furnace.
One survey line yields high values and an adjacent line yields lower values: Check to see if two samplers were collecting samples using different techniques.
Erratic bark analyses: Some inner bark might have been mixed-in with the outer bark (i.e., check the techniques of the samplers). Inner bark has lower concentrations than outer bark of most commodity elements of interest to mineral exploration.
Erratic ash yields from the same sample medium may indicate that there is dust contamination. However, samples that contain high concentrations of Fe commonly have a high ash yield.
Erratic values for a particular element in twigs: This may indicate that there is a mixture of old with new growth (e.g., 10 years of growth from one site and only 3 years from another). As an example alder, for which old twig growth has relatively high levels of Ba, REE and Fe, whereas new growth has relatively high levels of Mo and Co. Sampling procedures of field crews need to be verified.
Isolated sample with a different pattern of element concentrations would probably indicate that the wrong species was collected.

Contamination of the natural environment

Lead: Lead anomalies near roadsides are likely to be related emissions of leaded petroleum. Although unleaded petroleum is now widely used, Pb persists for many years.

Vanadium and Nickel: Enrichments around oil-fired power plants have been recorded. Oils commonly contain V- and Ni-enriched porphyrins.
Zinc: Samples collected downstream from galvanized iron culverts or other galvanized iron containers may give rise to elevated Zn levels in vegetation. Sampling should be conducted upstream and upslope from potential sources of contamination.
High levels of lead and antimony: If these occur together at a single site, they could be the result of someone having fired a rifle at the tree. This has been known to occur. See section on Hg in Chapter 9.

Species-specific enrichments

Arsenic – Douglas-fir and western hemlock have an unusual ability to concentrate As. Some ferns, too, can be highly enriched in As.
Barium – Douglas-fir in western North America and the Brazil nut (*Bertholletia excelsa*) in the Amazon can accumulate high levels of Ba.
Chromium – Hazelnuts (*Corylus spp.*) may be enriched in Cr.
Molybdenum – Alders are able to concentrate high levels of Mo.
Rare Earth Elements (REE) – Ferns can accumulate high levels of REE. Other species may accumulate REE in certain environments, such as oxidized black shales.
Silver – Trunk wood of spruce and pine concentrates Ag.
Zinc – Birch and willow accumulate high concentrations of Zn.

Vascular plant species are known to hyperaccumulate about a dozen elements, but few of the several hundred hyperaccumulator plant species are sufficiently common to be of general use in a biogeochemical exploration surveys.

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Chapter 11

CASE HISTORIES

The following pages contain a selection of case history studies of biogeochemical surveys that have been conducted over known and/or potential zones of mineralization. There are many case history studies in the literature – enough to warrant a separate volume to do them all justice. Some of the richest sources of such studies can be found in the *Journal of Geochemical Exploration*, and for Russian studies that have been translated into English there is a book by Kovalevsky (1987).

The format adopted in the following pages is similar for each case history, with basic information on geology, mineralogy, climatic and botanical environment, the scope of each study, analytical results and data interpretation. Where available, a summary of follow-up exploration results are provided. Except for one contribution from the University of Barcelona, each account is a synopsis of studies that have had personal involvement. By so doing, this permits a full and detailed appraisal of the full procedures from planning a survey through to data interpretation.

These case histories focus on Au, PGEs, Ni, U and, because of their diamond-bearing potential, kimberlites. Certain aspects of case history results from surveys for other commodities are summarized as examples throughout this book. On the accompanying CD, the publication on halogens includes full accounts of additional surveys over several zones of Au and porphyry Cu mineralization in the forests of British Columbia. Additional case histories can be viewed by referring to the many complete papers for which abstracts are provided on the CD with this book.

The intention here is not to provide a comprehensive account of each study, but to put results in context of geological substrate and concealed mineralization, and in some examples provide consideration of potential processes that might have contributed to a positive biogeochemical signature. As such, they are aimed at demonstrating the role of biogeochemical surveys in mineral exploration programmes. The scope of the surveys for which examples are presented cover the spectrum from a few samples representing an orientation survey, to surveys involving a thousand or more samples collected with expensive airborne support.

GOLD

Canada: La Ronge and Glennie Domains, Northern Saskatchewan

Gold occurs mainly within volcanic-derived supracrustal and felsic intrusive rocks of the La Ronge and Glennie Domains. Native gold may be associated with pyrite, pyrrhotite, chalcopyrite, galena, sphalerite and tourmaline. Molybdenite, stibnite, marcasite, hematite, magnetite and arsenopyrite may be present locally (Coombe, 1984; Delaney, 1995). Bedrock has a veneer of glacial deposits (mostly tills) upon which soil development is primarily podzol. Many of the Au deposits in this region are associated with quartz veins and/or shear zones. In the following, the descriptions of the geology and production statistics are summarized from the Mineral Deposit Index on the website of the Saskatchewan Department of Industry and Resources. Further details are provided in the description of each individual deposit.

The area experiences a cold continental climate with temperatures typically in the range of −40 °C–+35 °C with 40–60 cm of precipitation occurring mainly in the summer months. These conditions sustain a boreal forest (see Chapter 3 for details). Principal biogeochemical sample media are the dominant black spruce (*Picea mariana*), with jack pine in drier areas and the understory shrubs mountain alder (*Alnus crispa*), river alder (*Alnus rugosa*), paper birch (*Betula papyrifera*) and Labrador tea (*Ledum groenlandicum*).

Rod Zone – Jolu Mine – La Ronge Domain

Exploration of this deposit in the 1980s was conducted under several names, mostly Mahogany Minerals Resources, Royex and Corona Corp.

Geology and mineralization

Located in a northeast-trending linear belt of greenstones of the La Ronge Domain (Central Metavolcanic Belt) in the Star Lake area 120 km north of the town of La Ronge, Au deposits are underlain by an Aphebian (1875 Ma) metasedimentary-volcanic assemblage intruded by the Hudsonian Star Lake Pluton. Metamorphism is lower amphibolite facies resulting in chlorite-biotite-hornblende schists, locally rich in tourmaline. Gold occurrences are located within a system of regional shear zones in the metavolcanic-sedimentary succession or with shear zones in the diorite-monzonite members of the Star Lake Pluton. Shears form mylonitic zones trending 060°. The surrounding metasediments have been fractured to form a system of intersecting fractures trending 060° and 035° into which quartz veins have been emplaced. Tourmalinization and chloritization of the wall rocks to the veins are well developed.

In 1985, the Rod Zone was a trenched occurrence of a quartz vein containing visible Au and molybdenite. Field observations indicated that the quartz veining was likely to follow the regional trend of the shears, extending north-eastward beneath glacial material covered by black spruce forest rooted in moist to wet boggy

ground. The challenge at the time for Geoff Burrill, working for International Mahogany Corp., was to discern where along this trend the quartz might be Au-bearing, without going to the expense and environmental disruption of extensive stripping of the ground and trenching of the bedrock. It had been ascertained that the glacial till comprising the overburden was variable in thickness up to a maximum of ~3 m.

Biogeochemical survey

Samples of outer bark scales (about 50 g) were scraped from black spruce at 20 sites spaced at 50 m intervals along the projected strike of the quartz vein. This took the field crew of three a little more than an hour to accomplish. Samples were dried, reduced to ash by controlled ignition at 475 °C and 0.5 g portions analysed by INAA. In the following account concentrations are shown in ash with dry weight (DW) equivalents in parentheses.

In the boreal forests of Canada background levels of Au in spruce bark are typically 5–10 ppb in ash (0.1–0.2 ppb Au DW). In sharp contrast, at the trench the bark ash yielded 230 ppb Au (3.7 ppb Au DW), and levels greater than 100 ppb Au (2 ppb Au DW) were recorded in bark from four additional sites reaching a maximum of 690 ppb Au (20 ppb Au DW). Alder twigs from selected sites contained 25 times usual background levels of Mo (maximum of 61 ppm Mo in ash [2.5 ppm Mo DW]) and more than 5 times background levels of Co (maximum of 28 ppm Co in ash [0.6 ppm Co DW]).

Post survey exploration

Subsequent drilling at these sites yielded Au in each drill hole (Table 11-I). In a letter from Geoff Burrill advising of the results summarized in this table, he also stated that another 'anomalous result [200 ppb Au in bark ash] that you obtained in the muskeg … also appears to correlate with a new zone which has been found … values over 1 oz [per tonne] were intersected in shallow drill holes very close to your sample location. Overburden is about 2.5 to 3 m'.

After a decline was excavated at the Rod Zone, Corona Corp. identified sufficient reserves to establish the underground Jolu Au mine that went into production in 1988. Reserves were exhausted by 1991 at which time the mill had processed 520,000 tons of ore and recovered 205,000 oz of Au at an average grade of 0.42 oz/t (14.4 g/t).

Jasper pond

Geology and mineralization

Located in the La Ronge Gold Belt, the Jasper North Showing was discovered in the summer of 1987 during prospecting by Rick Kusmirski of the Saskatchewan Mining and Development Corporation (later renamed Cameco Corp.). The area is

TABLE 11-I

Relationship between gold concentrations in the outer bark of black spruce (*Picea mariana*) and results of a subsequent drilling programme, La Ronge Gold Belt, Saskatchewan. Drill results courtesy of G. Burrill. Modified after Dunn (1986a) and Dunn et al. (1990)

Gold (ppb) in outer bark from black spruce in ash	Dry weight	Overburden thickness (m)	Drill results
230	3.7	1	Near main trench
120	2.4	1	Erratic mineralization: 1 m of 0.85 oz/t (29 ppm) Au at depth of 50 m
690	14	2.5	Subcropping mineralization: 0.11 oz/t (3.8 ppm) Au over 60 cm
450	11.3	1	Well mineralized shear zone: 0.3–0.7 oz/t (10-24 ppm) Au over 4 m
200	3.5	3	Mineralization, locally over 1 oz/t (35 ppm), at shallow depth

underlain by an intercalated series of intermediate to felsic tuffs and andesitic flows, and minor turbidites intruded by (a) gabbros (Fork Lake gabbro) and ultramafic rocks; followed by (b) emplacement of the felsic Island Lake pluton; then (c) shear-hosted mafic dykes and quartz veins. The rocks in the area have been metamorphosed to upper greenschist facies.

Mineralization is pyrite, minor marcasite, sphalerite, galena, chalcopyrite and visible gold. The gold occurs predominantly in silicate aggregates, but also occurs within the pyrite. Quartz flooding and mineralization were accompanied by hydrothermal alteration of the wall rock. A narrow sericite $\pm$ tourmaline halo encloses the mineralized zone.

Biogeochemical surveys

Prior to development of the showing, geochemical surveys were undertaken by Cameco that included sampling of alder twigs and Labrador tea stems. Their data showed that northward from the showing into a boggy area Labrador tea yielded up to 1000 ppb Au in ash ($\sim$20 ppb Au DW) and alder twigs contained up to 750 ppb Au in ash (12 ppb Au DW). The following year soon after commencement of a drilling programme, the Geological Survey of Canada conducted a more extensive biogeochemical sampling programme through the area, using the same sample media, and recorded a maximum of 779 ppb Au in ash of Labrador tea stems ($\sim$15 ppb Au DW) and 289 ppb Au in ash of alder twigs (5.6 ppb Au DW). Multi-element INAA of the

TABLE 11-II

Jasper Pond. Concentrations in ash of Labrador tea twigs from nine sites in a bog adjacent to the discovery outcrop of Au mineralization at Jasper Pond (Dunn et al., 1990)

	Range	Average	Background	Concentration factor (average:background)
Au (ppb)	38–389	129	20	6.5
As (ppm)	2–4	3	2	1.5
Co (ppm)	5–65	25	5	5
Cr (ppm)	12–37	25	10	2.5
Th (ppm)	1–3.6	1.8	0.2	9
U (ppm)	<0.1–3.3	1.4	<0.1	>15
W (ppm)	<1–22	7.3	<1	>7

ashed Labrador tea stems provided information on the relative concentrations of several elements – notably Au, As, Co, Cr, Th, U and W (Table 11-II).

Post survey development

During 1990 and 1991 when the mine was in production, the Jasper Mine produced 83,700 oz of Au from 140,000 tons of ore at an average grade of 0.55 oz/ton, including some very high grade sections.

Seabee Mine, Laonil Lake

Laonil Lake is located within the Glennie Domain 125 km northeast of the town of La Ronge. Gold mineralization was discovered in 1947 and drilled by Cominco in 1949, but exploration languished until 1985 when Placer Development Ltd. entered into a joint venture agreement with Claude Resources Inc.

Geology and mineralization

The area underlying the Seabee deposit consists of hornblende gabbro intruded into metasediments and metavolcanic rocks. Mineralization occurs in a series of 070°-trending narrow shear zones which parallel the main foliation in the gabbro. A conjugate set of shear zones trend at 140°–150°. Both sets of shear zones host the auriferous quartz-tourmaline that forms the deposit. The pyrite, pyrrhotite and associated Au mineralization appear to postdate deformation and may be associated with high-grade metamorphism. Ultramafic-layered lenses lie at the base of some of the gabbroic sheets that host the deposit.

The vein mineralization consists of fine-grained Au-bearing pyrite and chalcopyrite and minor visible Au. Other minerals present are sphalerite, pyrrhotite, minor arsenopyrite, pentlandite, tellurides, magnetite, barite and molybdenite in a matrix dominated by sericite and actinolite-tremolite.

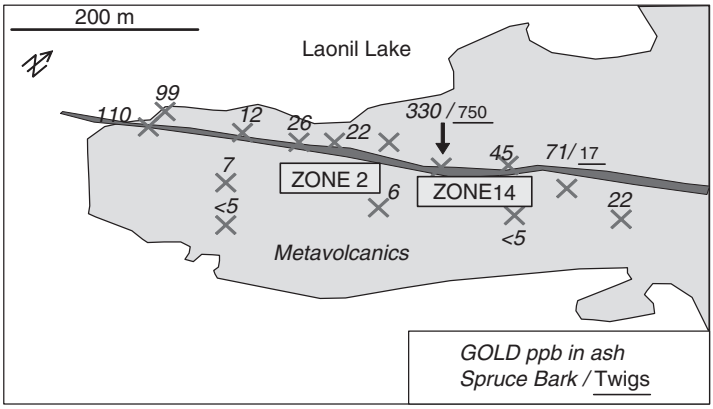

Fig. 11-1. Gold in ash of black spruce bark and twigs. Seabee Mine, northern Saskatchewan.

Biogeochemical survey

In 1986, an orientation survey was undertaken along a shear zone with the purpose of assessing any biogeochemical response. Thirty-eight samples were collected from 18 sites across and along the V2 shear zone, which forms a well-drained northwesterly facing ridge. The most common species in the area is black spruce. Outer bark scales were collected at all sample stations. Spruce twigs (most recent 10 years of growth) were obtained from the 12 sites where branches could be reached. In addition a few samples of jack pine outer bark and 3-year growth of alder and birch were collected.

Figure 11-1 shows strongly anomalous Au concentrations in both spruce bark and twigs (330 ppb and 750 ppb Au in ash [6.5 and 15 ppb Au DW]) at Zone 14, and elevated levels at several additional sites. Of note is that several elements concentrated in vegetation at other Au deposits in the La Ronge Belt are also concentrated at Seabee. Examples are, with dry weight equivalent in parentheses, 450 (9) ppm Mo in alder twigs, 66 (1.3) ppm Co in birch twigs, 3 (0.06) ppm W in spruce bark and a feature noted in Chapter 9 in the section on Nd is that high Nd values flank the Au anomaly.

The biogeochemical signatures indicated that near the mineralized veins in the Laonil Lake area there are enrichments of Au, Co, Mo and W with what appears to be some chemical differentiation of the REE giving rise to Nd enrichment flanking elevated Au concentrations. The brief study concluded that multi-element biogeochemical surveys in this area may assist in outlining mineralized zones, and could help in differentiating between auriferous and barren shear zones (Dunn, 1986c).

Post survey developments

Subsequent to this study, exploration programmes that included extensive drilling to outline resources resulted in the delineation of substantial Au reserves. Claude

Resources took over development of the site in the late 1980s and the Seabee mine that opened in 1991 is still in operation. Early in 2005 reported reserves were 730,000 tons grading 0.20 oz/t (6.9 g/t) and a resource of 406,200 tons grading 0.24 oz/t (8.16 g/t) Au.

Canada: Temperate Forest of British Columbia

QR (Quesnel River) Deposit

Geology and mineralization

Propylitic gold skarns are contained within a 300 m-wide hornfelsed aureole around a diorite stock. Gold occurs in and along the contact between a calcareous basalt and overlying siltstones and argillites. Gold occurs in five known zones in association with pyrite and pyrrhotite. There is minor chalcopyrite, sphalerite and galena. A detailed account of the geology and soil geochemistry of the area is given by Fox et al., 1987.

Environment

The QR deposit is located in central British Columbia 140 km southeast of Prince George, adjacent to the steep banks of the Quesnel River. Outcrop is mostly masked by glacial tills of variable thickness, and the extensive forest cover is dominated by interior Douglas-fir and lodgepole pine.

Scope of survey and analysis

In 1988, a helicopter-borne treetop-sampling programme was undertaken by the Geological Survey of Canada over the QR Au deposit. At the time of sampling there was little ground disturbance. Douglas-fir tops were collected during a 2-h period in late April, at intervals of approximately 200 m (100 m on one line) along 12 lines spaced 200 m apart. The length of line varied from 500 to 2300 m. In total 103 samples were collected, including a few from distant areas to determine background levels of elements. After drying and separation of needles from stems, the stems were reduced to ash and the ash analysed by INA.

Results

A substantial and intense Au anomaly in the treetops was outlined (Dunn and Scagel, 1989). Subsequently, the Main and West Zones were mined by Kinross Gold Corporation between 1994 and 1998. During that period the mine produced 118,000 oz of Au (approximately 30,000 ounces per year) from 1,060,000 tonnes of ore milled at an average grade of 4.1 g/tonne Au.

In 2005, Douglas-fir needles from the samples that were collected in 1988 were retrieved from archive storage. Dry needles were milled to a powder, 1 g portions

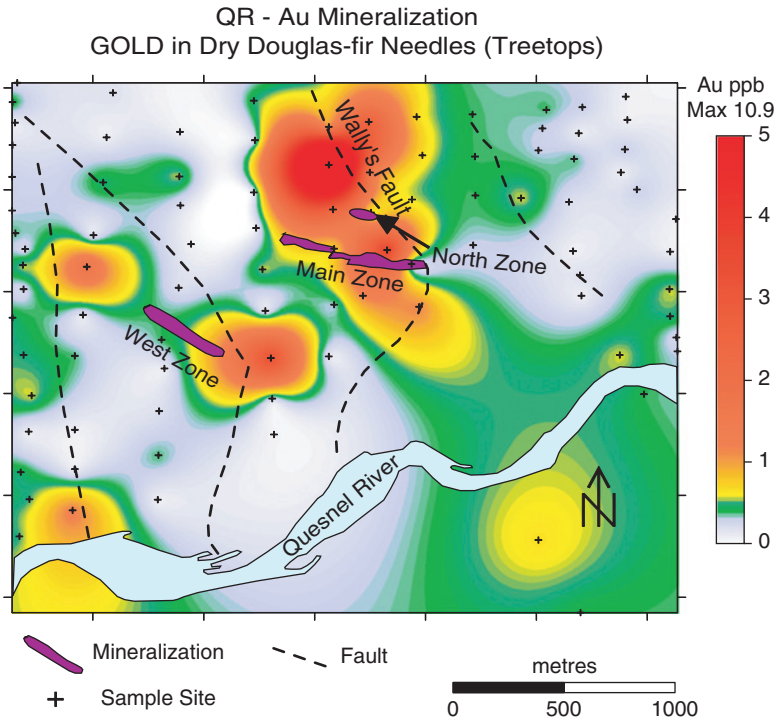

Fig. 11-2. Gold in dry Douglas-fir needles from QR Deposit, central British Columbia.

dissolved in nitric acid followed by aqua regia and analysed by ICP-MS (Dunn et al., 2006a). Results confirmed and refined the original anomalies identified from analysis of stems validating the robustness of the geochemical signature (Figure 11-2). In 2006, Cross Lake Minerals announced that they were restarting the mine after significant new reserves were discovered at the North Zone (possibly 200,000 oz Au). The North Zone is a broad area of propylitically altered basalt located below the Main Zone in the footwall of Wally's Fault. This is the largest zone of gold mineralization on the property with a drill indicated strike length of at least 1 km. Average grade for this deposit is estimated at approximately 6 g/tonne Au.

South America: Peru-Ecuador border

Cordillera del Cóndor

Geology and mineralization

Host rocks comprise

- Triassic to Jurassic marine clastic and carbonate rocks, continental red-beds and volcanic units of the Piuntza and Chapiza Formations.

- Felsic to intermediate intrusions (Jurassic).
- Sub-aerial volcanics of the Chinapintza Fm (Cretaceous and Tertiary) with coeval felsic to intermediate stocks. The lowermost strata along the Nangaritza River are Cretaceous shales with abundant fossil ammonites, overlain by karst limestone. Sandstones form flat-topped ridges and table-mountains.

Known styles of mineralization include the following:

- Porphyry Cu+/−Mo+/−Au associated with the Zamora batholith.
- Garnet-scapolite skarn.
- Epithermal carbonate, base metal-sulphide veins and breccias.
- Epithermal Au, Hg, As and Bi mineralization at Chinapintza.
- Low sulphide, low sulphidation epithermal veins north of Chinapintza.

Environment

The Cordillera del Cóndor forms the border between Ecuador and Peru and extends from 3° to 5° south of the equator. Located in the eastern foothills of the Andes at elevations from 800 to 2100 m, it comprises the upper reaches of the rain-forested Amazon basin and experiences wet, cool, tropical weather. Dense sub-tropical vegetation grows in the rugged terrain that is deeply incised by streams. Except for access from the Ecuadorian side of the border, roads are virtually non-existent.

Vegetation cover has been described as 'perhaps the richest flora of any similar-sized area anywhere in the New World' (Schulenberg and Awbrey, 1997) and it has one of the highest concentrations of vascular plant species of any place on earth.

Based on results obtained from orientation surveys in a similar environment, the selected sample media were foliage from the commonly occurring tree fern *Cyathea spp.* (local name 'Chonta con Espinas') and foliage of the evergreen liana *Clusia cf. hammeliana* (local name 'Churgun').

Scope of survey and analysis

At each of 366 sample stations, samples of fern fronds and liana leaves were collected at intervals of 500 m on an evenly spaced square grid covering approximately 100 km^2. Samples were oven-dried in Lima and shipped to Canada for milling. Coarse ribbed veins from both ferns and leaves were removed, and the leafy tissues milled to a fine powder. Analysis was by ICP-MS for 37 elements after a nitric acid/aqua regia digestion. Appropriate QC samples were inserted that included standards, duplicates of field samples, splits of prepared samples and analytical solutions. Data were statistically evaluated and kriged for plotting as gradational coloured contour maps. The 98th percentile was selected as the maximum value for plotting, so that outliers did not distort the data distribution patterns, and the structure of the bulk of the dataset could be observed.

With few exceptions, the ferns yielded higher concentrations of elements than the liana foliage. Table 11-III provides a comparison of the mean, median and maximum

TABLE 11-III

Western Amazon: Element concentrations in foliage of ferns and lianas at 366 sample stations within an area of approximately 100 km^2

	Fern foliage			Liana foliage			Concentration factors	
	Mean	Median	Max.	Mean	Median	Max.	Mean	Median
							Fern:Liana	Fern:Liana
Ag (ppb)	67	9.5	2748	3.1	2	88	21.6	4.8
Al (%)	0.592	0.19	3.3	0.008	0.005	0.77	74.0	38.0
As (ppm)	0.28	0.2	2.9	0.18	0.1	1	1.6	2.0
Au (ppb)	0.81	0.5	16.8	0.46	0.3	6.8	1.8	1.7
B (ppm)	17.4	17	56	18.5	17	55	0.9	1.0
Ba (ppm)	25	14	208	26	12	404	0.9	1.1
Bi (ppm)	0.011	0.01	0.12	0.01	0.01	0.03	1.1	1.0
Ca (%)	0.302	0.25	1.19	0.983	0.75	3.44	0.3	0.3
Cd (ppm)	0.16	0.07	2.53	0.18	0.06	4.18	0.9	1.2
Co (ppm)	0.21	0.14	1.69	0.08	0.03	1,84	2.8	4.5
Cr (ppm)	2.37	2.1	20.7	1.67	1.63	3.86	1.4	1.3
Cs (ppm)	2.013	0.99	20.07	0.161	0.105	2.807	12.5	9.4
Cu (ppm)	29.3	22	164	4.47	4.2	19.4	6.6	5.2
Fe (%)	0.012	0.008	0.07	0.004	0.003	0.21	3.0	2.7
Ga (ppm)	0.11	0.1	1.7	0.067	0.05	0.3	1.6	2.0
Hg (ppb)	108	76	3943	51	39	725	2.1	1.9
K (%)	1.702	1.48	4.01	0.888	0.82	2.72	1.9	1.8
La (ppm)	15	2	267	0.06	0.02	1.71	255.1	102.3
Mg (%)	0.379	0.335	1.015	0.316	0.284	0.82	1.2	1.2
Mn (ppm)	490	366	2973	1446	1132	8197	0.3	0.3
Mo (ppm)	0.22	0.06	12.3	0.16	0.06	4.23	1.4	1.0
Na (%)	0.003	0.002	0.014	0.001	0.001	0.011	3.0	2.0
Ni (ppm)	2.38	1.3	29.4	0.48	0.2	28.4	4.9	6.5
P (%)	0.171	0.141	0.604	0.062	0.059	0.158	2.8	2.4
Pb (ppm)	2.9	0.8	99.6	0.34	0.26	1.42	8.5	3.0
Rb (ppm)	89	80	244	23	20	272	3.9	4.1
S (%)	0.247	0.22	0.81	0.09	0.08	0.24	2.7	2.8
Sb (ppm)	0.018	0.01	0.21	0.013	0.01	0.05	1.4	1.0
Sc (ppm)	0.0327	0.2	5.2	0.11	0.1	0.6	3.0	2.0
Se (ppm)	0.156	0.1	1	0.096	0.1	0.3	1.6	1.0
Sn (ppm)	0.037	0.02	1.12	0.022	0.01	0.27	1.7	2.0
Sr (ppm)	26	18	146	81	61	484	0.3	0.3
Te (ppm)	0.01	0.01	0.02	0.01	0.01	0.04	1.0	1.0
Th (ppm)	0.007	0.005	0.16	0.005	0.005	0.08	1.4	1.0

Continued

TABLE 11-III *Continued*

	Fern foliage			Liana foliage			Concentration factors	
	Mean	Median	Max.	Mean	Median	Max.	Mean	Median
							Fern:Liana	Fern:Liana
Ti (ppm)	4.2	4	22	1.6	1	25	2.6	4.0
Tl (ppm)	0.047	0.01	0.78	0.012	0.01	0.25	3.9	1.0
U (ppm)	0.006	0.005	0.09	0.005	0.005	0.02	1.2	1.0
V (ppm)	1.15	1	4	1.1	1	4	1.1	1.0
W (ppm)	0.05	0.05	0.1	0.05	0.05	0.2	1.0	1.0
Zn (ppm)	43	39	241	17.8	16	79	2.4	2.5

concentrations obtained from each sample medium, and the relative enrichment expressed as the ratio of the average concentration in fern versus liana and also as the median value for fern versus liana. The only elements more highly concentrated in the liana leaves were Ca, Mn and Sr. With respect to median values, there was little or no difference between ferns and lianas for B, Ba, Bi, Cd, Mg, Mo, Sb, Se, Te, Th, Tl, U, V and W. However, for many of this latter group of elements, concentrations were mostly below detection.

The two species did not exhibit a linear relationship in element concentrations. Results indicated that for exploration purposes the fern was the preferred sample medium, because it had the demonstrated capability to absorb higher concentrations of most elements, especially precious metals and their pathfinder elements, and it was therefore the more sensitive plant with fewer barriers to element uptake.

Unusually high concentrations of several elements were present in the ferns. The following numbers are the maxima in dry tissue with the median in parentheses: Ag 2748 (10) ppb, Au 16.8 (0.5) ppb, Cu 164 (22) ppm, Hg 3943 (76) ppb, La 267 (2) ppm, Mo 12.3 (0.06) ppm, Ni 29.4 (1.3) ppm, Pb 100 (0.8) ppm, Rb 244 (80) ppm. From these numbers it is evident that there are unusual enrichments of Au and base metals within the survey area, with a very high background level of Cu. The high La concentrations are characteristic of REE enrichments commonly found in ferns.

Figure 5-4 (Chapter 5) shows plots of Au and As in the ferns from this survey. All elements were plotted in a similar manner, resulting in more than 70 element distribution plots for which the patterns needed to be summarized. In the lianas the patterns of a few elements were more informative than their corresponding elements in the ferns – notably Co, Cu, Ga, Mn, Mo, Pb, Sb, Sn and Tl. For example, Fig. 11-3 shows an area to the north that stands out as enriched in Pb and Sb. Relative enrichment of Zn, Cd and Se occurred, also, in the eastern part of this

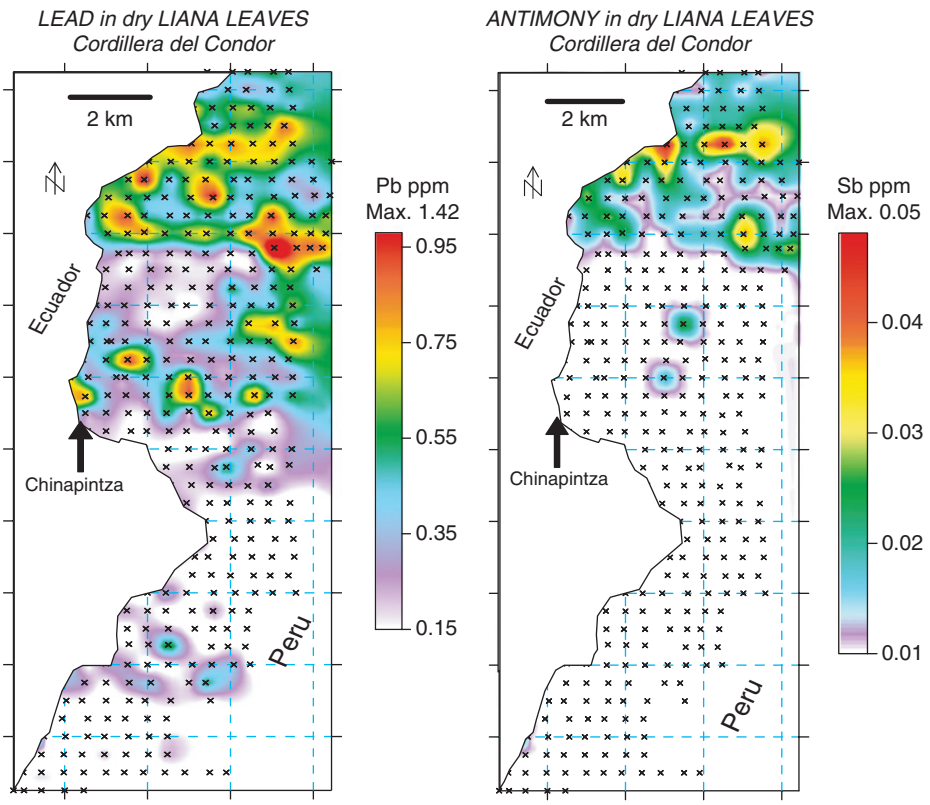

Fig. 11-3. Western Amazon – Pb and Sb in dry liana leaves ('Churgun').

area. In the ferns, patterns for these elements were less apparent. Consequently, although the ferns were the more sensitive to Au and its pathfinder elements (As, Hg, Bi and Ag) amongst others, the lianas were more suitable for defining the base metal signature.

A method to summarize patterns from a large number of plots is to take a percentile value (e.g., 95th) for each element and superimpose the limits of these values using lines with patterns each representing a different element. For example, Au > 95th percentile could be plotted as a polygon with a solid line, As with a dotted line, and Sb with a dashed line. However, when there are many elements that need to be superimposed, it is usually necessary to draw a polygon or an ellipse outlining the approximate limits of the anomalous suite of elements and the elements can be listed alongside the map in a legend. For the Cordillera del Cóndor survey area the situation is complex in that there are several known and anticipated styles of mineralization, each with a different suite of minerals and therefore different element signatures. For this survey, the dataset from the ferns was examined and areas of anomalous suites of elements

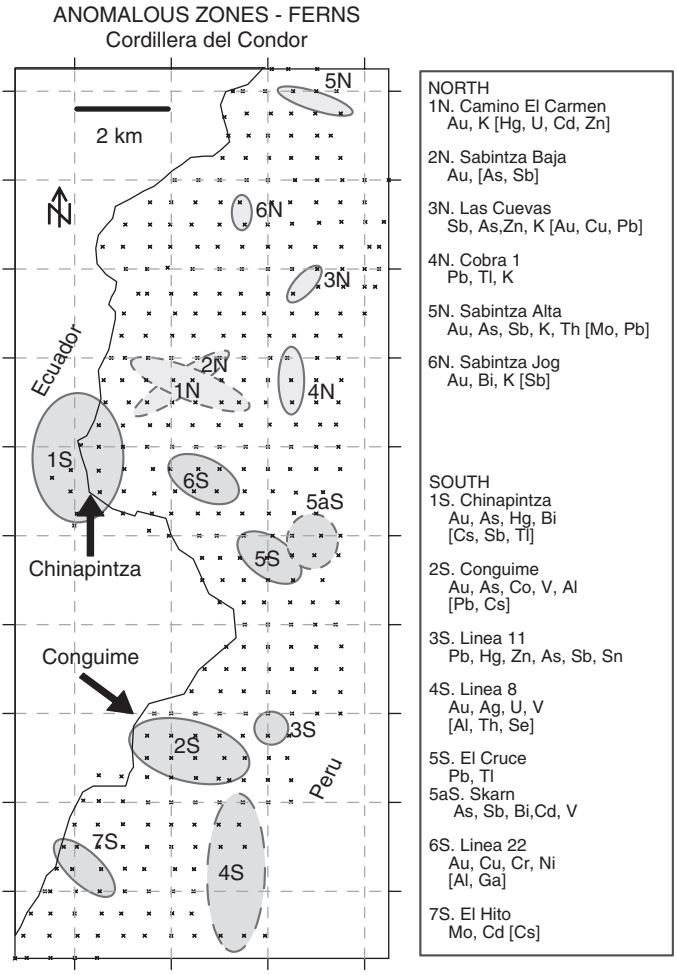

Fig. 11-4. Western Amazon – Zones of relative element enrichments in dry fern foliage ('Chonta').

plotted on a single summary map (Fig. 11-4). A similar map could be produced for the liana, showing the base metals noted above, and ultimately a single map can be produced that can rank the anomalies. In the present example only the fern anomalies are shown, and the two most significant are identified by arrows (Chinapintza and Conguime). The liana foliage also delineated these zones from most of the same elements, but yielded lower concentrations.

At this time much of the exploration in this general area is taking place on the Ecuadorian side of the border, notably northward from Chinapintza, westward from Conguime, and westward from El Hito (anomaly 7S).

Permission from AngloGold Ashanti to reproduce the essence of this study is gratefully acknowledged.

South America: Bolivia and Argentina

This section has been contributed by Manuel Viladevall[2] and Ignasi Queralt[3]. It should be noted that these are orientation surveys based upon only a few samples from each of several areas of significant metal enrichment that have been worked, in some cases, for centuries. As such it can be expected that an historic 'imprint' of the metals has been left in the soils and subsequently transferred into the vegetation. Therefore, the extraordinarily high concentrations of Au, Sb and Cs may be in part related to an anthropogenic signature. However, regardless of what that anthropogenic component may be, the plants under discussion indicate a phenomenal ability to accumulate and tolerate these metals and therefore represent excellent candidates as biogeochemical sampling media for mineral exploration throughout vast areas of South America.

Geology and mineralization

1. *Bolivia*: Saddle reef-type Sb-Au ore deposits in siliciclastic materials. Epithermal Au with Sb – (a) Cevada Mayu and (b) Chilcobija.
2. *Bolivia*: Placer Au (Khollpana) derived from conglomerates and epithermal Au deposits associated with acidic volcanic rocks.
3. *Argentina*: Epithermal Au deposits – Aguas Calientes caldera.

Environment

Andean highlands of Bolivia and Argentina (Fig. 11-5). Arid to semi-arid regions of the Altiplano ('Puna').

Biogeochemical surveys

'Thola' is the popular name for an intricately branched indigenous bush that grows at an altitude of between 3350 and 4250 m and covers an area of about 210,000 km^2 in Bolivia and Argentina. There are three genera that are included in the generic term 'Thola': *Baccharis spp.* (Compositae Family), *Parastrephia lepidophylla* (Asteraceae Family) and *Fabiana densa* (Solanaceae Family). Of these, the first is the more common and includes several species selected for the biogeochemical surveys. Many additional species of *Baccharis* are found at lower altitudes throughout the Americas. *Baccharis* has a network of shallow roots with deep roots penetrating 5 m or more allowing the plant to live in arid to semi-arid areas deficient in soil.

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Fig. 11-5. Locations of biogeochemical surveys.

From preliminary studies it was ascertained that leaves absorb higher levels of the elements of interest than the stems (Fig. 11-6), and that seasonal variations occur.

Subsequently, three orientation surveys for Au deposits using the Thola bush were carried out in the Andean highlands of Bolivia and Argentina using two species of *Baccharis* and the species *Fabiana densa*.

Samples were rinsed in running water to eliminate adhering dust particles, dried at 40 °C and leaves separated from stems. Tissues were milled and the dry leaves were analysed by INAA at Activation Laboratories Ltd. (Ontario, Canada) for Au, As, Ag, Ba, Br, Ca, Co, Cr, Cs, Fe, Hf, Hg, Ir, K, Mo, Na, Rb, Sb, Sc, Se, Sn, Sr, Ta, Th, U, W, Zn, La, Ce, Nd, Sm, Tb, Yb and Eu.

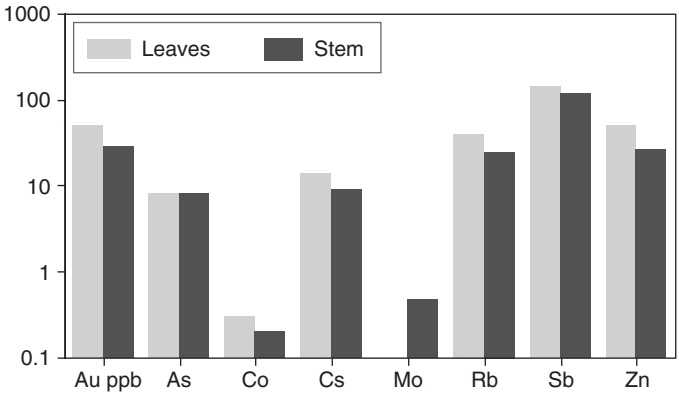

Fig. 11-6. Comparison of element concentrations in dry leaves and stems of the Thola bush *Baccharis incarum* at the Sb-Au Antofagasta mine near Oruro, Bolivia (Viladevall, 1994; Marti, 1996).

A. Saddle reef Au-Sb deposits. In the southern part of Bolivia, 37 Thola samples (*Baccharis incarum*) were collected along traverses in the proximity of the old Cevada Mayu mine located at Sora Sora (55 km SW of Oruro), and from near the Chilcobija mine in the Tupiza district (Fig. 11-5). Limited sampling at Cevada Mayu showed that strong enrichments of Sb and Au in *Baccharis incarum* are present and that the highest concentrations appear to be located on the anticlinal limbs of the Ordovician Llallagua Formation (Fig. 11-7).

In the vicinity of the Chilcobija mine concentrations in dry Thola foliage ranged from 49.2 to 101 ppb Au and from 330 to 540 ppm Sb. Although samples were rinsed prior to analysis, it is suspected that these exceptionally high values are partially due to contamination from the large Sb processing plant nearby. Airborne particulates can precipitate on the ground, become dissolved by groundwater and then absorbed through the root systems. Of note is the ability of Tholas to concentrate these extreme amounts without exhibiting signs of significant stress.

It was found that both species of Thola that were sampled (*Baccharis incarum* and *B. leptophylla*) yielded identical profiles for most elements indicating that their elemental uptake is such that these species could be mixed without compromising the integrity of a survey. The Sb content of soils ranged from 3440 to 4400 ppm, yielding a plant-soil coefficient of approx 0.13 (Fig. 11-8).

B. The Khollpana Formation Placer Au Deposit (Caracollo). The placer Au deposits associated with the Khollpana Formation are located in the Caracollo and Soledad areas of western Bolivia (Fig. 11-5). These deposits have been mined since pre-Colombian times. The Khollpana Formation comprises Tertiary and Quaternary conglomerates composed of quartzite and sandstone pebbles derived from Ordovician to Devonian rocks. The matrix is Tertiary acidic volcanic tuffs and sandstone pebbles cemented by iron oxides.

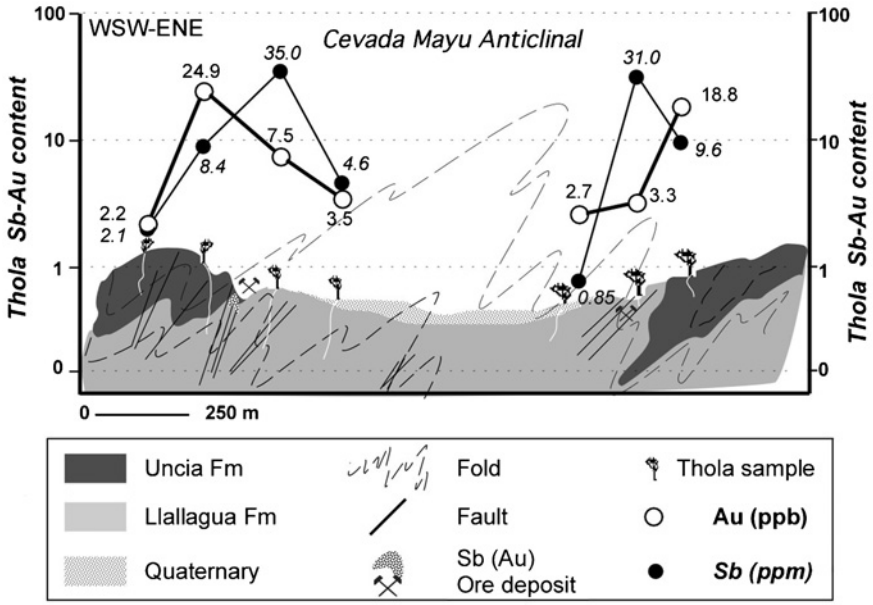

Fig. 11-7. Cevada Mayu Sb-Au deposits. Profiles of Au and Sb in dry leaves of *Baccharis incarum*.

Of the different Tholas (*Baccharis incarum, Baccharis leptophylla, Parastrephia leptophylla* and *Fabiana densa*) growing on the Kollpana Formation, the most common is *Fabiana densa*. In light of the similarities in response shown by two species of Thola (Fig. 11-8), the orientation sampling was performed by compositing *Baccharis incarum* and *Fabiana densa* at each sample station. Gold in these composite samples ranged from 5.9 to 90.5 ppb. Although data from this orientation survey look promising, in order to fine tune the responses of the individual Thola species to mineralization additional work needs to be undertaken to establish the relative uptake of each species to a wide range of elements.

C. Epithermal gold deposits of Bolivia and northern Argentina. The response of Tholas to epithermal Au mineralization was tested in areas of acidic volcanic and sub-volcanic rocks.

In Bolivia, *Baccharis incarum* grows on Neogene lavas and tuffs in the Korikoya, Tarikoya and Nueva Esperanza areas in the La Joya Au district (near Caracollo, approximately 200 km southeast of La Paz). Ten samples contained high levels of Au (5–54 ppb) and the pathfinder elements As (0.1–11 ppm) and Sb (0.8–29 ppm).

Other Thola tests were carried out at the 'Aguas Calientes' caldera in the San Antonio de los Cobres district in the Salta district in the Argentinean highlands (Fig. 11-5). In that area a volcanic caldera contains mesothermal Pb-Ag ore (Poma Mine) and an epithermal Sb-Au vein ore system exploited as the Incachule mine

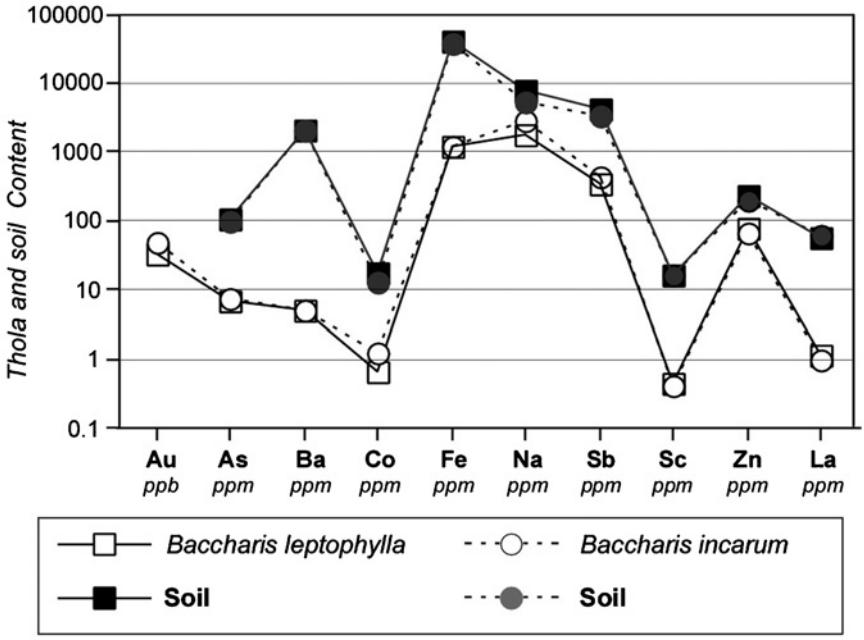

Fig. 11-8. Chilcobija Mine: plot of Plant:Soil coefficients (PSC). All values in ppm except Au (ppb).

(Fig. 11-9). These veins are situated in the chlorite – sericite alteration zone with an extension of 15 km^2 into surrounding ignimbrites.

The most common Thola bush in this area is *Baccharis incarum*. Forty-one composite samples were taken from over the epithermal and mesothermal ore deposits. Samples yielded anomalous Cs–Sb values in the argillic and silicified zones, and anomalous Pb–Zn values in the chlorite-sericite zone. Of particular note are Cs concentrations up to 1500 ppm in samples from a silicified zone and up to several hundred ppm Cs in samples from over chlorite-sericite schists. From discussion with the analyst there is no possibility that these concentrations could be either analytical artefacts (analysis by INAA) or from laboratory contamination. Even though the underlying soils had several hundred ppm Cs thereby accounting for elevated levels in the plant tissues, it is apparent that *Baccharis incarum* has a strong propensity to accumulate Cs.

Summary of results from the orientation surveys

- Gold and Sb contents are lower in stems than in leaves of *Baccharis incarum*.
- There is a marked increase in the Au content of leaves in spring (October).
- In spring, Tholas exhibit an increase in As and Sb whenever concentrations of these elements are high. Where concentrations of As and Sb are low an increase occurs in autumn (June).

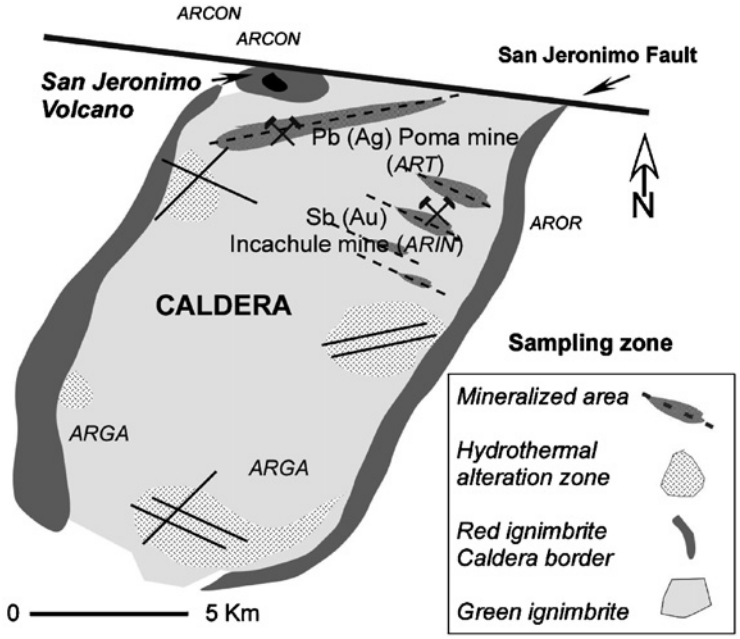

Fig. 11-9. 'Aguas Calientes Caldera' in San Antonio de los Cobres (Salta, Argentina).

- Other elements (e.g., Zn, Mo, Cs and Br) yield higher contents in autumn than in spring.
- High Cs, Sb and As anomalies occur in the argillic and silicified zones of epithermal volcanic areas.
- For the different varieties of 'Thola', *Fabiana densa* can accumulate more Au, As, Ba, Co, Fe, Hf, Sb, Sc, W and U than *Baccharis incarum*. Conversely, *Baccharis incarum* contains more Br, Ca, Mo, Na, Sr and Zn than *Fabiana densa*.

In all three survey areas Thola leaves proved to be a useful medium for delineating anomalous zones of Au, Sb, As, Pb and Zn in the Andean highlands.

The authors acknowledge, with thanks, the support of the University of San Andres (La Paz, Bolivia); the University of Salta (Argentina); and the European Union International Cooperation for Contracts CT1*-CT90-0784 and CE93-0021.

PLATINUM GROUP METALS/NICKEL/COPPER

Canada: Rottenstone Lake, Northern Saskatchewan

Geology and mineralization

Harzburgite-orthopyroxenite sill with unknown stratigraphic continuity (Hulbert et al., 1988). Surrounding rocks are primarily highly metamorphosed (granulite facies) metasediments and metavolcanics.

The dominant mineralization is contained within magmatic Ni-Cu-PGE (Au) net-textured massive sulphide, and includes the following:

- Violarite ($FeNi_2S_4$) and pentlandite $(Fe,Ni)_9S_8$.
- Chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4) and pyrrhotite ($Fe_{1-x}S$).
- Sphalerite (ZnS).
- PGEs: contained mostly in sperrylite ($PtAs_2$), moncheite ($PtTe_2$) and kotulskite (PdTe).
- Many additional mineral phases, including those with Au, Ag, Bi, Se and Te.

Environment

The survey area experiences a cold continental climate that supports boreal forest (see Chapter 3), dominated by black spruce, with minor jack pine. Understory shrubs are mostly alder and Labrador tea.

The area is remote, located 130 km NNE from the town of La Ronge and 55 km from the nearest all-weather road. It is only accessible by float-plane or by ground during the winter over frozen ground, bogs and lakes. Consequently, except for some minor exploration activities during a 30-year period after mining operations ceased in 1968, exploration activities were mostly on a small scale until Uravan Minerals Inc. commenced a heightened level of exploration in 1999. The remoteness of the area has permitted natural vegetation to regenerate, and it now comprises an excellent biogeochemical field laboratory for examining the PGE and multi-element content of a comprehensive range of common boreal forest plant species. Whereas controlled laboratory studies are useful for solving some biogeochemical problems, they cannot fully emulate the natural environment in which the plants are subjected to the rigours of nature. At Rottenstone winter temperatures of $-40\,^{\circ}C$ are not uncommon, and in the summer $+35\,^{\circ}C$ may occasionally be reached.

Exploration history

The Rottenstone deposit (formerly the Hall deposit) was discovered in 1928 and mined between 1965 and 1968. It produced approximately 40,000 tons of high-grade ore yielding 3.28% Ni, 1.83% Cu and 9.63 g/t PGE. The extremely high Ni-Cu-PGE grades in association with high-contained sulphides (40–60%) in a small ultramafic body indicate that the Rottenstone deposit is an extension of a much larger ultramafic intrusive body hosting a high-grade Ni-Cu-PGE-Au deposit in the area or at depth.

Prior to mining activities the deposit outcropped as a gossanous rust-coloured hill. Although small (approximately 50 m × 50 m × 10 m), it was richer in PGEs than any other Ni-Cu deposit in Canada. During the early stages of mining in 1965, the deposit yielded 4.79 ppm Pt and 3.92 ppm Pd. Subsequent analyses of scattered boulders have yielded up to 38 ppm Pt, 13 ppm Pd, 0.27 ppm Rh, 0.18 ppm Ir, 0.2 ppm Os, 0.14 ppm Ru with 4 ppm Au (Hulbert and Slimmon, 2000).

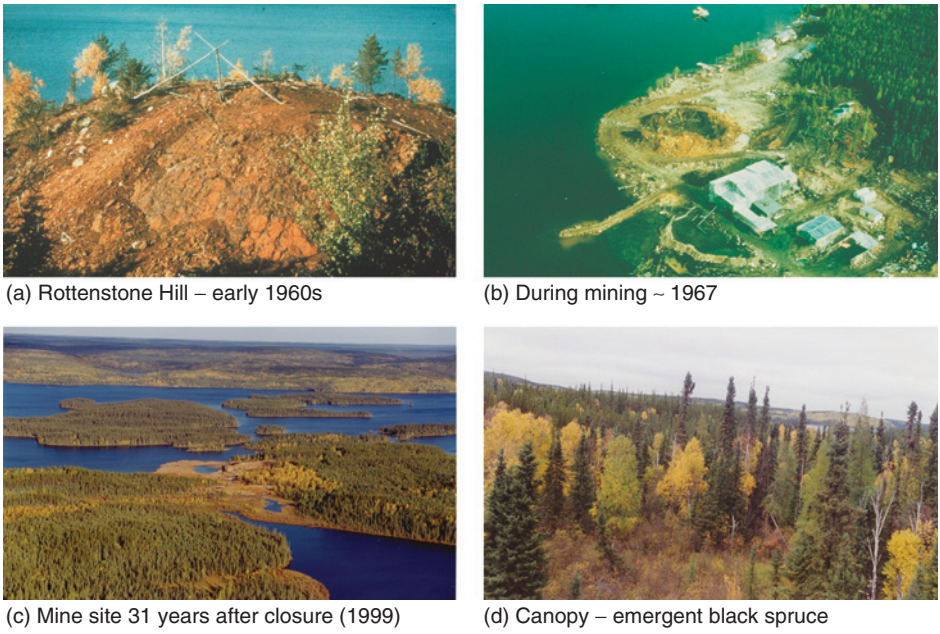

Fig. 11-10. Rottenstone Lake. Photos (a) and (b) provided by the late Dr. J.J. Brummer – (a) discovery outcrop of PGE-Ni-Cu-Au-rich ultramafic rock prior to mining operations; photo taken in early 1960s; (b) the scene during mining operations; (c) the view 30 years later; (d) the forest canopy showing black spruce as the primary emergent species.

Figure 11-10 shows (a) the original orange gossanous hill of friable oxidized rock prior to mining operations; (b) a view of the partially mined hill taken about 1967; (c) a view of the site taken over 30 years later, with its setting among heavily forested terrain and abundant lakes; and (d) a view of the forest canopy showing black spruce crowns that provided the sample medium collected by helicopter in the autumn of 1999.

Biogeochemical surveys

The first small-scale multi-species and multi-tissue vegetation sampling programme at Rottenstone took place in the early summer of 1983. In the ash of black spruce twigs maximum concentrations (with dry-weight equivalent in parentheses) around the old mine site were 1350 (42) ppb Pd, 880 (28) ppb Pt and 240 (7.6) ppb Au (Dunn, 1983a). Subsequent visits over the following 5 years permitted the collection of tissues from all common tree and shrub species growing over and adjacent to the mine tailings, and also from more distant locations to provide an estimate of background concentrations of metals in the vegetation (Dunn, 1986b; Coker et al., 1991). Table 4-VII (Chapter 4) shows elemental concentrations in various tissues collected in the mid-1980s. The data show that twigs and outer bark of black spruce accumulate PGE more than the other tissues that were examined. Labrador tea is also

sensitive to the presence of most of these elements. Since the first collection in 1983 several more visits have confirmed these values and in the summer of 2006 newly collected samples added further weight to the long-term stability of the biogeochemical signature, yielding concentrations in the most recent 10 years growth of black spruce twigs that were very similar to those obtained 23 years earlier.

On a regional scale the terrain is difficult to cover without air support, because of the abundance of lakes, streams and bogs, and moderately dense forest. Small-scale detailed biogeochemical surveys over three geophysical conductors, primarily using outer bark of black spruce, were conducted by Uravan Minerals in 1999 and yielded anomalous concentrations of PGE, Au and Ni. Maximum concentrations in ash were 46 ppb Pd, 107 ppb Pt, 52 ppb Au and 218 ppm Ni. These anomalous levels were substantiated by analysis of twigs from the same trees.

The same year, armed with the knowledge that black spruce is an effective sample medium, black spruce tops of 1000 trees within an area of 130 km^2 were collected from a helicopter. The primary grid involved 794 trees, mostly at 500 m spacing on offset lines with infill sampling at 250 m over and around the old mine site. Figures 11-11 and 5-6 (Chapter 5) show the sample coverage that was achieved during a period of approximately 5 days and, once procedures had been established, at a collection rate of more than 30 samples per hour of flying.

After samples had been dried the tissues were separated and twigs were reduced to ash by controlled ignition at 475 °C. Ashing was necessary in order to concentrate the PGEs and other ultra-trace elements to detectable levels. A bulk sample of vegetation was reduced to ash for use as a control on the analytical precision. Determinations were made by ICP-MS for 61 elements after an aqua regia digestion, resulting in more 60,000 data bits.

The predominant strike of the bedrock in the area is northeast–southwest, and the direction of glacial transport was primarily from the northeast. Consequently, it was anticipated that biogeochemical patterns might exhibit similar trends. However, the tree top data revealed strong *northwest* trends of many elements (i.e., normal to the anticipated direction) suggesting a structural and lithological control to the element distributions. Control samples and sequence of analyses were carefully scrutinized to ensure that these trends were not analytical artefacts.

Figure 11-11 shows concentrations of Ni and Pd as kriged plots, with the maximum value set at the 98th percentile in order that undue weight is not given to outlier values, and also that the structure of the bulk of the data can be seen. Therefore, outlier values greater than the 98th percentile are all shown as the same intensity of colour.

The plots show that Ni is concentrated around the mine site (up to 1730 ppm Ni) and toward the southeast, with an additional zone in the north near Albert Lake where 705 ppm Ni in ash was recorded. The Pd data indicate a 4 km by 6 km elliptical ring of anomalous concentrations with highest values on the south western side of the annulus, centred upon the Rottenstone mine site. Plots of Pt, Te, Au, Fe, Th and Eu are coincident with this pattern around Rottenstone, and with slightly elevated levels of Pt, Te and Au near the zone of Ni enrichment at Albert Lake. Plots of Be, Li and

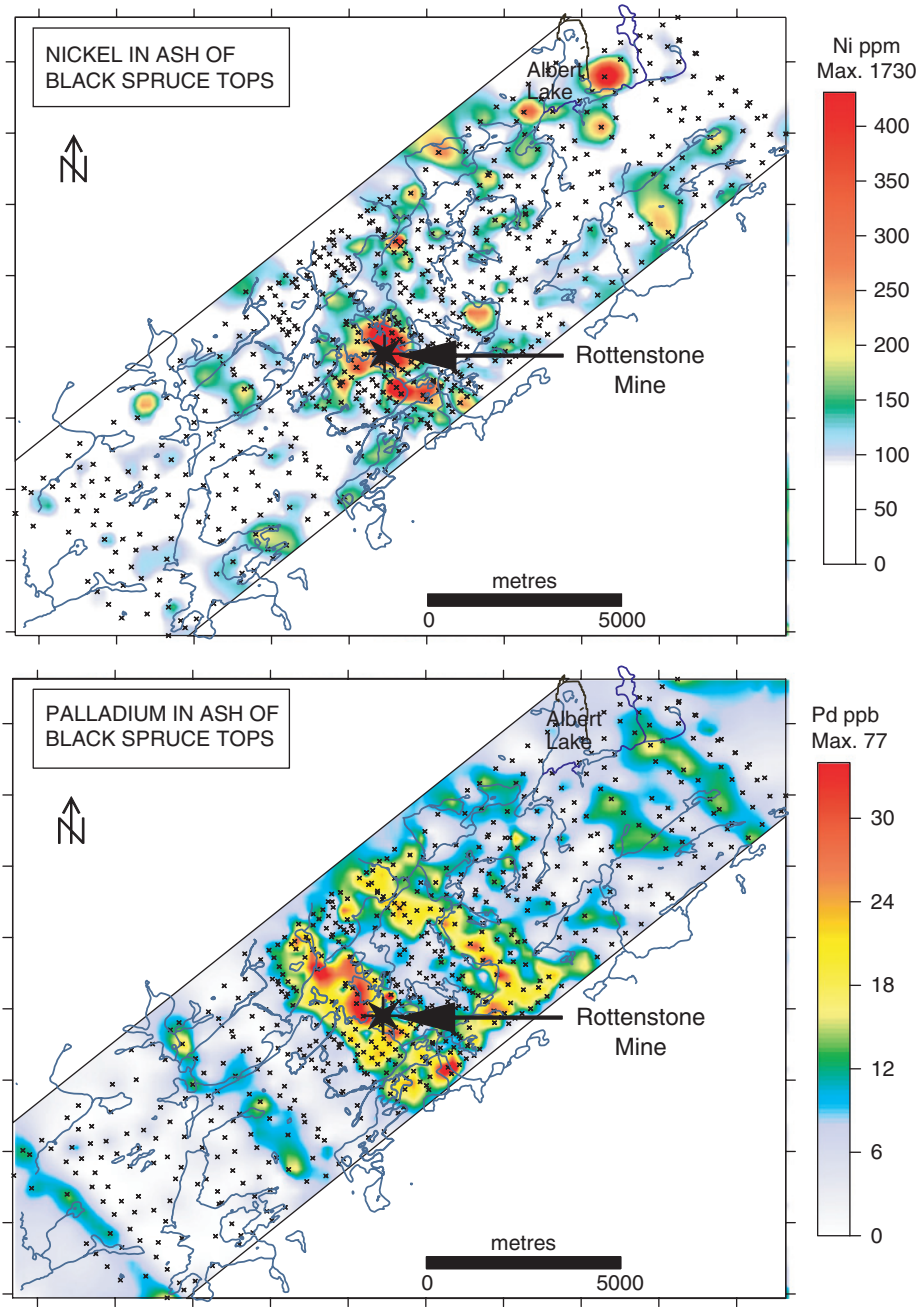

Fig. 11-11. Rottenstone, Saskatchewan. Patterns of Ni and Pd distribution in twigs from the tops of black spruce trees collected from a helicopter. Concentrations in ash determined by ICP-MS on an aqua regia digestion. Data released with permission of Uravan Minerals Inc.

Bi indicate sub-parallel zones, displaced to the northeast, which might define either shear zones or structure. Additional elements indicate metal zoning (e.g., Re peripheral to Pd, as shown in section on Re in Chapter 9).

Post survey exploration

In a late 2004 company report by Uravan Minerals, it was stated that up to 2002 reconnaissance drilling of a number of targets that were based on a combination of geological, geophysical and biogeochemical results had encountered ultramafic apophyses, some of which were mineralized. Shallow drilling of a coincident biogeochemical and gravity anomaly remains unresolved, although elevated Ni, Au and Cr concentrations in pelitic rocks suggest an ultramafic intrusive nearby. Petrophysical determinations of the net-textured ore indicate that it will not provide a good conductive EM geophysical response but a good IP response should be expected. A drill programme in 2003 intersected numerous 1–4-m-thick sulphide-bearing ultramafic dykes(?) or apophyses yielding high levels (maxima in parentheses) of Ni (1%), Pt + Pd (1341 ppb) and Cr (7300 ppm). It was considered that the results were highly supportive for a larger intrusive body nearby or at depth.

Summary notes

In this terrain of difficult access the spruce tops were acquired in less than a week for a cost of about two-thirds of that required to conduct a regional lake or stream sediment sampling programme. The multi-element data provide substantiation of the concept that significant undiscovered Ni and PGE mineralization may be present in the Rottenstone area. The biogeochemical anomalies are under investigation as exploration continues.

Uravan Minerals Inc. is gratefully acknowledged for permission to release these data.

NICKEL

Canada: Thompson Nickel Belt, Northern Manitoba

Geology and mineralization

In the survey area Precambrian metasediments of the Ospwagan and Burntwood Groups host Ni-bearing ultramafic intrusions within barren Archaean gneiss. Mineralization is dominated by massive sulphides that are locally Ni-bearing. These are expressed as geophysical conductors.

Environment

Boreal forest – black spruce with minor jack pine and tamarack. The understory is dominated by shrub alder and Labrador tea. The area is flat with low rolling hills interspersed with many lakes, streams and bogs (Fig. 11-12). There is extensive forest

Fig. 11-12. Typical mixture of open bog, lakes, rivers and forest covering the survey area. Water bodies frozen at the time of sample collection.

cover with open areas of bogs and patches of trees burnt by forest fires. There are no all-weather roads into the area; access is by winter trail over frozen ground or by float-plane in the summer.

Biogeochemical survey

During a 5-day period toward the end of winter in April, 2003, prior to complete spring thaw, a helicopter was used to undertake a tree top sampling program involving 670 black spruce tops. Targets were geophysical conductors representing 17 separate zones within an area of 600 km^2. Total helicopter time was 20 h, including all ferrying and a reconnaissance flight, generating an overall sampling rate of almost 35 samples per hour. The all-inclusive average sample collection (i.e., sample collection and flight to the next sample site) time was 1 min 48 s, although during the course of the main part of the survey this was reduced to 40 s. The sample design involved flights normal to the strike of the conductors. Typically, there were two flights over each conductor with sample spacing at 250 m, closing to 100 m at a few sites over the conductors (Fig. 11-13). In a few areas with more complex groupings of conductors addition lines were flown.

A practical divergence from the planned flight line was set at 30 m in order to obtain a suitable sample. This tolerance was required because of lack of coverage of black spruce and/or appropriate canopy structure (i.e., uniform height to the stand canopy which would preclude safe collection of a sample). Averaged across all

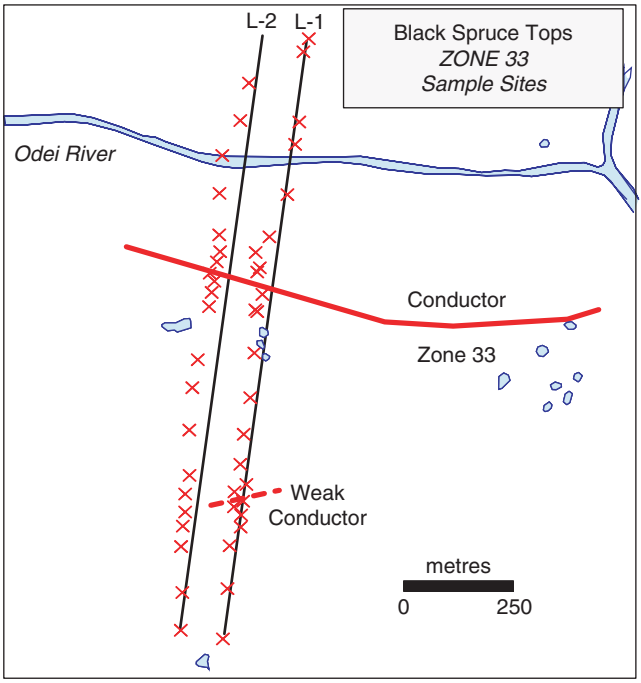

Fig. 11-13. Typical design of flight lines and spacing of samples over geophysical conductor.

samples, the departure from planned flight line was about 6 m. Each sample consisted of about 30 cm from the top of each tree.

While in the air there was no time for the two-person sampling crew to undertake reduction of sample material to the size and consistency required for analysis. This step was undertaken immediately after each flight (Fig. 11-14). The material needed to be trimmed down to a consistent composition with respect to age of growth and, while still fresh, notes made on the appearance of the sample (e.g., signs of chlorosis) and the number of years of growth collected. A greater volume of each sample was collected than was likely to be needed for analysis in order that there was plenty of material for any additional or repeat analyses that may be required.

Samples were oven-dried at 70 °C for 24 h, and the latest 3 years growth of twig was separated for milling and analysis of dry tissue by ICP-MS for 53 elements (after a nitric acid/aqua regia digestion). This consistency in years of growth was critical to the meaningful interpretation of the analytical data. Tests of twig chemistry of three years of growth compared to samples of random age (mostly a greater number of years of growth) demonstrated that for some elements concentrations were similar (e.g., Cd, Cu, Ni), but for other elements there were moderate to substantial differences. For this suite of samples, three years proved to be a practical amount to process. Whereas five years of growth would have produced some different concentrations, the

Fig. 11-14. Sample preparation in the field – samples spread out for examination, determination of age of growth and culling to the appropriate size.

patterns of element distribution would have been the same. Consistency of sample medium is of paramount importance.

Over all, element concentrations were not unusual except for Ni values that were considerably higher than typical background levels for conifer tops. Elsewhere in the boreal forests of Canada median concentrations of Ni in spruce tops are usually between 0.5 and 1.5 ppm Ni. In the survey area the median concentration was 6.2 ppm Ni, reaching a maximum of 24 ppm Ni over some conductors.

Examination of element profiles with respect to each flight line and known geophysical conductors indicated the presence of several multi-element anomalies. Some, mostly the commodity metals and their pathfinder elements, were confined to the immediate vicinity of the conductors, whereas other element assemblages indicated possible faulting and/or lithological changes. Figure 11-15 provides an example that compares element profiles along two parallel flight lines that passed over a conductor, such as shown in the example comprising Fig. 11-13.

Over conductors yielding positive responses in Ni, zones of enrichment varied from single point anomalies directly over conductors, to dispersed zones of enrichment several hundred metres wide. Of relevance to the exploration programme was that the five zones with strongest Ni signatures followed a northeast trend, indicating probable stratigraphic control to the strata that represent the most prospective zones for Ni exploration.

Over other conductors, trees yielded no positive response in Ni, but were slightly enriched in Zn, Pb, Cd and S suggesting that the conductors represented enrichments of these elements in the substrate. Additional zones contained insignificant element

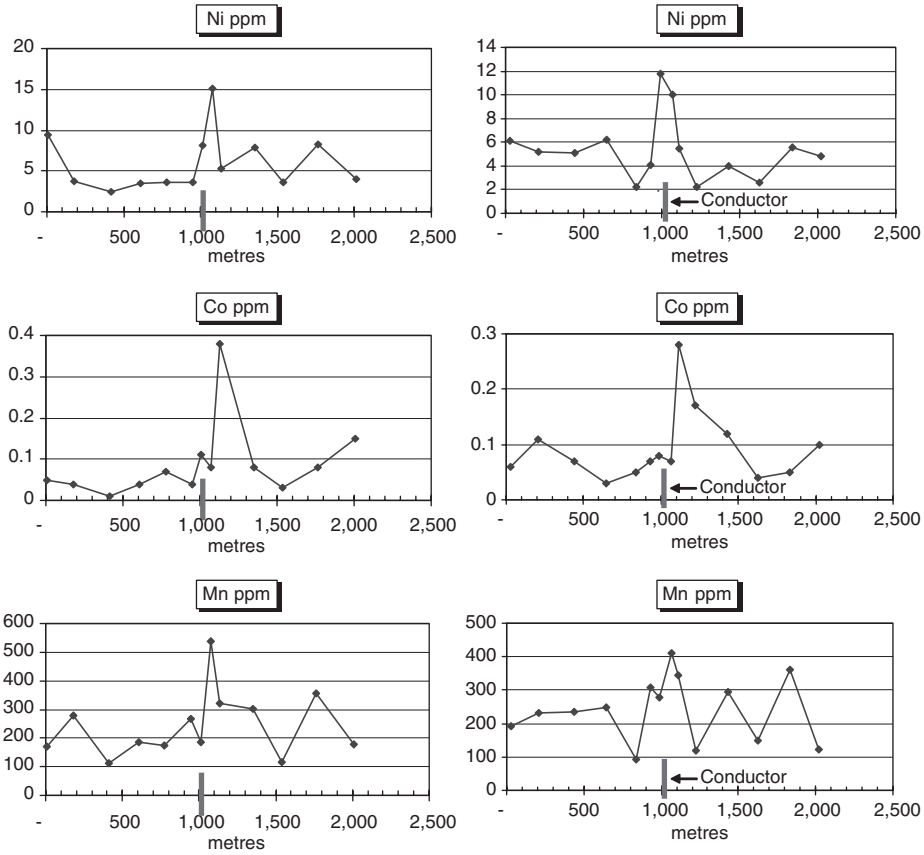

Fig. 11-15. Profiles of Ni, Co and Mn in dry spruce twigs along two parallel lines (100 m apart) over a known conductor.

enrichments, especially of base metals, except for local Fe and S which were interpreted to reflect the presence of barren sulphides (pyrite +/−pyrrhotite). Barium and Sr enrichments were considered to indicate the presence of stratigraphic zones with elevated carbonate contents. A summary of the 17 zones upon which this survey focussed is given as Table 11-IV. Four of the target zones (5, 9, 13 and 16) exhibited positive indications of Ni enrichment over conductors. Several additional zones yielded less definitive signatures. Typically, elements associated with the Ni were Co, Mn, S, Hg and locally Mg.

The data indicated that the treetop sampling may be of use in differentiating barren from base metal-rich sulphides, and the primary metal content of the latter. Used in conjunction with geophysical and geological information, the technique was considered to have the potential for screening conductors and optimizing the chances of defining which geophysical targets might host Ni-bearing mineralization.

TABLE 11-IV

Tabular summary of the element responses in black spruce twigs from each of the 17 conductive zones

Zone	Elements over conductor		Interpretation
	Enrichment	Depletion	
1			No indications of mineralization other than possible trace of ZnS.
2	Traces of Au, Ag, Bi, Cd, Co, Fe, Hg, Mn, Ni, Pb and REE over conductor	Fe, REE, Nb, Ni REE: Ba, Sr, depleted in SW	Possible minor precious/base metal zone coincident with conductor in SW.
3	Fe, Mn, Hg, Ni, and Co		Conductors may delineate Fe-rich zone (stratigraphic feature) and have some sulphide mineralization associated.
4	S, Sr, Ba, Cs	Pb, Cd	Probably barren sulphide with carbonate adjacent to S side of conductor.
5	Ni, (Co), Mn, REE	Ba, Sr, Rb, Cs	Ni-rich zone, associated with carbonate depletion.
6	Co (As, Mo, Cr, Se, Sn)		Barren sulphides – only traces of Ni. Probable change in stratigraphy at ~1000–1100 m.
7	Hg, (Cd)	P, Cs, Rb	No Ni associated with conductor. Possible stratigraphic change S of conductor.
8	Hg either side of low	P, Cs, Rb, Mo, Fe, Cu, Co, S, Hg	
9	Ni, Co, Mn, Fe, S, Pb, Hg, Ca, Rb, REE	Mo, Ag, Sr (P)	Ni-rich zone and /or faulting.
10	Ni, Co, Mn, Pb (Li – Ba zone)	Hg, Mo, S, Au	Ni/Co peak over conductor in east – less prominent moving west.

Continued

TABLE 11-IV *Continued*

Zone	Elements over conductor		Interpretation
	Enrichment	Depletion	
11	Ba, Sr, Mo, Cr	Cs, Rb	No discrete Ni anomaly. Probable carbonate at conductor.
12	Pb, Zn, Ag, Sr, (Ba), (S)	Mn	Possibly Pb/Zn with carbonates. No Nickel.
13	Ni, Co, Mn, Hg, Rb, Li, Mg, (Fe, REE, P, Pb, & Au, Ag, Cd, Cu)	Ba, Sr, Cs, As	Good Ni, Co, Mn signatures. Fe-rich, carbonate poor.
14	S, Zn, (Mn)	Co, Au, Cd, REE, Li, Mo	No nickel. Conductor may reflect carbonates with Fe sulphide and minor Zn/Pb.
15			No nickel. Possible stratigraphic change south of ~1600 m (more carbonate?) and some Pb and Zn.
16	Ni, Co, Hg	Mo, Pb, Ag, Mn, Cr, Cs, Cu	Both conductors indicate Ni-enrichment – especially that to S. Ni-rich zone between conductors. Different lithology (probably carbonate) north of conductors.
17			Little of significance. No obvious mineralization.
18	Ni, Co, Mn with some Ag, Zn, Cd		Low Ni, but positive signatures over conductor suggest some enrichment. Probably stratigraphic change at ~1200–1400 m along lines (Fe-rich rocks to south).

Post survey exploration

Subsequent drilling of some of these anomalous Ni zones has resulted in the intersection of several intervals with elevated Ni levels. At this time details are confidential.

Anglo American Exploration (Canada) Ltd. is gratefully acknowledged for permission to release the information contained within this summary.

URANIUM

Canada: Athabasca Basin, Northern Saskatchewan

Geology and mineralization

World-class U deposits occur at the unconformity between Helikian (Precambrian) clastic deposits of the Athabasca Group (Athabasca Sandstone) and underlying crystalline basement rocks (Aphebian). Outcrop of Athabasca Sandstone is extremely sparse because of the extensive blanket of sandy glacial tills that are commonly about 1 m thick. Peat bogs (muskeg) and lakes fill depressions, and soils are thin podzol.

Locally, the mineralization is complex, but the primary minerals are

- Uraninite (UO_2) as massive pods, veins and disseminated aggregates.
- The massive variety of uraninite, pitchblende (UO_2), fills extensional features in reactivated fault zones and locally replaces the clay-mineral matrix of the Athabasca Sandstone.
- Associated elements include all or some of Ni (notably gersdorffite – NiAsS), Co, Cu, As, B and REE.

Exploration history

In 1935, two small pitchblende-bearing veins were discovered in northernmost Saskatchewan, near Uranium City. The first recognition of U associated with the Athabasca Sandstone was by Kermeen (1956), but it was not until the mid-1960s that a major exploration programme commenced in the Athabasca Basin. Initially, exploration took place in the west of the Athabasca Basin in and around a large circular structure (the Carswell Dome) considered to be a meteorite impact crater. In 1968, Gulf Minerals Ltd. discovered the Rabbit Lake U deposit at the eastern edge of the Athabasca Basin prompting extensive staking. The 1970s witnessed an intensive period of mineral exploration resulting in the discovery of several more U deposits near the eastern margin of the Athabasca Basin. A more detailed account of the exploration activity is given by Beck (1977) and Schiller (1978).

By the end of the 1970s many exploration techniques for U had been tested with varying levels of success, and in 1979 the Geological Survey of Saskatchewan and the Geological Survey of Canada initiated a joint study to assess the relative effectiveness of all known U exploration methods by concentrating studies on an area encompassing 1450 km^2 on the eastern side of the Athabasca Basin, to be designated the 'Athabasca Test Area' (Fig. 11-16). Cameron et al. (1983) wrote that

> The discoveries of the Midwest and McClean Lake deposits … were not 'easy' discoveries, but required, in equal measure, prescience, skill, determination and money, plus a

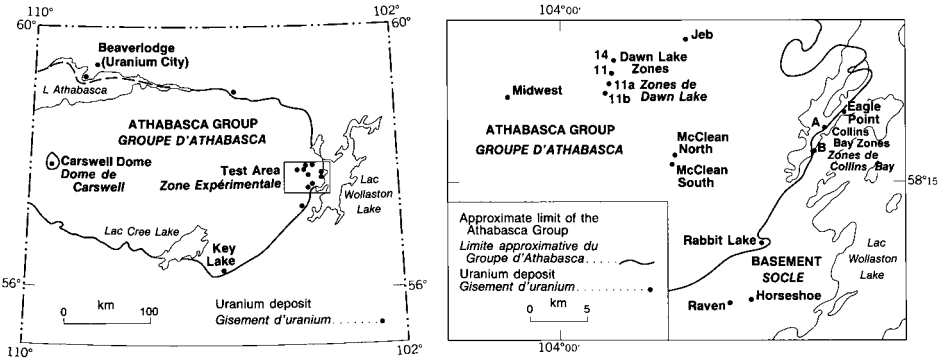

Fig. 11-16. Location map: Uranium deposits in northern Saskatchewan (as known in 1982) and limit of Athabasca Basin Test Area (Cameron et al., 1983).

> little bit of luck. The technical difficulty of discovering these deep deposits was stimulus for the program of comparative studies of exploration methods …

Included in the gamut of techniques were some orientation surveys to test the sensitivity of biogeochemical exploration methods to the presence of U mineralization in the Athabasca environment, because Walker (1979) had reported positive indications from over the Key Lake U deposit, located 150 km to the southwest.

Environment

The continental climate of the Athabasca Basin experiences a temperature range of −50 + 35 °C, with 40–60 cm of precipitation that occurs mainly during summer. Winds are generally light (8 kph) and dominantly from the west. These conditions sustain a boreal forest in a region of discontinuous permafrost. Dominant tree species are black spruce and jack pine, with an understory of mainly shrub alder and Labrador tea.

Biogeochemical surveys

The primary area for testing the biogeochemical methodology was in the vicinity of the McClean Lake mineralization (Fig. 11-16) that had been discovered only a few weeks prior to the first field investigations. The discovery occurred at the end of the 1978/1979 joint partnership between Canadian Occidental Petroleum Ltd. and Inco Metals Inc. when the last hole of the winter drilling programme intersected remarkably high-grade mineralization (up to 27% U_3O_8) at the basal unconformity beneath 150 m of unmineralized sandstone (Dunn, 1981, 1983b,c).

At the time that the biogeochemical surveys commenced in the late spring of 1979 an open pit comprising the Rabbit Lake U mine, located 10 km southeast from McClean Lake (i.e., downwind), was the first and only operating mine within a radius

of several hundred kilometres. Except for a rough road that reached Rabbit Lake from the south, there were no roads and access was by floatplane. Consequently, the environment was pristine except for some local disturbance by drilling operations.

The first surveys found that samples of black spruce twigs contained unusually high concentrations of U at McClean Lake and elevated levels at Midwest Lake. Background concentrations of U in plant ash are typically less than 1 ppm U, whereas these first samples locally yielded over 100 ppm U. These results provided the impetus for a more thorough investigation of other species and of a broader area during subsequent visits over the following three summers. These studies sought to establish variations in U content that might be attributed to a wide array of variables, including the following.

- Local variation in U content by sampling all 10 black spruce trees growing within an area of 200 km^2. This is a typical tree density for this area.
- The effect of tree height; tree health (as noted from any visible differences, such as chlorosis of the needles); live versus dead twigs; any variation in sampling procedures (two people collected samples).
- Variations around individual trees (e.g., twigs from the north side of the trees versus those from the south side; twigs from chest height versus those near the top).
- Seasonal variations in U content by re-sampling the same individuals in the spring and summer.
- Annual variations in U content by re-sampling the same individuals over three years.
- Differences in age of twig growth (by dissecting twigs into their respective last 10 years of growth).
- Differences in U content of trees from boggy areas compared to dry ground.
- Comparisons of U content of twigs with the U content of the underlying soils (see Table 1-V in Chapter 1)
- Comparisons of analytical determinations on dry tissue versus those on ash.

Whereas some variability in the data was observed for many of these parameters, over all it was evident that the patterns were robust and reproducible, attesting to the validity of the method to delineate U distribution patterns that provided focus for exploration activities. The fundamental tenets of maintaining consistency in sampling methods and not mixing plant species were the underlying protocols that were of paramount importance. Details of these studies are summarized in Dunn (1983b,c).

Among the results obtained were data from a comprehensive set of samples that permitted an evaluation of the relative sensitivity of each of the common species and tissues from a small area in the vicinity of the McClean Lake deposits. By determining the relative concentrations of U in each sample medium it was confirmed that black spruce twigs represented the sample medium yielding the highest concentrations of U (Table 9-VIII).

By subdividing 10 years growth of spruce twig tissues according to their age it was determined that highest U concentrations occurred in the 2–6-year-old growth. However, in that cold continental climatic regime there are only small annual

increments of growth. For efficient field operations it is impractical for a biogeochemical survey to isolate small growth increments and so 10 years of growth, a length of approximately 25 cm in the Athabasca region, was obtained at each sample station thereby providing an integrated U signature over that time period. Throughout the remainder of the 3-year biogeochemical survey all spruce twig samples were of consistent age (10 years) and diameter. The consistency in diameter was important because, as shown earlier (Fig. 3-5) U is concentrated mostly in the bark, hence for meaningful data interpretation it is necessary to maintain a consistent twig wood to twig bark ratio.

The optimal analytical method at the time was neutron activation/delayed neutron counting of 1 g samples of ash. This required the collection of approximately 100 g of fresh twig, since the moisture content was about 50% and the loss on ignition was 98% of the dry twig. Therefore, values reported here are concentrations in ash. Dry-weight equivalent concentrations can be approximated by dividing the ash values by 50 (i.e., 2% ash yield). Details of other analytical procedures involving multi-species determinations by ICP-ES and INAA are given in Dunn (1981). For this case history only the U data are presented, because for most samples in the subsequent regional survey U was the only element determined.

Detailed surveys

At McClean Lake black spruce twig samples were collected at 30 m intervals (with a few at 15 m) normal to the strike of the ore bodies. The criterion for the 30 m intervals was simply one of practicality, since survey pickets for the drilling programme were set at these intervals. A more conventional spacing of 25 m would have been equally appropriate. At a distance of 1 km on either side of known mineralization the sampling interval was increased to 60 m. Most traverse lines were 60 m apart, with a few at 30 m.

Figure 11-17 shows contoured concentrations of U in the ash of the spruce twigs in relation to known zones of U mineralization, established by later drilling, at a depth of up to 150 m beneath barren sandstone. The McClean North U deposit (between McClean and Candy Lakes) has biogeochemical anomalies which follow the strike of the mineralization, but they are displaced to both sides with relatively strong anomalies on the north-western flanks of the ore zones, giving rise to a cross section profile of classic 'Rabbit's Ears' type of geochemical anomaly (i.e., enrichments on either side of a zone of mineralization). A second sub-parallel zone of mineralization representing the McClean Lake South zone has less intense anomalies in spruce twigs. An additional zone of strong biogeochemical anomalies occurred at the south-eastern end of the detailed survey area, south of Bena Lake (see following section on 'Subsequent Discoveries').

In the vicinity of the McClean North deposit normal background concentrations of U are present in surface groundwater, soil and peat. Formation waters exhibit an upward hydraulic gradient and some artesian flow from drill holes has been reported. These waters locally contain several tens ppb U near mineralization (Dyck, 1983). Relatively permeable zones (flow rates of 10^{-3}–10^{-4} cm/sec) in the Athabasca

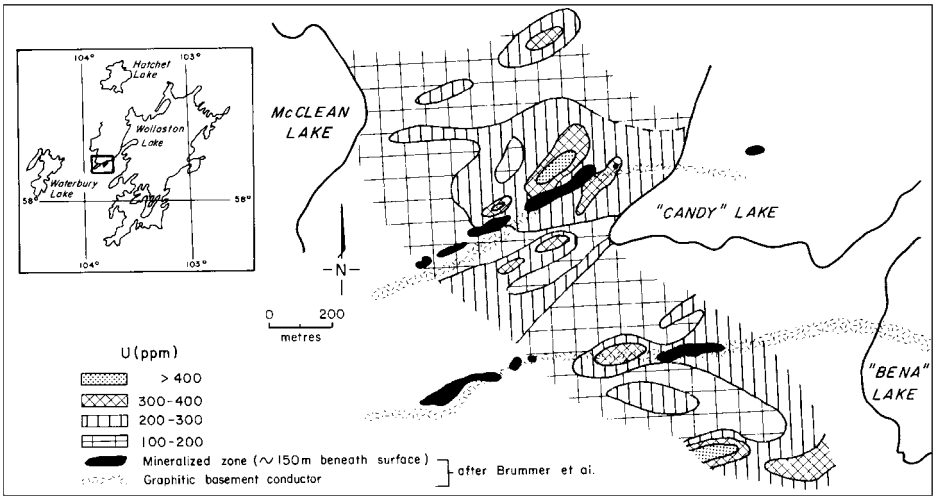

Fig. 11-17. McClean Lake – contoured concentrations of U in spruce twig ash in relation to known zones of U mineralization.

Sandstone give horizontal continuity to water flow, and pathways for upward flow are provided by an abundance of near vertical fractures. Within the Athabasca Sandstone ample evidence of upward movement of fluids is provided by the presence of secondary alteration and oxidation products that locally reach the surface (up to 200 m above the uranium-rich unconformity), and enrichment of trace elements within the alteration zones and along steeply dipping faults and fractures (Sopuck et al., 1983).

Possible explanations for the 'Rabbit's ears' include (a) the ore bodies have created a natural galvanic cell similar to those discussed by others (e.g., Govett et al., 1976; Smee, 1983); and/or (b) there is a 'shadow-zone' of silicified breccia above the ore in which there are relatively few steeply inclined fractures. In the latter situation any upward movement of formation waters containing dissolved U would pass around the silicified zone and be tapped by the plant roots from trees on either side of the vertical extension of the ore (Fig. 11-18).

Soon after first the biogeochemical survey was carried out at McClean Lake in 1979, samples were collected also from the vicinity of the Midwest Lake deposits, located 14 km northwest from McClean Lake (Fig. 11-16). At this location U mineralization is present beneath 200 m of Athabasca Sandstone with secondary U oxidation products close to the surface, above the ore zone. Spruce twigs were sampled from 69 sites at mostly 150 m spacing (i.e., only one-fifth of the sample density at McClean Lake) and concentrations ranged from 20 to 136 ppm U in ash, with highest values occurring above the southern part of the mineral deposit. Over all, it was considered that the data from this area were not conclusive, and that a more detailed survey would be required to establish a clear relationship to U mineralization. However, the situation was complicated in that most of the Midwest mineralization is located beneath a lake.

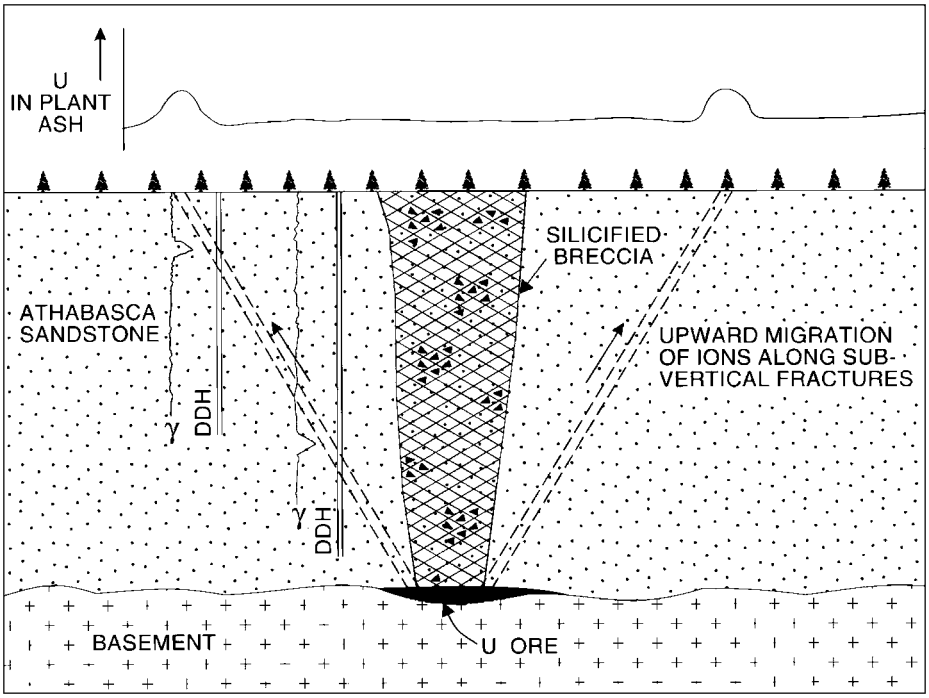

Fig. 11-18. Conceptual model of U migration from ore beneath the Athabasca Sandstone to the surface, giving rise to biogeochemical anomalies.

Adjacent to the Rabbit Lake open pit operation a maximum of 2270 ppm U was recorded in spruce twig ash. Nearby sites locally yielded in excess of 1000 ppm U.

Regional survey: Eastern part of the Athabasca Basin

The recognition of U enrichment over zones of mineralization prompted increasingly wider investigations in an attempt to delineate the size of the anomalous zone, since during the first two years of surveys it was apparent that all trees that were sampled contained considerably more U than the usual background concentrations of <1 ppm U in ash. Samples were collected at increasing intervals along the road leading southward from Rabbit Lake. Spacing was at intervals of 2 km southward from Rabbit Lake, then increasing to 5 km for about 50 km and finally at intervals of 10 km for a further 150 km (Dunn, 1981). Sample sites to the north were accessed by boat and to the west, southwest and northwest by float plane. This succeeded in establishing that the 'Wollaston Uranium Biogeochemical Anomaly', defined as the 10 ppm U-in-ash contour, extends over an area of approximately 10,000 km^2 (Fig. 11-19). Within this immense area the 50 ppm U contour encompasses 3000 km^2 and within a central core of 1000 km^2 almost all spruce trees sampled (more than 1000) contained in excess of 100 ppm U in the ash of their twigs.

Exhaustive checks on analytical precision, accuracy, sample preparation, analytical procedures, and resampling of trees confirmed the validity of the data. Although the Rabbit Lake open-pit U mine was in operation at the time, no other U mine existed within 300 km of Wollaston Lake. However, the possibility of airborne contamination was investigated with the following results.

- Environmental baseline studies conducted in the Wollaston area at around that time demonstrated that the air was very pure and had lower than normal levels of radioactivity. For example, the gross beta level near the McClean deposits was 0.22 mCi/km/30 days during the autumn of 1979, several months after first recognition of U enrichment in the vegetation. This level was less than half of that measured two years earlier over the Prairie Provinces.
- Gross alpha levels of dust-fall at McClean lake were 0.018 mCi/km/30 days, representing about one-twentieth of the alpha levels that had recently been recorded in northern Ontario.
- Thirteen air-monitoring stations around Rabbit Lake repeatedly reported U levels to be less than 6% of the maximum permissible concentrations set by the

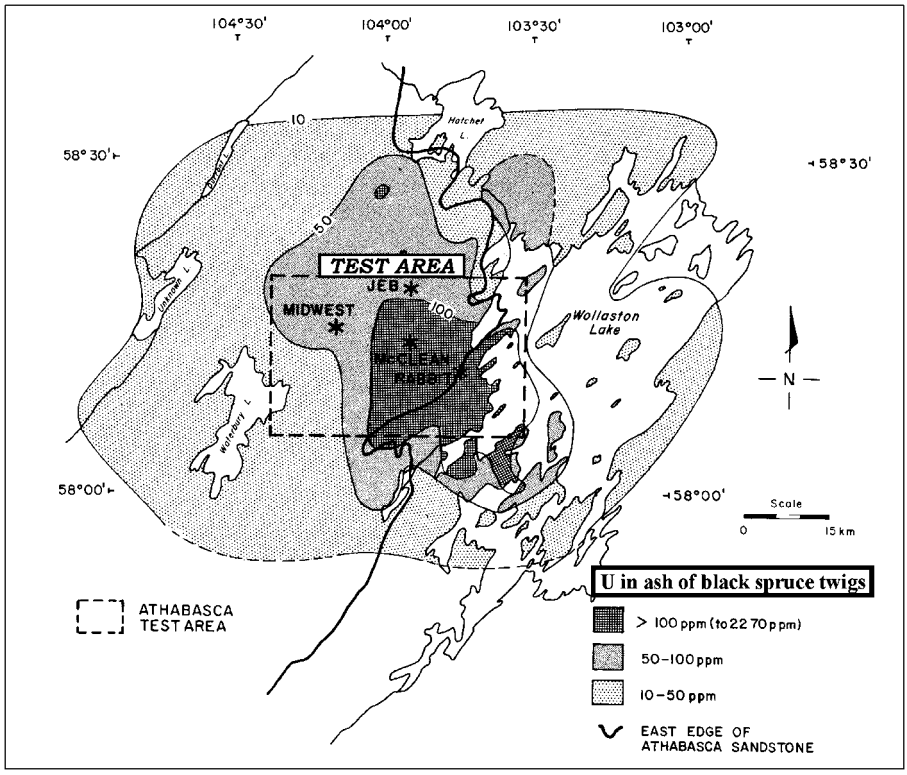

Fig. 11-19. 'Wollaston Biogeochemical Uranium Anomaly', with U deposits referred to in the text and outline of Athabasca Test Area.

International Commission on Radiological Protection (i.e., $<6\%$ of $6 \times 10/\mu Ci/cc$ of insoluble U). Furthermore, McClean Lake is upwind from Rabbit Lake.

The 'Wollaston Uranium Biogeochemical Anomaly' remains the world's largest known U biogeochemical anomaly. Discussions with Alexander Kovalevsky in 1984 indicated that the only other comparable biogeochemical anomaly of this size and intensity was one of Mo from an undisclosed location in the former Soviet Union. Subsequently, a Mo biogeochemical anomaly of similar magnitude has been defined around the Endako Mo mine in British Columbia (Dunn, 1999).

Subsequent discoveries

Within the large, zoned Wollaston anomaly there are several areas where intense local U in twig anomalies were delineated. Some of these local anomalies were sampled in sufficient detail to determine their relationships to locations of U deposits discovered since the 1979–1981 surveys. Others were too regional in their spacing to provide more than a general 'signature' to buried mineralization. The following observations are pertinent:

- The JEB deposit (announced in May, 1982) yielded 'Rabbit's Ears' anomalies toward the eastern extent of concealed mineralization (Dunn, 1983c). Maximum concentration was 264 ppm U in ash. The grade announced at the time was 9.72% U_3O_8 over 7 m at a depth of 90 m for a tested strike length of 190 m.
- Over the Dawn Lake deposits, spruce twigs collected by the Saskatchewan Mining and Development Corporation in 1983 yielded maximum concentrations of 80 ppm U.
- A linear trend of U enrichment, locally over 400 ppm U in ash, was located 200 m west of the Sue deposits that were discovered in 1988. The Sue A, B, C, CQ and D deposits lie on a 2 km long north-trending segment of graphitic gneisses at the west contact with the Collins Bay dome.
- Intense U anomalies occur east and north of the Sue deposits. The strongest of these is 1360 ppm U (Dunn, 1983c) recorded from a site at the north end of Tut Lake, located 2 km northeast of the Sue and 5 km northeast of the McClean Lake deposits. To date the source of these anomalies has not been identified. Re-sampling of several of the same trees in 1990 confirmed that U enrichments persist.
- South of Bena Lake (Fig. 11-17) in 2005 Denison Mines Ltd. reported 0.53% U_3O_8 over 5 m, and in October, 2006, their website indicated that 'work during 2006 is focused on evaluating the recently discovered Bena Lake alteration system'. It remains to be seen if the zones of anomalous U enrichments in the spruce twigs shown in Fig. 11-17 could be related to the new discovery.
- Since the biogeochemical sampling programme was completed in 1981, significant new discoveries (>1 million pounds U_3O_8 and up to 70 million pounds) within the area outlined by the 100 ppm U in ash contour (Fig. 11-19) include Collins Bay A-zone, Collins Bay B-zone, Collins Bay D-zone, Eagle Point, Raven-Horseshoe, Sue A, B, C, D and E and West Bear. Rabbit Lake and the Dawn Lake and McClean deposits had already been discovered, but only Rabbit Lake was being

mined. Within the 50 ppm U-in-ash contour, where mineralization is mostly at greater depth, the Midwest Lake deposits were already known; subsequently, JEB was discovered and mined, and recently high-grade mineralization has been reported at La Rocque (up to 31.9% U_3O_8 over 7 m reported) and Bell Lakes. Drilling at an additional zone 3 km north of the main Midwest mineralization known as the Mae Zone has recently intersected 31.3 m of an average weighted grade of 3.21% eU308 from 191.8 to 223.1 m.

- The world-class Cigar Lake and McArthur River deposits are located to the south of the Wollaston Biogeochemical Anomaly. Any biogeochemical data that might have been acquired from over these deposits is not in the public record. However, both deposits occur at depths greater than 400 m and, unless there is considerable upward flow of groundwater from the basement unconformity (where the deposits are located) to the surface, it is considered unlikely that a significant biogeochemical response occurs. From the evidence accumulated to date, it appears that a cover of 200 m of Athabasca Sandstone is about the maximum through which a biogeochemical signature from basement-related U deposits can be detected.

Summary notes

These U biogeochemistry case histories recount the results of survey programs completed more than 25 years ago. Extraordinarily high concentrations of U in vegetation were encountered, and the world's largest U biogeochemical anomaly was delineated. During the intervening years there was a lull in the exploration activities because of the depressed prices for U. Over the past 2–3 years the pace of U exploration has picked up considerably and old results can start to be put in context of new activities and discoveries.

It can now be demonstrated that the Wollaston Uranium Biogeochemical Anomaly encompasses an area containing many significant zones of mineralization with more than 20 deposits outlined that each contain well in excess of one million pounds U_3O_8 and up to 70 million pounds. However, the widely spaced sampling of the regional survey is inadequate to define zones of mineralization. Semi-detailed traverses (200 m spacing) over large parts of the Athabasca Test Area are sufficient to define trends of U enrichment, but it requires detailed surveys, such as those at 30 m pacing over the McClean deposits, to establish patterns that might be useful for establishing drill programmes.

KIMBERLITES

Canada: Ekati Trend, Lac de Gras, Northwest Territories

Geology

Eocene kimberlite diatremes (including crater and hypabyssal facies) emplaced in metamorphosed Archaean sediments, granites and diorites. Minimal dilution of the kimberlites by country rock xenoliths.

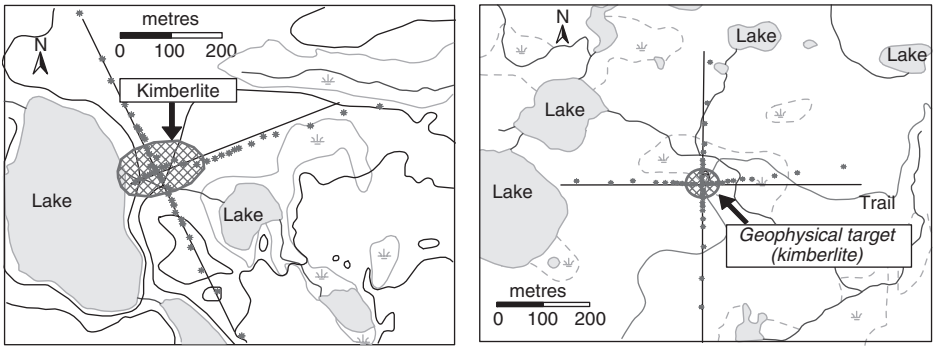

Fig. 11-20. Sample locations with respect to surface features and the Impala and Bighorn kimberlites – Ekati Trend, NWT, Canada.

Environment

Arctic tundra – dwarf birch (*Betula nana* and *B. glandulosa*), Labrador tea (*Ledum groenlandicum* and *L. palustre*). Typically associated with very stunted black spruce and willow (*Salix spp.*), and an understory dominated by crowberry (*Empetrum nigrum*), bearberry (*Arctostaphylos alpina*) and blueberry (*Vaccinium spp.*).

Biogeochemical survey

Combined samples of twigs and leaves from dwarf birch were collected at 96 sample stations that traversed the Impala and Bighorn kimberlites. Figure 11-20 shows the location of the orientation sampling programme with respect to geographic features.

After oven drying at 70 °C, leaves were separated from twigs and the leaf tissue was milled to a fine powder. Control samples were inserted and 1 g portions of each sample were digested in nitric acid then aqua regia, and analysis was by ICP-MS for 66 elements.

A. Impala (Fig. 11-20a)

Figure 11-21 shows profiles of several elements with respect to the location of the Impala kimberlite. The left column shows the north–south traverse, and on the right are profiles along the west–east traverse, which was limited to the west by the presence of a lake.

These profiles for selected elements show that relative enrichment with respect to the location of the kimberlite occurred either over the kimberlite or at its margins. The boggy area in the east has given rise to increased uptake of Mn and Co with

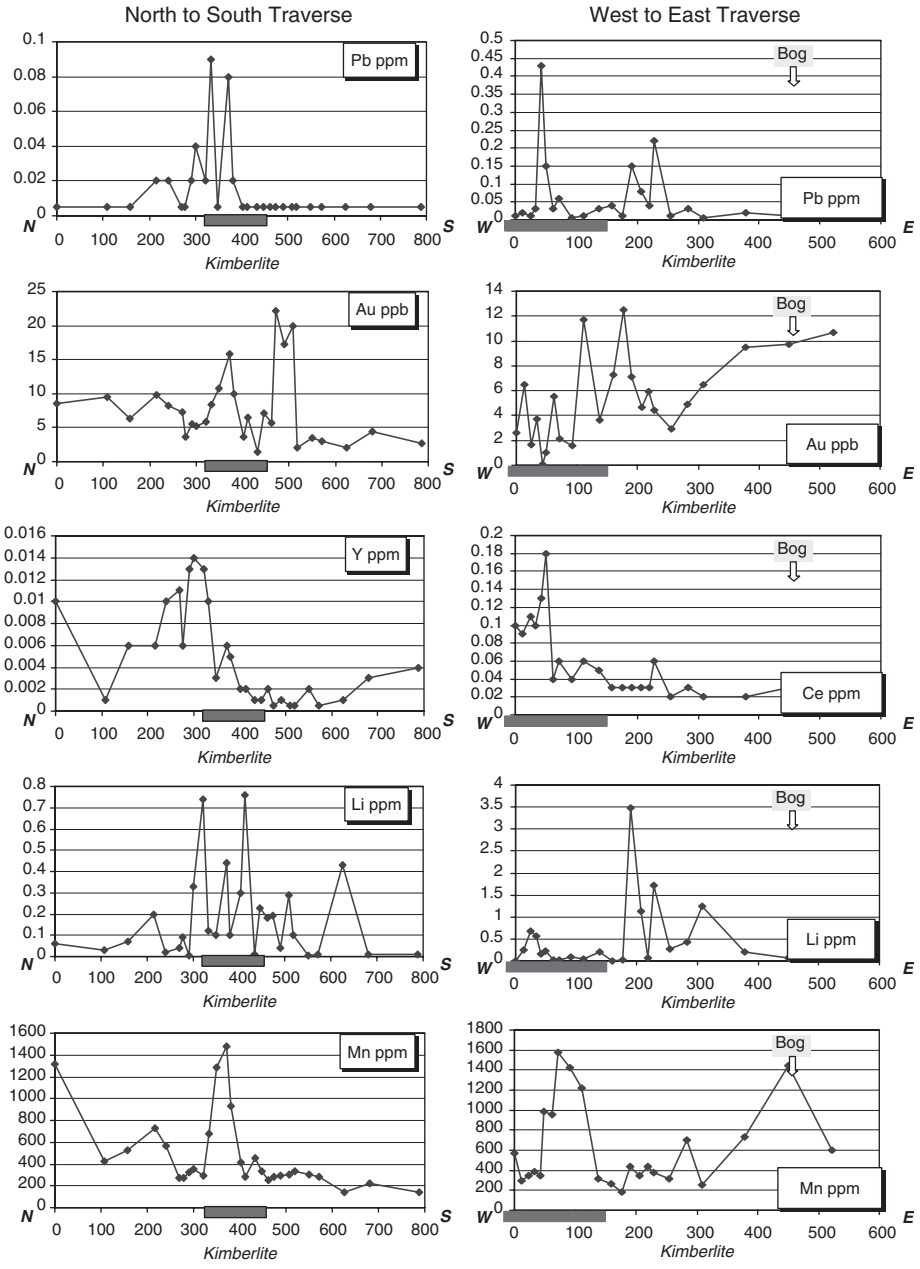

Fig. 11-21. Profiles of element concentrations over the Impala kimberlite. Left column shows the north–south traverse; right column the west–east traverse. Concentrations in dry leaves.

coincident Au enrichment. In summary, the concentrations of elements with respect to the kimberlite were as follows:

- Strongest signatures – Pb, Au, REE, Li, Mn and Co.
- Weaker response – Rb, Hg, Ba, Sn and S.
- Slight response – Ca, Cs, Cu and Sb. There was possibly a weak response, too, in Ta, but concentrations were close to the detection limit.

B. Bighorn (Fig. 11-20b)

Profile plots of the data from two traverses over the Bighorn kimberlite show a similar suite of elements to those at the Impala kimberlite that are enriched over or at the margins of the kimberlite (Fig. 11-22).

Similarly to the Impala kimberlite, the two traverses over the Bighorn kimberlite showed relative enrichment either over the kimberlite or at its margins. In summary the concentrations of elements were as follows:

- Strongest signatures –Au, Co, Fe, Mn, Mo, Pb, REE and Zn.
- Weaker response – Ba, Hg, Li, S, Sn and Sr.
- Slight response – Cs, Rb and Ta (Ta concentrations were close to detection).

Table 11-V compares the multi-element signatures at the two kimberlites.

South Africa: Kimberley

Geology

Volcanic and sedimentary rocks of the Precambrian Karoo Supergroup. The Perdevlei diatreme intrudes through Palaeoproterozoic dolomites and is covered by red soil and a calcrete cap varying in depth from 2 to 7 m. It is an intensely carbonated, partly clay-degraded and oxidized volcaniclastic diatreme that has been

TABLE 11-V

Comparison of element signatures along traverses over the Impala and Bighorn kimberlites. Elements in parentheses indicate weak signatures

Location	Over and peripheral to kimberlite (dominant)	Over and peripheral to kimberlite (lesser)	Over and peripheral kimberlite (minor)
Impala	Au, Co, Mn, Li, Pb, REE and Zn	Ba, Cs, Hg, Rb, S, Sn and Sr	Ca, Cu, P, Sb and Ta (Cd)
Bighorn	Au, Co, Fe, Mn, Mo, Pb, REE and Zn	Ba, Hg, Li, S, Sn and Sr	Ca, Cs, (Cu), (P), Rb, Sb and Ta

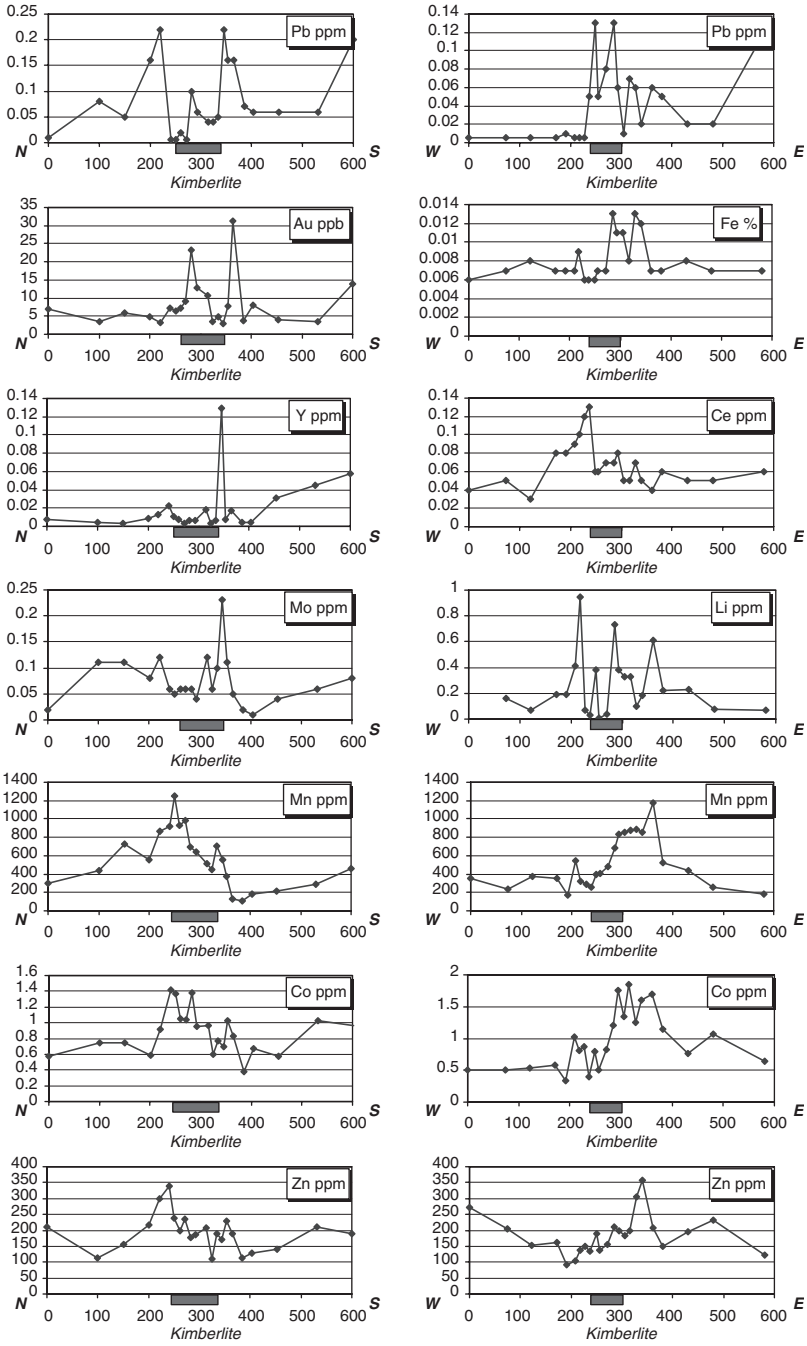

Fig. 11-22. Profiles of element concentrations in dry birch leaves from sites over the Bighorn kimberlite. Left column shows the north–south traverse; right column the west–east traverse.

mined and no longer in operation. The Welgevonden kimberlite has a calcareous cover. The Kouewater kimberlite has oxidized iron-formation cover.

Environment

Warm, temperate, arid scrubland.

Biogeochemical surveys

Combined samples of twigs and leaves were collected from the following:

- *Perdevlei*: 'Vaalbos' or camphor bush (*Tarchonanthus camphoratus*) from 16 sample stations.
- *Kouewater*: Black Thorn Acacia (*Acacia mellifera*) from 23 sample stations. Black Thorn Acacia is also one of the most common plant species in the Kalahari.
- *Welgevonden*: a shrub identified as 'Hak en Steek' (27 samples of an unknown genus, possibly a member of the Asparagus family).

The locations of the samples selected for the orientation surveys are shown in Fig. 11-23.

At Perdevlei, the stems and foliage of the vaalbos samples were combined and milled to a fine powder. At the other localities the foliage was separated from the tough and fibrous twigs and only the foliage was analyzed. A 1 g portion of each milled sample was digested in nitric acid then aqua regia and the solution analysed by ICP-MS for 66 elements.

Perdevlei (Calcrete)

No samples could be taken from over the kimberlite because it had been mined-out. With respect to all samples collected along this line, relative enrichments of elements occurred mostly at the northern rim of the mined-out kimberlite. This suite of elements included Ca, Li, Na, Nb, Ni, P, Rb, S, Se, Sn and Ta. At the southern rim of the open pit the elements with highest concentrations were Au, B and Mo.

Kouewater (Fe-Formation cover)

Element enrichments occurred at the margins of the kimberlite – especially REE and Cs (Fig. 11-24). All REE exhibited the same patterns with the exception of Ce that was more enriched than the other REE over the kimberlite, although it, too, had higher concentrations at the kimberlite margins. The implication is that there was some differentiation of Ce. Also enriched at the kimberlite margins were Co, Fe, Nb, Ni and to a lesser degree Ca, B, Mn, Cu, Te, Th and Zn.

Welgevonden (Calcareous sediment cover)

Elements that exhibited the highest concentrations over the kimberlite in relation to the surrounding terrain were Hg, Li, Mn, Mo, P, Rb, Sn and Ta. There were

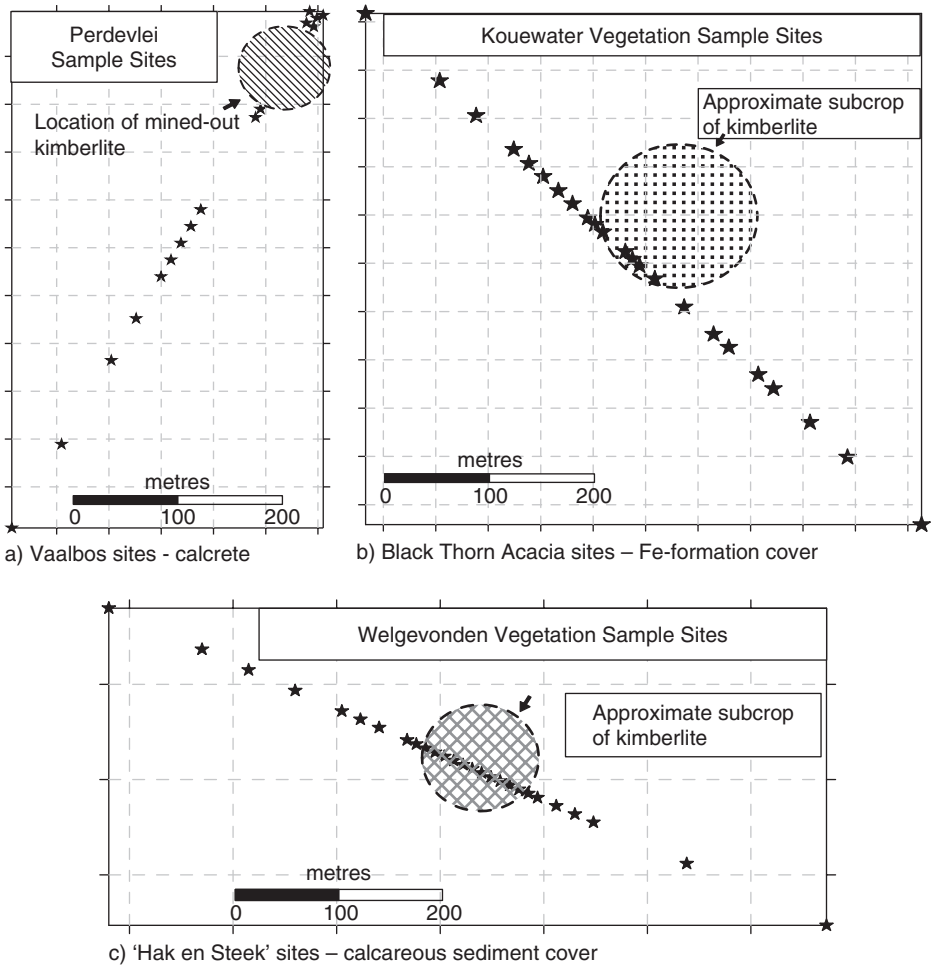

Fig. 11-23. Sample sites over three kimberlites in South Africa (Kimberley area). Orientation survey locations.

slightly depleted levels of Cs, Mg, Se and Sr over the margins of the kimberlite. Lead and Nb were enriched at the northwest margin, whereas Ni, Zn and REE were enriched to the southeast.

Expanded surveys – summary observations

Samples for the orientation survey comprised one third of the complete collection. The purpose was to examine the element distribution patterns along one traverse over each of the three kimberlites. The remaining samples were kept in reserve pending the outcome of the initial results. This is a sound approach in that there is

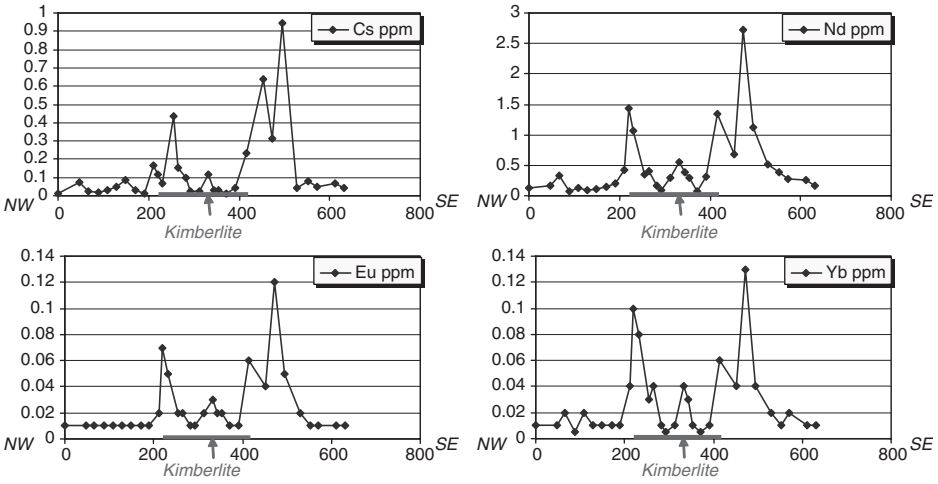

Fig. 11-24. Kouewater – Cs and REE (represented by light [Nd], medium [Eu] and heavy [Yb] REE) enrichments in relation to kimberlite beneath Fe-formation cover.

considerable expense involved in launching a sample collection programme, whereas once in the field there is relatively small additional cost in obtaining more samples. Furthermore, since seasonal changes in plant chemistry are likely to occur it is preferable to collect samples within a short time span (two to three weeks), obviating any doubts with regard to anomalies being related to collections made later in the season.

In light of the encouraging orientation survey results, the remainder of the complete suite of 277 samples was prepared for analysis and analysed in the same manner by the same laboratory.

In summary, the over all results indicated that approaching and over the kimberlites there were consistent enrichments of Sr, Ca, Ni, REE and locally Cu, Hg, Mo, Nb, Re, Se, Te and Zn. Elements that were notably enriched at the margins of the kimberlites were Cs, Li, Pb, REE, Sn, Ta and Zr.

Thanks are extended to Dr. W. B. Coker and BHP Billiton for permission to release data from the Ekati area and South Africa.

Canada: Buffalo Head Hills, North-Central Alberta

Geology

During the late Cretaceous, kimberlites were intruded through Phanerozoic strata with contemporaneous marine clastic sediment deposition. Boyer et al. (2003c) reported the following:

> The Cretaceous (~85 Ma) Buffalo Hills kimberlite province presently consists of thirty-six bodies distributed over 250 square kilometres in northern Alberta, Canada. Of

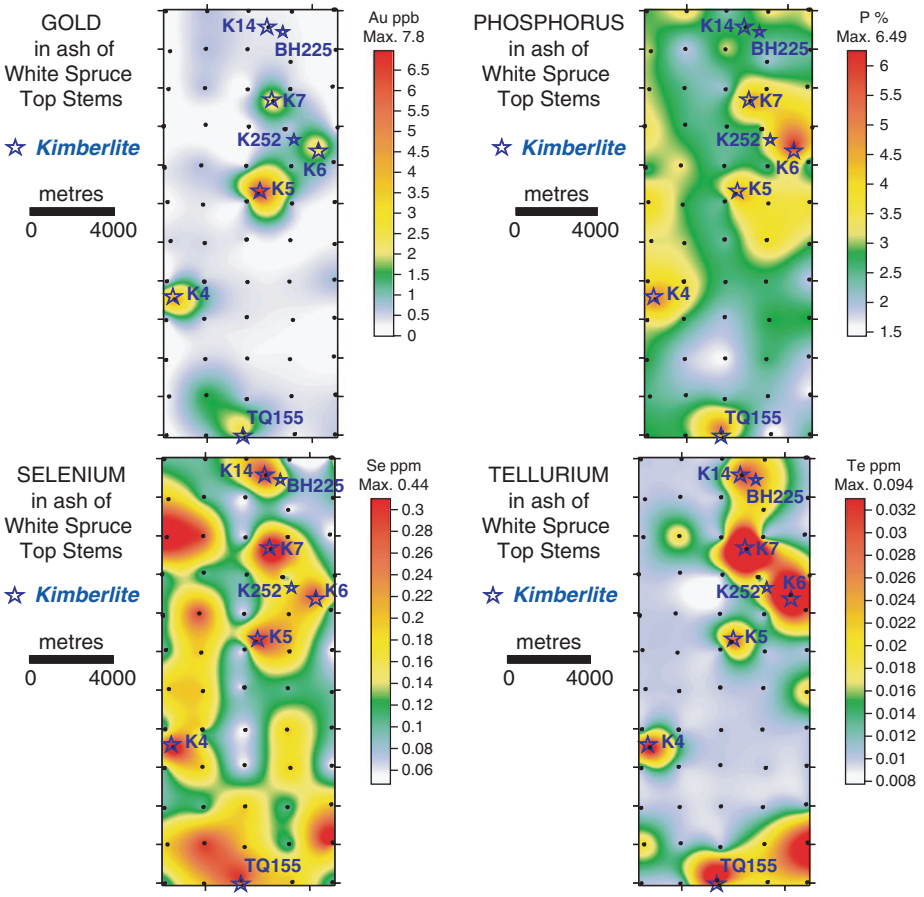

Fig. 11-25. Elements in top twigs from white spruce – Buffalo Head Hills kimberlite cluster of north-central Alberta.

> the thirty–six kimberlites identified to date all but one are classified as crater facies at the level of outcrop or to the depth of the exploration drilling … (they) are predominantly fine-bedded olivine crystal tuff with coarser horizons rich in juvenile magmaclastic volcaniclastic kimberlite. Xenocrysts include olivine, chromian pyrope garnet, eclogitic garnet, spinel, enstatite, and chromian diopside. Diamond was recovered from 24 of the kimberlites. The mesostasis is usually serpentine, carbonate minerals and/or chlorite.

Exploration history

Kimberlites were first identified during geophysical exploration for oil and gas in the late 1990s. In 1997, Ashton Mining of Canada Inc., in conjunction with Alberta Energy Company and Pure Gold Resources Ltd., discovered kimberlites on the

southeastern flanks of the Buffalo Head Hills. Within the survey area the only outcropping kimberlite is K5 (Fig. 11-25). Post-emplacement sedimentary cover up to 130 m thick can be found over the remainder of the bodies. Despite the cover, some of the kimberlites form subtle topographic highs as a consequence of preferential erosion of the softer country rock. The kimberlites range in size from less than one hectare up to 47 Ha, with circular to irregular shapes based largely on the outlines of their magnetic anomalies (Boyer et al., 2003a,b,c).

Environment

Boreal forest. Mostly white spruce (*Picea glauca*); trembling aspen (*Populus tremuloides*); and in the wetter areas black spruce (*Picea mariana*), willow (*Salix spp.*) and tamarack (*Larix laricina*). The understory is dominated by alder and Labrador tea.

Biogeochemical survey

Airborne tree top reconnaissance survey over the kimberlite swarm comprising the Buffalo Hills Kimberlite Province, with semi-detailed sampling focusing on several known kimberlites.

Two areas were surveyed in the autumn of 2000. This account deals with the larger of these areas, where white spruce is the dominant species and represents the only treetop sample medium discussed. The geochemical responses of other vegetation species and tissues, and other sample media are discussed in Seneshen et al. (2005).

Figure 5-3 (Chapter 5) shows the sample sites and the locations of known kimberlites of which K5 is the only occurrence that outcrops in the area. The others have a till cover ranging from 7 to 35 m in thickness. The original survey plan was to use a helicopter to collect white spruce samples at 1 km intervals on an offset grid in accord with the pattern shown in Fig. 5-3. However, budgetary constraints necessitated that the numbers of samples were reduced to a 2 km grid within an area of 176 km^2, resulting in a collection of 60 samples for the reconnaissance component of the area surveyed. In addition, crews were asked to collect an additional five samples over each of the six known kimberlites (K4, K5, K6, K7, K14 and TQ155) in order to establish signatures that might be unique to the kimberlites. Including all field duplicates, a total of 120 treetops were collected.

Samples were air-dried for two weeks, and the needles then removed from the twigs. The twigs were reduced to ash by controlled ignition at 475 °C for 24 h, the ash dissolved in aqua regia and the solution analysed by ICP-MS. Results for this survey are reported as concentrations in ash. The ash yield of the samples average 3.3% and so data should be divided by 30 to obtain an approximate dry-weight equivalent concentration.

Element concentrations were on average similar to the background concentrations that would be expected for white spruce twigs. There were no unusually high concentrations of any elements. However, a notable feature was that although concentrations remained low, trees growing over the locations of the buried kimberlites yielded slightly elevated levels of several elements, especially Au, P, Se and Te (Fig. 11-25). For Te, in particular, anomalous concentrations (with respect to the whole dataset) occurred over all the known kimberlites. The Au values were barely above the usual background values for Au in plant ash, but probably because of the very low Au content of the Cretaceous clastic sediments that form the surrounding host rocks, there is sufficient contrast for the kimberlites to emerge as slightly enriched in Au. Presumably, the small traces of Se and Te represent a geochemical association with the Au. Phosphorus enrichment with kimberlites is a common association. Other elements were locally enriched over one or more kimberlites (e.g. As, Ba, Cr, Nb, Ni, Pb, Rb, REE, Sr and V).

This biogeochemical survey comprised part of a much larger exploration programme involving the comparison of many different geochemical sampling media. Of these, the media that provided the best indications of deeply buried (i.e. >30 m) kimberlite pipes were white spruce-top twigs, C-horizon, till, peat, and sub-peat sediments. The partial extraction method of enzyme leach ^{TM} provided a good signature from the C-horizon soils (Seneshen et al., 2005).

Kimberlites – summary

The unusual abundance of many trace elements in kimberlites is striking (Dawson, 1980). Kimberlites in general are characterized by high content of (a) elements compatible with ultramafic rocks – Mg, Cr, Ni, Co; and (b) 'incompatible' elements that are considerably more concentrated than are usually found in ultramafic rocks – especially K, P, Zr, Nb, Sr, Ba, Rb, Cs and the light REE.

A biogeochemical study conducted over the crater-facies kimberlite at Sturgeon Lake, Saskatchewan, pointed to the possible relevance of Cr, Mn, Nb, Ni, Rb and Sr distribution patterns (Dunn, 1993). It was considered that Sr may be derived from the carbonates associated with the kimberlite. The Rb probably occurs in phlogopite from which it is easily solubilized by weakly acidic groundwater and transported until it is absorbed, through plant roots, and translocated to the aerial parts, providing a halo of enrichment around and above a kimberlite that may be concealed by sediments.

Several subsequent studies have noted positive Rb and Sr signatures in various types of vegetation growing over kimberlites, as demonstrated in some of the above examples, and by McClenaghan and Dunn (1995), Dunn and McClenaghan (1996). At this time it appears that the rather large list of elements that most commonly are enriched in vegetation growing either over or marginal to kimberlites is, in alphabetical order Au, Ba, Cs, Co, Hg, Li, Mn, Mo, Nb, Ni, P, Pb, Rb, REE, Se, Sn, Sr, Ta, Te and Zn. The ultimate objective of discriminating barren from diamondiferous kimberlites remains a challenging goal.

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Chapter 12

EXPLORATION GEOMICROBIOLOGY – THE NEW FRONTIER

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INTRODUCTION

Mineral deposits characterized by outcropping hard-rock mineralization are well explored and currently being depleted. Consequently, mineral exploration is increasingly focussing on areas where thick layers of overburden conceal potential mineralization (Smith, 1996). In Australia, significant parts of the landscape are covered by deeply weathered residual regolith as well as transported cover of up to 100 m, which may mask underlying mineralization (Taylor and Eggleton, 2001). Critical to the development of successful predictive exploration techniques in these areas is an understanding of processes that lead to trace element dispersion and re-concentration, and thus mediate the formation of surface and near surface expression of buried mineralization.

Microbial processes are known to drive processes involved in the mobilization, distribution and speciation of many trace metals under a wide range of environmental conditions (Ehrlich, 1996a,b). Understanding the relationship between microbial mineral solubilization and precipitation and trace element bioavailability in the rhizosphere (i.e., zone immediately surrounding the plant root) is critical in the application of biogeochemical exploration using plant materials, because the biogeochemical activity of micro-organisms and plants is closely interlinked. Bacteria and fungi living in the rhizosphere of plants have been shown to increase the mobility and solubility of commodity metals and pathfinder elements, such as As, Cd, Cu, Hg, Ni, Pb, Se and Zn, which in turn leads to a increased uptake of these elements by the plant (de Souza et al., 1999a,b; Whiting et al., 2001; Khan, 2005). However, other studies indicate that rhizosphere microbiota may under different conditions reduce plant metal uptake via surface adsorption and related processes such as ion-exchange, complexation, precipitation and crystallization on and within the cell wall (Mattuschka and Straube, 1993; Cotoras et al., 1992; Morley and Gadd, 1995).

The structure and activity of the microbiota resident in the soil are strongly influenced by the prevailing geochemical conditions such as pH, redox conditions and element content (Brock et al., 1996; Ehrlich, 1996a,b; Kizilkaya et al., 2004). Previous studies have shown that some micro-organisms indicate the presence of buried mineralization (Parduhn et al., 1985; Parduhn, 1991; Parduhn, 1995). In particular, the use of *Bacillus cereus* as an indicator organism for Au and other metals has been successfully applied in studies of different terrains in Belgium, China, Argentina and Mexico (Neybergh et al., 1991; Melchior et al., 1994, 1996; Wang et al., 1999). Cell numbers of *Bacillus cereus* in polymetallic soils, especially those with high Au concentrations, were up to several orders of magnitude higher compared to soils containing background concentrations, indicating an unambiguous association of bacterial population with polymetallic soils (Watterson, 1985; Neybergh et al., 1991; Melchior et al., 1994, 1996; Wang et al., 1999).

To generate an understanding of the relationship between micro-organisms, their geochemical environment and processes relevant to mineral exploration, 'exploration geomicrobiology' has emerged as a new area of research. Studies in this developing field provide the understanding of microbial populations and processes that is required to successfully develop bio-prospecting tools and predictive biogeochemical (*sensu stricto*) models. Figure 12-1 is a schematic diagram that demonstrates the links between geomicrobial and biogeochemical processes (in grey) and mineral exploration and bioprocessing of ores.

The aim of this summary is to introduce the field of exploration geomicrobiology to readers with a background in exploration geosciences by (i) providing an overview of microbial processes relevant to trace element dispersion and re-concentration in the soil; (ii) introducing methods to study activities and structures of complex microbial communities; and (iii) highlighting how these methods are applied in three case studies relevant to mineral exploration.

SIGNIFICANCE OF MICRO-ORGANISMS AS BIOGEOCHEMICAL AGENTS

Microbes are the oldest and most diverse forms of life on the planet (Ehrlich, 1996b). Fossil records demonstrate that the oldest group of micro-organisms, the single-celled prokaryotes (bacteria and archaea) inhabited the planet as early as 3.8 billion years ago, and that these early ancestors of today's prokaryotes display cellular structures similar to their modern descendants (Ehrlich, 1998). Other groups of micro-organisms that are important in environmental systems are fungi and algae, which belong to the eukaryotes and whose earliest ancestors have been around for approximately 2.1 billion years (Han and Runnegar, 1992).

Micro-organisms are highly diverse and abundant in soils with population sizes of up to 10^{12} cells per gram of material (Brock et al., 1996; Paul and Clark, 1996). They occur in 'extreme' environments such as deep marine sediments (Parkes et al., 1994), deep-sea hydrothermal vents (Juniper and Tebo, 1995), deep rock fractures

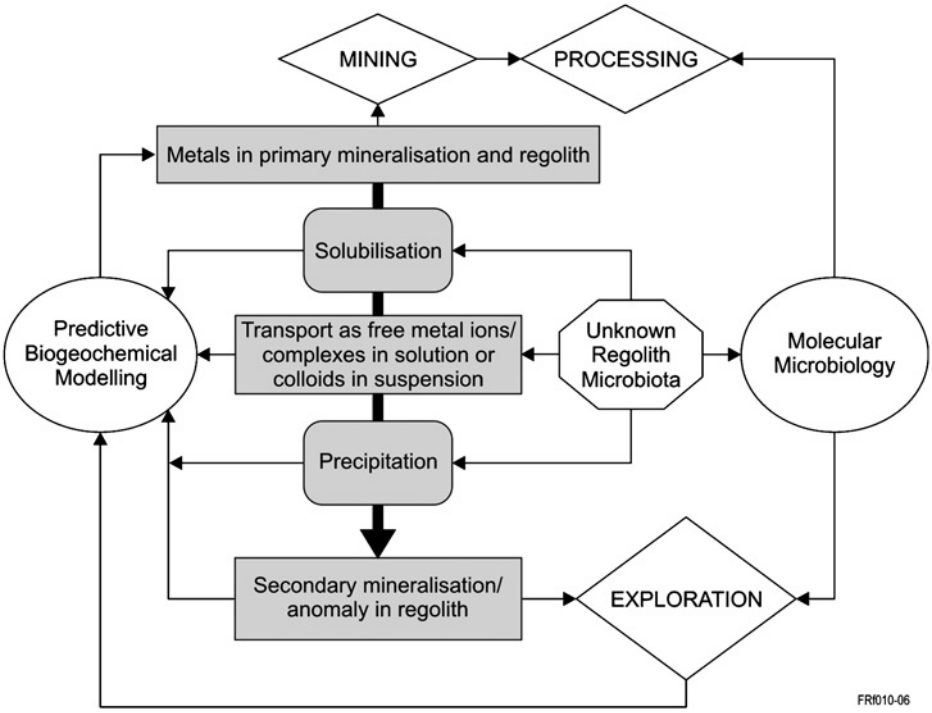

Fig. 12-1. Schematic diagram showing the integrated approach used in exploration geomicrobiology to link geomicrobial and biogeochemical processes (in grey) with areas of research and the requirements of the minerals industry.

(Pedersen, 1993), deserts (Adams et al., 1992), polar-regions (Vincent and James, 1996) and acidic (pH < 1) heavy metal-polluted mine wastes (Ledin and Pedersen, 1996; Baker et al., 2004). Besides being genetically and ecologically extremely versatile, micro-organisms and in particular bacteria have developed a wide spectrum of metabolic capabilities including the ability to utilize inorganic elements in the production of energy (Brock et al., 1996; Nealson and Stahl, 1997). Elements that bacteria are known to oxidize or reduce in order to gain metabolic energy include H, C, P, S, V, Mn, Fe, Co, Cu, As, Se, Br, Mo, Sn, Sb, Te, Hg, W and U (Woolfolk and Whiteley, 1962; Silverman and Ehrlich, 1964; Ehrlich, 1996b; Nealson and Stahl, 1997; Ehrlich, 1998). Furthermore, bacteria are known to concentrate extraordinarily high amounts of many metals (see Table 1-I).

In the 19th century, Louis Pasteur demonstrated the existence of bacteria, and their importance in disease and in agricultural fertility has been recognized for over 100 years. However, only in recent years has their significance for geological processes, such as rock weathering and secondary mineral formation, been recognized (Ehrlich, 1996b). The particular importance of micro-organisms in processes relevant to mineral exploration lies in their ability to promote mineral dissolution and

diagenesis as well as control major and trace metal mobilization, transport and precipitation that may lead to the formation of secondary mineralization and anomalies in and around mineralized zones (Ehrlich, 1998). Thus, it is increasingly recognized that many mineral transport and transformation processes, previously considered to be purely chemically driven, are in fact controlled by microbes (Ehrlich, 1998).

Micro-organisms are able to alter the composition and structure of many minerals, among them metal sulphides, silicates and carbonates (Garcia-Vallès et al., 2000). Iron- and sulphur-oxidizing bacteria and archaea mediate the direct oxidative breakdown of sulphides, and thus contribute directly to the dispersion of metals associated with these minerals. Several hundred different species of iron- and sulphur-oxidizing bacteria and archaea are known and have been shown to promote the breakdown of numerous economically important metal sulphides such as pyrite, bornite, covellite, arsenopyrite, gallium sulphide, stibnite, cinnabar, cobalt sulphide, galena, millerite and sphalerite. Thus, the mineral processing industry uses iron- and sulphur-oxidizing bacteria in industrial bio-leaching processes to assist in the extraction of metals from sulphide ores (Krebs et al., 1997).

Other bacteria, archaea and fungi promote rock weathering and trace metal mobilization by excreting metabolites that corrode minerals through chemical interaction (Ehrlich, 1998; Sterflinger, 2000). The compounds that these micro-organisms excrete include inorganic acids (such as HNO_3 or H_2SO_4), and organic acids such as acetic-, fumaric-, gluconic-, formic-, oxalic-, citric-, succinic-, malaic-, pyruvic- or amino acids and complex molecules such as siderophores (Ehrlich, 1996a; Sterflinger, 2000). Many of these extracellular organic molecules readily form complexes with free metal ions, and thus control their speciation and mobility in soils. These microbial processes are particularly important in the rhizosphere, where they are stimulated by plants excreting organic compounds as root exudates (Curl and Truelove, 1986; Khan, 2005). Root exudates directly increase metal mobilization, and provide nutrition for rhizosphere micro-organisms which, due to their then higher metabolic activity, further increase the turnover of metals. These microbe-plant interactions may result in increased mobilization of trace metals in the rhizosphere, which may lead to increased uptake and accumulation of trace metals by plants (Khan, 2005).

Micro-organisms have also been shown to promote the formation of minerals at moderate temperatures ($<50^{\circ}C$) and atmospheric pressure that previously were thought to form only at high temperatures and pressures (Ehrlich, 1998). In particular, carbonate- and silica minerals are precipitated by bacteria, archaea, fungi and algae under a wide range of biogeochemical conditions (Castanier et al., 1999a,b). Biogenic sulphides, which react with metal cations to form sulphide minerals, are formed anaerobically by sulphate-reducing bacteria (Brock et al., 1996). Bacteria and fungi also promote the formation of metallic minerals at their cell surface by binding metal cations to negatively charged groups of the cell wall or cell envelope (Ehrlich, 1998); through this process the authigenic formation of secondary Au grains in soil has been attributed to microbial processes, as shown in Case Study 1 (Reith et al., 2006).

METHODS FOR IDENTIFYING MICRO-ORGANISMS AND MICROBIAL PROCESSES

The task of developing a mechanistic understanding of element dispersion and re-concentration in soils, which display complex inorganic and organic matrices and distinct abiotic and biotic phases, is challenging. Understanding the biotic phases in these systems is particularly demanding, because one gram of soil may contain several thousand different microbial species (Paul and Clark, 1996). To assess the diversity of these complex microbial populations as well as their function in soil, numerous methods have been developed (Fig. 12-2). A synopsis with respect to their usefulness for exploration geomicrobiology is given in the following section, and for an in depth look into techniques introduced in this section the reader is referred to references given in the text.

Generally, methods to study microbial diversity are grouped into culture-dependent and culture-independent techniques (Barns and Nierzwicki-Bauer, 1997).

Culture-dependent techniques

Referred to as classical methods, these techniques use artificial nutrient-rich growth media to enrich and isolate micro-organisms from soil samples (Barns and Nierzwicki-Bauer, 1997). They require the inoculation of a solid or liquid growth medium with a small quantity (0.1–10 g) of the soil sample, and incubation under a variety of conditions such as different pH, temperature, composition of gases, nutrient contents or metabolic inhibitors (Brock et al., 1996). Once micro-organisms are growing in the enrichment culture, the aim is obtain pure cultures of individual species for a comprehensive analysis of their biochemical, physiological and genetic characteristics. Using selective media and conditions, a number of cell-counting methods (e.g., most-probable-number counts and counting of colony-forming-units) have been developed to assess the cell numbers of particular micro-organisms in soil samples (Atlas, 1984).

Community-level physiological profiling (CLPP) is a culture-dependent technique commonly used to assess the diversity and function of complex microbial communities. This technique is based on the fact that differing bacterial and fungal populations have differing abilities to utilize particular carbon sources such as sugars, amines, organic acids, amino acids and complex organic molecules. CLPP can thus be used to link bacterial and fungal diversity with their function in soils (Garland and Mills, 1991; Garland, 1996a,b; Garland, 1997).

All culture-dependent methods share one main bias: they rely on having to culture the organisms in a growth medium in vitro. Depending on the type of soil sample only 0.001 to 10% of the total microbial species contained therein can be successfully cultured using existing culturing techniques (Alexander, 1977). This is a reflection of the difficulty in accurately reproducing conditions of the natural micro-environments that the organisms require, with the result that less than one percent of the total number of bacterial species estimated to exist in the environment have been cultured and described

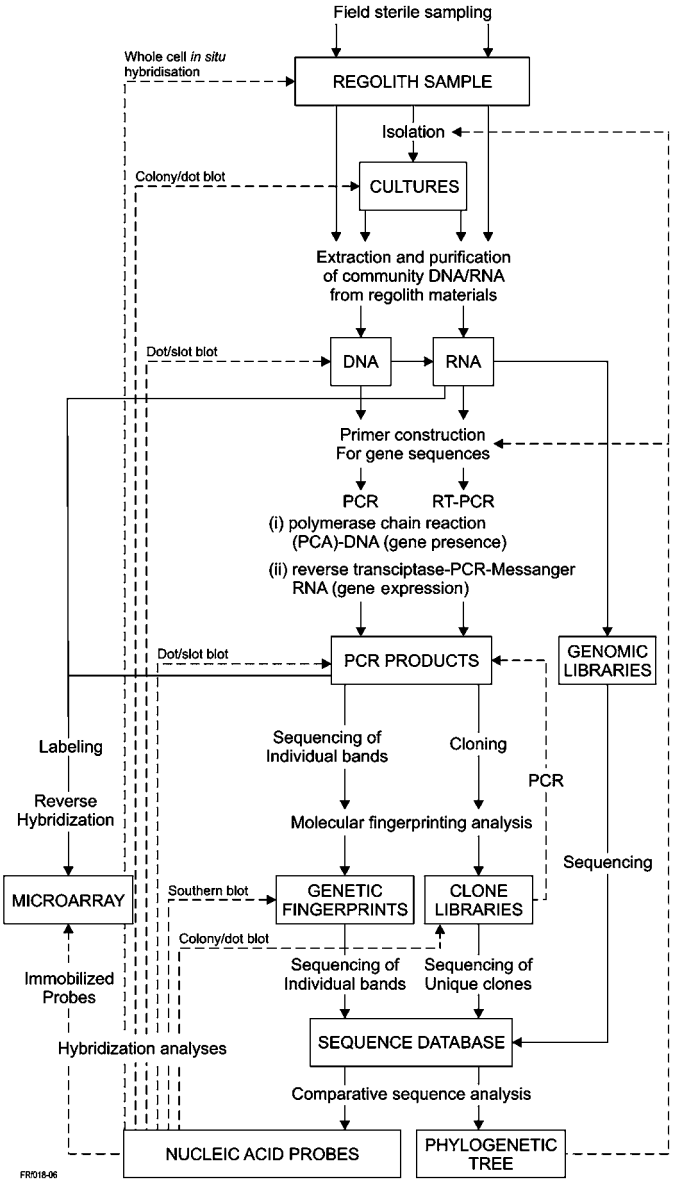

Fig. 12-2. Molecular approaches for detection and identification micro-organisms involved in cycling of heavy metals and their catabolic genes from environmental samples (adapted from Widada et al., 2002).

to date (Amann, 1995; Macalady and Banfield, 2003). In spite of this bias, the use of the classical culture-dependent methods has led to the isolation and characterization of thousands of micro-organisms from natural environments, and classical methods, especially in combination with molecular techniques, will continue to be crucial for

exploration geomicrobiology, because they allow comprehensive analyses of biochemical processes and pathways in species important for geomicrobial processes.

Culture-independent methods

These methods for characterizing microbial populations in soil samples have been developed in recent years. They are based on the analyses of cellular components such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or phospholipid fatty acid (PLFA) (Olsen et al., 1986; Pace et al., 1986; Barns and Nierzwicki-Bauer, 1997). These cellular components are extracted directly from the cells in the soils without prior cultivation. The main advantage of molecular methods is that they do not rely on culturing the organisms, and therefore provide a more accurate insight into the *in situ* composition and activity of microbial communities in soil samples. Figure 12-2 shows how they are used in combination to obtain a detailed representation of the phylogenetic (i.e., evolutionary) and functional relationships in microbial communities. Phylogenetic methods aim to identify key-organisms, community structures and genetic relationships (similar to DNA fingerprinting used in criminal forensics) and are based on the amplification, fingerprinting, sequencing and analyses of ribosomal DNA and RNA (Barns and Nierzwicki-Bauer, 1997). Methods assessing the functional aspects of microbial communities target genes that encode proteins/enzymes responsible for key biogeochemical transformations. This can involve measuring the presence (DNA-based) and expression (RNA-based) of the particular functional gene, e.g., the genes responsible for Fe and S oxidation or reduction or metal resistance (Torsvik and Øvreås, 2002; Widada et al., 2002). These methods can be summarized as follows:

- DNA-based molecular methods allow detection of the presence or absence of a particular gene in a soil sample, and thus establish if a microbial community is capable of catalyzing a certain reaction (Barns and Nierzwicki-Bauer, 1997).
- RNA-based methods are used to study the expression of a gene by the microbiota in the sample, and establish if the function is utilized by the microbial community at the time of sampling (Barns and Nierzwicki-Bauer, 1997).

MOLECULAR PROCEDURES

The steps required for the molecular procedures are as follows.

Extraction and purification of nucleic acids from soils

The first and most important step for any DNA- or RNA-based technique is the isolation and clean-up of the nucleic acids (DNA or RNA) from the soil samples, because all methods used in the following steps rely on the quality and the quantity

of extracted nucleic acids. The ideal procedure for recovering nucleic acids from soil samples was summarized by Hurt et al. (2001) and should meet the following criteria:

- Nucleic acid recovery efficiency should be high and not biased so that the nucleic acids extracted are representative of the nucleic acids of the naturally occurring microbial community.
- Extracted RNA and DNA fragments should be as large as possible so that molecular studies such as community gene libraries or gene expression analyses can be carried out.
- Extracted RNA and DNA should be sufficiently pure for reliable enzyme digestion, hybridization, reverse transcription and polymerase chain reaction (PCR) amplification.
- RNA and DNA should be extracted simultaneously from the same sample so that direct comparative analyses can be performed.
- Extraction and purification protocols should be as simple as possible so that the extractions are rapid and inexpensive.
- Extraction protocols should be robust, reliable and generate reproducible results from a variety of soil samples.

Most of the commercially available DNA or RNA extraction kits have been designed to fulfill these criteria. However, preliminary experiments to test extraction efficiency and reproducibility are recommended when applying commercial kits to new groups of soil samples, because organic matter and clay have been shown to significantly influence their performance.

AMPLIFICATION OF TARGET GENES FROM EXTRACTED DNA OR RNA

Target genes have to be amplified, because their concentrations in soil samples are too low to visualize on an electrophoresis gel without prior amplification (Giraffa and Neviani, 2001; Widada et al., 2002). For DNA-based methods the target gene is amplified using the PCR. In the process of PCR-amplification, the target DNA is doubled during each of the 20–60 cycles, so that by the end of a PCR run several millions of copies of a particular area of DNA have been produced (Fig. 12-3).

To assess if a PCR-amplification was successful the final product is visualized on an agarose electrophoresis gel (Fig. 12-4). By using agarose electrophoresis gels it is possible to separate DNA fragments based on molecular sizes (i.e., number of bases). A different approach is used for RNA-based methods, because RNA has a different molecular structure (Brock et al., 1998). Thus, the first step of most RNA-based techniques is the conversion of the RNA molecule to its DNA analogue using reverse transcription polymerase chain reaction (RT-PCR) (Widada et al., 2002). To specifically amplify only the target DNA or RNA of interest, primers specific to the genes are used in the PCR reactions (Figs. 12-2 and 12-3). Primers are short sequences of

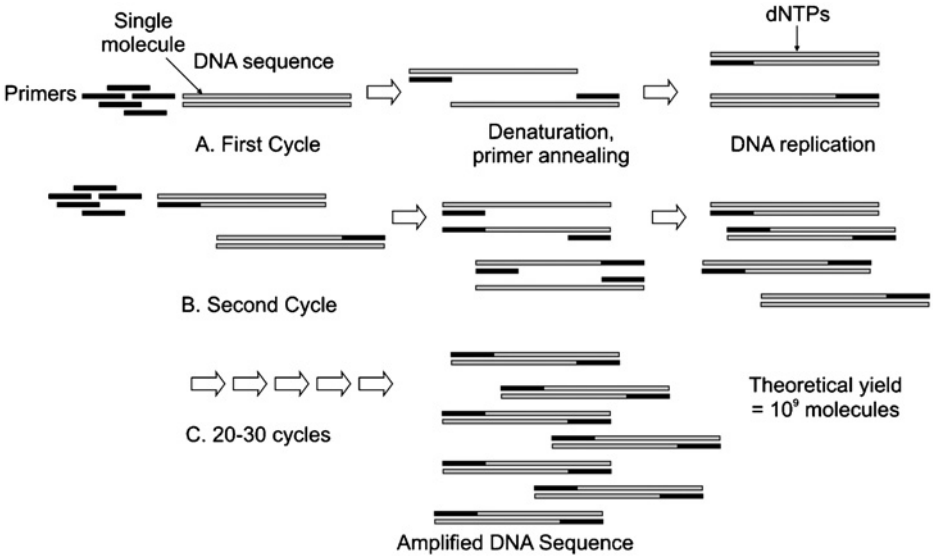

Fig. 12-3. Scheme showing the selective amplification of target DNA using polymerase chain reaction (PCR).

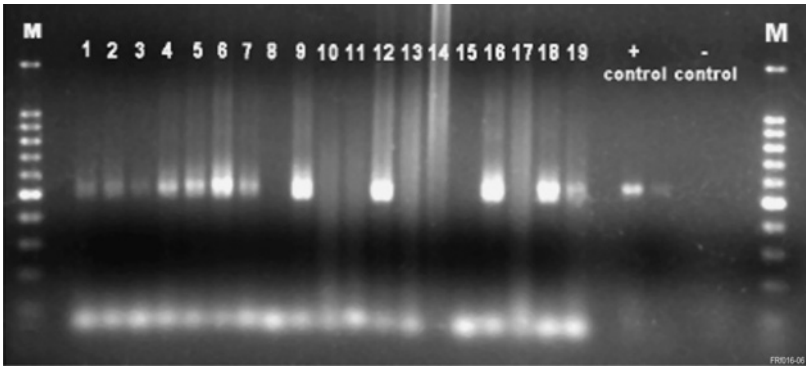

Fig. 12-4. Agarose electrophoresis gel of the ~510 bp (base pair) PCR-product from the primer pair 27-GC and 534R amplifying 16S rDNA from Au grains obtained from the Hit or Miss Gold Mine. Lanes marked with M contain a 100 bp ladder, fragment sizes (1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100). Lanes 1–19 contain PCR-product of the amplification of bacterial DNA associated with the Au grains. *E. coli* DNA was used as positive (+) control during amplification, sterile MilliQ water was used as negative (–) control.

DNA that bind to the ends of extracted DNA and are the starting point from which PCR-amplification of a gene commences (Amann et al., 1995). Primers have been developed to assess ribosomal or functional genes of individual species, phylogenetic groups and whole domains; thus it is possible to assess the presence and activity of

individual species, groups or domains such as bacteria, archaea or eukaryotes in a soil sample (Amann et al., 1995; Barns and Nierzwicki-Bauer, 1997).

Recently, an accurate quantitative analysis has been developed of the target DNA from community DNA and RNA (so-called real-time PCR). This technique allows quantifying how many copies of a particular gene are present in the soil sample (Widada et al., 2002). These results can be correlated to the total amount of organisms that express this gene in a sample and the rates of biogeochemical processes in the soil samples. This makes real-time PCR a very powerful method to establish a mechanistic link between the genetic composition of a microbial community and the microbially mediated geochemical processes in soil materials that are relevant to exploration geochemistry (Widada et al., 2002).

Assessing genetic diversity

To assess genetic diversity and identify key-species of microbial communities in environmental samples, DNA libraries of unknown species are created (Fig. 12-2; Barns and Nierzwicki-Bauer, 1997; Giraffa and Neviani, 2001). The DNA sequences in these libraries can then be compared to other sequences deposited in global sequence databases, which are an increasingly accurate and fast way of identification, similar to the way that human DNA samples can be used to identify individuals in criminal forensics.

Genetic fingerprinting

A number of techniques have been developed to obtain genetic fingerprints of microbial communities in soil samples, and to relate these structures to environmental conditions of different samples or treatments. The most commonly used techniques, shown in Fig. 12-2, are as follows:

- DGGE – denaturing gradient gel electrophoresis
- TGGE – thermal gradient gel electrophoresis
- SSCP – single strand conformation polymorphism
- T-RFLP – terminal restriction fragment length polymorphism.

Initially, these methods were used to assess the phylogenetic relationships and genetic diversity of microbial communities based on 16S rDNA or 18S rDNA, but now they are also used to assess microbial communities based on functional genes (Giraffa and Neviani, 2001; Widada et al., 2002).

1. DGGE and TGGE are methods by which similar sized fragments of PCR-amplified DNA can be resolved electrophoretically by their nucleotide composition (Giraffa and Neviani, 2001; Widada et al., 2002). The double-stranded DNA

molecules have different melting behaviour and will stop at different positions along the gel, as shown in Fig. 12-5. The emerging banding pattern is stained to allow visualization and then analysed for similarity using cluster analysis (Fig. 12-6) or non-metric multidimensional scaling (nMDS, Powell et al., 2003). Thus, PCR-DGGE and -TGGE methods provide an ecological insight into the structure of microbial communities in environmental samples, and may also be used to identify key organisms (see Case Study 1) which can be cloned, sequenced and subsequently identified.

2. SSCP also detects sequence variations between different amplified target DNA fragments.
3. T-RLFP is a method to study mixed populations in soil samples that is based on restriction enzyme digestion of fluorescently marked PCR products.
4. Phospholipid fatty acid (PLFA) analysis is an additional fingerprinting technique, based on using cell constituents other than DNA or RNA (Barns and Nierzwicki-Bauer, 1997). PLFA are fatty acids present in the lipid bi-layer membranes of living micro-organisms that are unique for specific groups of micro-organisms. Thus, PLFA analyses can be used to assess the diversity of a microbial community and to evaluate changes in microbial community structures as a result of changing biogeochemical conditions.

CASE STUDIES

Three case studies are presented to demonstrate how classical and molecular tools enhance the understanding of micro-organisms and microbially mediated processes associated with the turnover of trace metals in soils and deeper soil materials, and how this increased understanding may lead to the following:

(1) The prediction of mineral transport and transformation pathways and the location of secondary mineralized zones in the soil.
(2) The development of biosensors for masked mineralization.

Case Study 1 – The geomicrobiological cycling of gold

This study assessed the ability of natural soil microbiota to mediate the solubilization, transport and precipitation of Au in the Australian regolith, and is taken from Reith et al. (2005), Reith and McPhail (2006), Reith et al. (2006) and Reith and McPhail (2007).

Whereas it is now well established that micro-organisms play a key in role in the cycling of major and trace elements in the environment, current evidence for the role of micro-organisms in the biogeochemical cycling of Au is at best equivocal (Mossman et al., 1999). Laboratory experiments using pure cultures of common bacteria such as *Bacillus subtilis* or *Bacillus megaterium* have shown that these organisms can solubilize

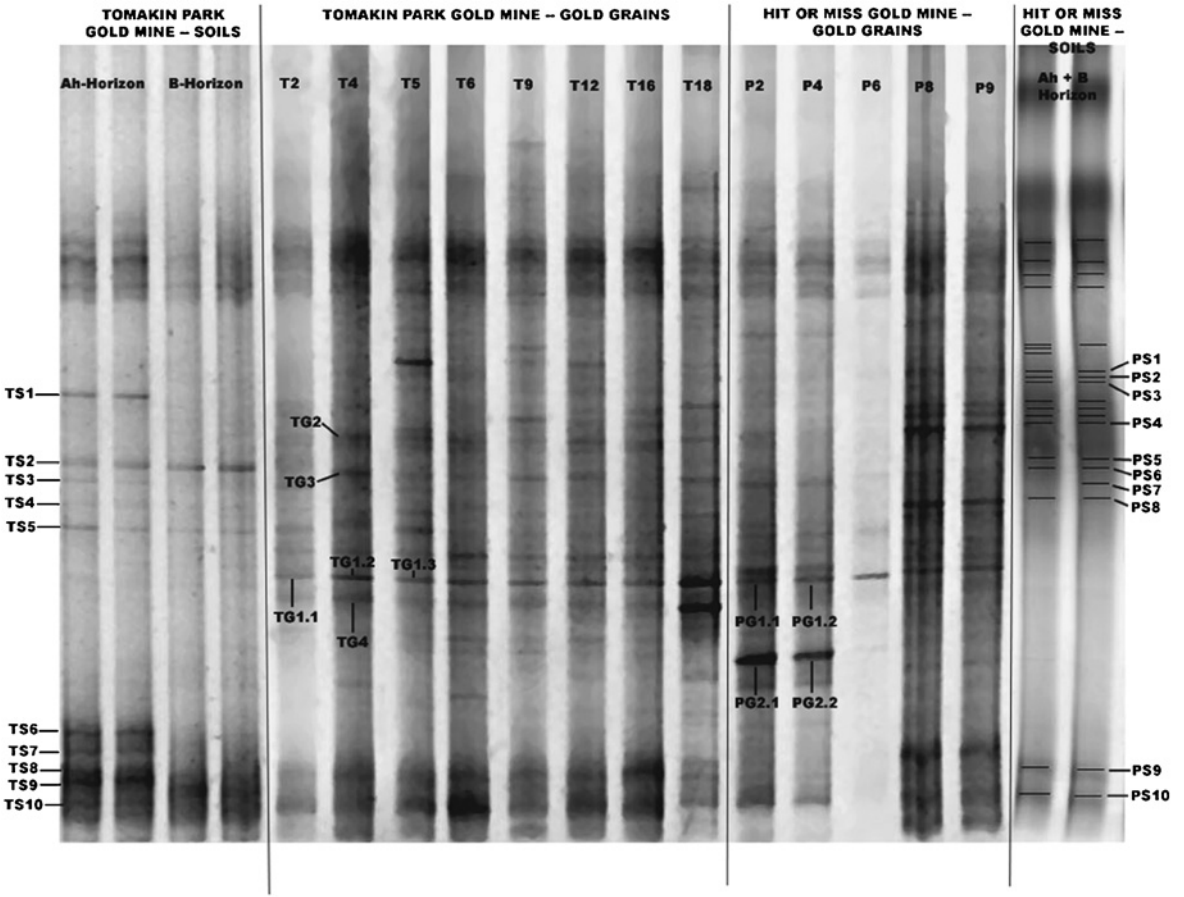

Fig. 12-5. DGGE (denaturing gradient gel electrophoresis) patterns obtained after amplifying the V3 region of the 16S ribosomal DNA extracted from Au grains and surrounding soils collected at the Tomakin Park- (T) and the Hit or Miss (P) gold mines. Bands designated with acronyms were excised from the gels, re-amplified and sequenced (Reith et al., 2006).

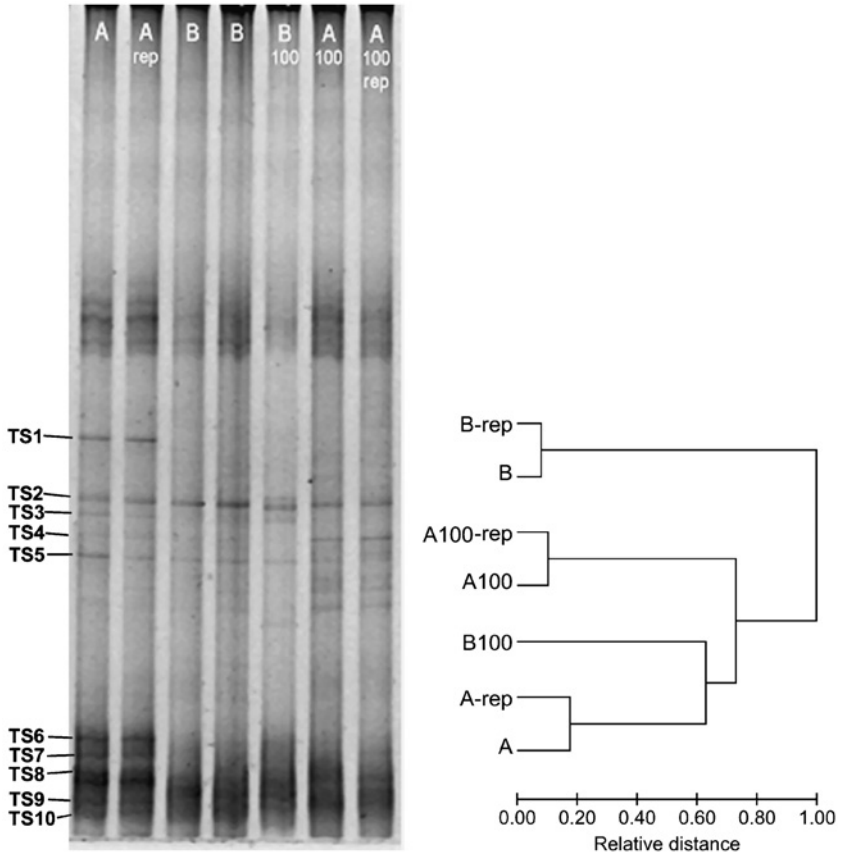

Fig. 12-6. Molecular fingerprinting analyses showing DGGE-patterns of 16S rDNA and dendrogram of subsequent cluster analysis from auriferous (A, B) and non-auriferous (A100, B100) soils from the Tomakin Park Gold Mine.

Au (Korobushkina et al., 1983). The ability of some bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*, *Penicillium chrysogenum*) to precipitate mobile Au complexes and colloids in vitro has also been demonstrated (Karthikeyan and Beveridge, 2002; Nakajima, 2003). Furthermore, morphological evidence for a microbially mediated formation of Au grains and nuggets in soils and deeper soil materials has been presented (Mann, 1992; Keeling, 1993; Bischoff, 1994, 1997). Using biochemical tools, Levchenko et al. (2002) demonstrated that a Au (I/III)-redox couple acts as a metal centre in the membrane-bound enzyme NADH-oxidase utilized by the common bacterium *Micrococcus luteus* during the oxidation of methane. Whereas this limited evidence suggested that microbial biogeochemical mechanisms may exist for the solubilization and precipitation of Au, the relative importance of these mechanisms in the regolith compared to abiotic processes and the organisms

driving these processes are not known. Thus, the aim of the research was to obtain evidence for the presence and significance of a biologically mediated cycle of Au in the regolith, and subsequently identify and quantify microbial processes that are important in mediating the dispersion and concentration of Au.

Figure 12-7 shows, from the sequence of observations, that microbiota resident in auriferous Australian soils and deeper regolith materials are capable of mediating a geomicrobiological cycle of Au. The indigenous microbiota in biologically active soil microcosms were able to solubilize up to 80 wt.% of the Au contained in these materials during the first 50 days of incubation, after which the solubilized Au was re-adsorbed by mineral- and organic soil fractions. In contrast, no Au was solubilized in sterilized microcosms incubated under otherwise identical conditions. Molecular (PCR-DGGE, cloning and sequencing of 16s rDNA) and physiological profiling (CLPP) of bacterial communities during the incubation of the microcosms combined with amino acids analyses indicated that changes in the structure of the bacterial community from carbohydrate- to amino acid-utilizing populations occurred concurrently with, and appear to be linked to, the observed solubilization and re-precipitation of Au.

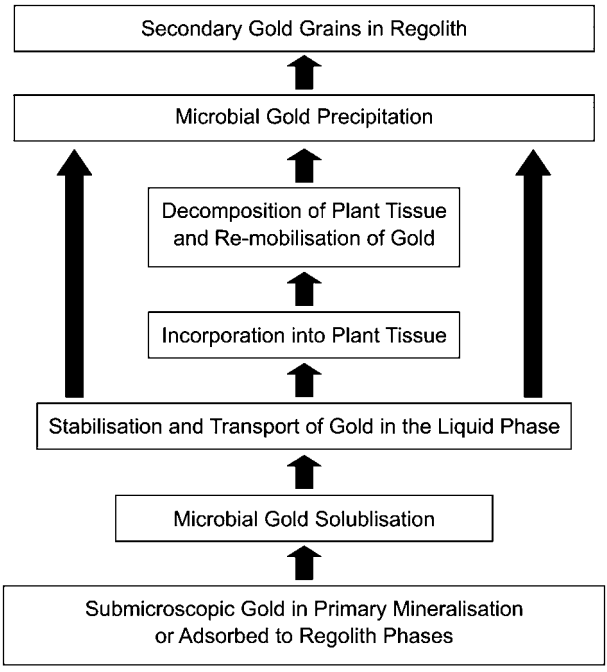

Fig. 12-7. Conceptual model linking the processes of Au solubilization, transport, precipitation and authigenic Au nugget formation to form a geo(micro)biological cycle for the behaviour of Au in the regolith.

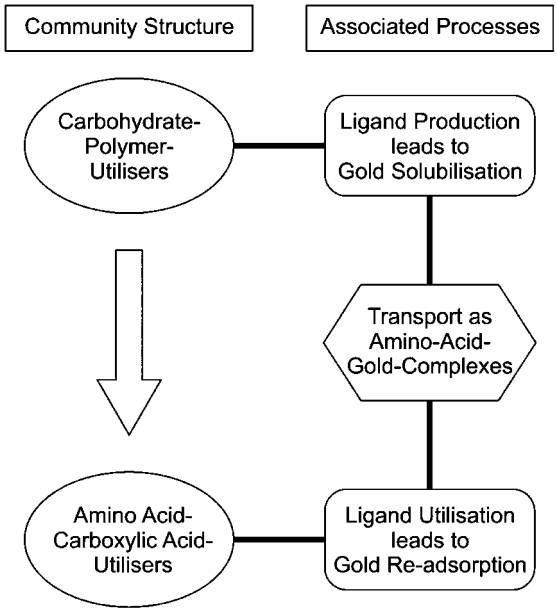

Fig. 12-8. Conceptual model linking Au solubilization and re-precipitation in microcosm experiments with auriferous soils to the results of microbial community structure analyses.

These results suggest the following model of Au solubilization and re-precipitation in the soil microcosms (Fig. 12-8).

- The bacterial community in the early stages of incubation apparently produced surplus amino acids, which are known to solubilize native Au and stabilize it in solution.
- The bacterial community in the later stages of incubation utilized these ligands, and as a result the Au complexes were destabilized and Au was re-precipitated to the solid soil fractions.

Molecular profiling also allowed the differentiation of bacterial communities from auriferous and adjacent non-auriferous soils (Fig. 12-6). These results in combination with results of *Bacillus cereus* spore counts (which were up to 1000 times higher in soils that displayed Au concentrations of 150–1000 ppb compared to soils with background Au concentrations), and microcosms amended with dissolved $AuCl_4^-$, suggest that the presence of highly mobile Au in the regolith as observed at many Australian sites may influence the composition of the resident microbiota. The results indicate that a geomicrobial exploration technique may be developed, where *B. cereus* spore counts are measured and used as a pre-screening method to target areas useful for further sampling and complete geochemical analysis (Reith et al., 2005).

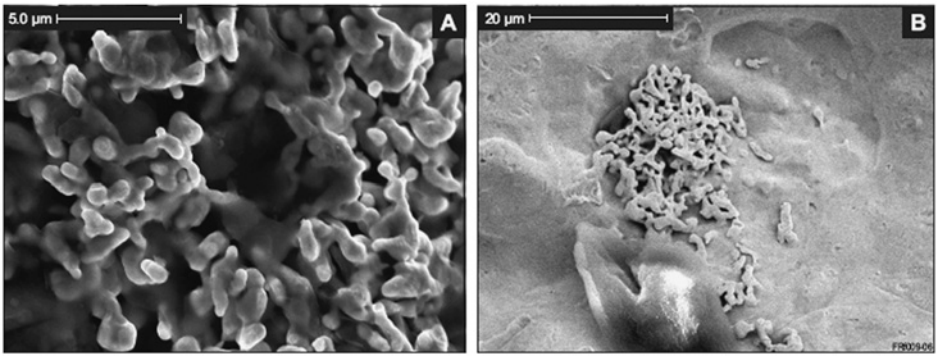

Fig. 12-9. Secondary electron micrographs of surface features of secondary Au grains from the Hit or Miss Gold Mine in the Palmer River Goldfields in north eastern Queensland, Australia. (a) 'Bacterioform' Au consisting of bacterial cell-like structures on the surface of the grain (scale bar = 5 µm) and (b) Initial stages of 'bacterioform' Au formation in a surface depression of a Au grain (scale bar = 20 µm).

Scanning electron microscopy (SEM) revealed bacterial pseudomorphs on untreated secondary Au grains from two field sites used in this study (Fig. 12-9). The presence of active bacterial biofilms on the surface of Au grains was confirmed using confocal stereo laser microscopy (CSLM) combined with nucleic acid staining. Molecular profiling showed that unique, site-specific bacterial communities are associated with these Au grains, which differed from those dominating the surrounding soils (Figs. 12.4 and 12.5). 16S ribosomal DNA clones belonging to the genus *Ralstonia* and bearing 99% similarity to *Ralstonia metallidurans* were present on all 16S rDNA positive Au grains from both locations, but were not detected in the surrounding soils (Fig. 12-5). The ability of *R. metallidurans* to actively accumulate Au from solution was then successfully tested suggesting that *R. metallidurans* may contribute to the formation of secondary Au grains and nuggets in the regolith.

These studies have shown for the first time that microbiota resident in Australian regolith are capable of actively mediating a biogeochemical cycle of Au in the environment, and that the microbially induced turnover of Au in the regolith may be rapid. Furthermore, there is evidence for a number of processes and organisms that may be associated with this cycle. Future experiments will use molecular microarrays to assess Au solubilizing and precipitating organisms, such as *R. metallidurans*, in order to understand the specific biochemical mechanisms regulating Au solubilization and precipitation. This may enable the development of specific gene probes for genes associated with the biogeochemical cycle of Au. Novel biosensor technology (as shown in Case Study 3) in combination with molecular characterization of Au-transforming organisms and associated biochemical processes will then have the potential to facilitate the development of molecular biosensors specific to Au for use in the field.

Case Study 2 – Microbial diversity in soils and sediments contaminated with Zn and Cu

Brim et al. (1999) examined bacterial communities of Zn-contaminated soils from Belgium using a combination of molecular and classical methods. From an exploration geomicrobiology perspective these results are interesting, because they show that the composition of the resident bacterial community was dominantly influenced by the concentration and speciation of Zn in these soils, as may be the case in soils overlying buried mineralization. The site had been monitored for two decades, and previous studies using cultivation-based techniques had shown that *Ralstonia eutropha*-like strains had dominated in the microbial community in the soil, i.e., in previous studies up to 40% of cultured cells belonged to the *Ralstonia* group.

In this study using a colony-forming-units technique, 10^4–$10^5\,g^{-1}$ culturable soil bacteria were counted in a number similar to results of previous studies. However, 23 of the isolates belonged to the *Arthrobacter* group of bacteria, but no *R. eutropha*-like isolates were detected. Most of the isolates were Zn tolerant but only seven were considered Zn resistant. Sequences obtained from 16S rDNA clones from a clone library, established from the soil community DNA, were affiliated with a number of different groups such as α- and β-*proteobacteria* and the *Cytophaga- Flexibacter-Bacteroides* group. Molecular profiling, using TGGE, of amplified 16S rDNA from isolates, soil clones and the extracted soil community DNA showed that isolates and clones only represented a part of the microbial population of these soils. *Arthrobacter* was the dominant band when 16S rDNA had been amplified from DNA extracted from the soil in a procedure with a bead-beating step, but was absent or faint when soil DNA was extracted without bead beating. From these results, Brim et al. (1999) concluded that the microbial community in previous studies was highly resistant to Zn toxicity concentration but was replaced with a less resistant bacterial microflora. They suggested that Zn toxicity levels had been reduced because of the activity of the Zn resistant *R. eutropha*-like bacteria that had led to the formation of insoluble Zn carbonates. The results also showed that molecular techniques such as TGGE in combination with classical techniques are powerful tools to assess microbial community structures, as they allow monitoring of the temporal and spatial changes of microbial communities and may facilitate the identification of key organisms, i.e., *R. eutropha*- and *Arthrobacter*-like strains.

In another study published by Konstantinidis et al. (2003) *Ralstonia*-and *Arthrobacter*-like strains were the only Cu resistant isolates cultivated from lake sediments contaminated with 200–5500 ppm Cu. T-RFLP showed that a number of OTUs (operational taxonomic units), among them *Ralstonia sp.,* were present universally the entire width of the sediment. A number of other studies also demonstrated the presence *Ralstonia sp.* and *Arthrobacter sp.* in samples with elevated concentrations of heavy metals and may as such be tried as bio-indicator organisms in geomicrobial mineral exploration.

Case Study 3 – Bacterial biosensors as alternatives for measuring heavy metals in soil extracts

This study tested the applicability of metal-specific whole-cell bacterial sensors for the analysis of arsenite and Hg in soil extracts from polluted soils by comparing them to results from ICP-AES analyses. The luminescence-based bacterial sensor strain *Pseudomonas fluorescens* OS8 (pTPT11) was used for Hg detection and *Pseudomonas fluorescens* OS8 (pTPT31) for arsenite detection. Petanen and Romantschuk (2002) spiked three different soil types (humus, mineral and clay) with 1100 or 500 $\mu g\, g^{-1}$ (ppm) of dissolved Hg(II) or As(III). They took samples after 1, 14 and 30 days of incubation and extracted with water, ammonium acetate, hydrogen peroxide and nitric acid to represent water soluble, bioavailable, organic matter-bound and residual fractions, respectively.

Concentration results with chemical and biosensor analysis were similar in the case of Hg-spiked samples. However, the lowest Hg concentration measured in the soil extracts using the bacterial sensor was 0.003 $\mu g\, kg^{-1}$ (ppb) which was considerably lower than by the chemical method 0.05 $\mu g\, kg^{-1}$ (ppb). The sensor strain with pTPT31 for arsenite had a useful detection range similar to that of chemical methods. Thus, they demonstrated that the bacterial sensors were sufficiently sensitive to measure concentrations of As and Hg that are relevant to mineral exploration in their soil extracts. Recently, other authors found similar low-detection limits in biosensors for Cu, Co, Zn, Pb and Cd (Tibazarwa et al., 2001; Rensing and Maier, 2003).

FUTURE TECHNOLOGIES FOR BIO-PROSPECTING

There are several emerging molecular techniques that have the potential to become the basis of future bio-prospecting technologies – these are nucleic acid probes, molecular micro-arrays, biosensors and immuno-assays. These techniques will provide the platform on which bio-prospecting test kits will be developed for the analyses of soil samples in the field. It is expected that within the next ten years field test kits will be developed that permit analyses of up to several hundred samples per day. Use of these methods to supplement traditional chemical methods will have a number of advantages for mineral exploration in that, apart from providing an additional layer of information, they are rapid, inexpensive, simple to perform, portable, highly sensitive and selective and thereby allow rapid assessment of potential mineralization in the field. These techniques will not replace traditional chemical analysis techniques but will provide an additional level of information and they represent an additional tool in the mineral explorationist's tool box. Molecular techniques will add value to existing techniques, most importantly 'green field' exploration where they can narrow down the final drilling target in relatively cheap and rapid ways.

The identification of bio-indicator organisms or genes present in elevated numbers in soil samples with anomalous trace metals concentrations may lead to the development of specific nucleic acid probes for these bio-indicators that can be used directly for bio-prospecting. Nucleic acid probes are complementary to signature sequences of functional or ribosomal DNA or RNA of particular species or groups of organisms and are fluorescently- or isotopically labelled allowing for their detection at very low concentration (Barns and Nierzwicki-Bauer, 1997). Nucleic acid probes can bind to bulk community DNA or RNA bound in filter membranes making it possible to quantify the amount and activity of particular bio-indicator organisms (Barns and Nierzwicki-Bauer, 1997).

Up to several hundred thousand nucleic acid probes can be used together as a molecular micro-array, as shown in Fig. 12-10 (Widada et al., 2002; Zhou and Thompson, 2002;, Zhou (2003); Bae and Park, in press). Molecular micro-arrays, which allow researchers to study complex microbial communities, comprise one of the new tools in molecular microbiology. Micro-arrays are powerful tools for detection of multiple genes from soil samples, giving a vast amount of information relating to the phylogenetic and/or functional structure of microbial ecosystems.

Expression micro-arrays are used to assess gene expression from microbial cultures or environmental samples. This type of micro-array provides information relating to the activity of the microbe(s), i.e., 'listening-in' to the message provided by the microbes. Furthermore, key genes associated with environmental attributes can be identified. For example, genes can be identified that are important when a species is subjected to elevated concentrations of heavy metals. Identification of genes that facilitate heavy metal transformations may then lead to the development of micro-arrays with specific probes for these genes. These can then be applied as exploration tools after RNA extraction from soil samples.

Heavy metal specific bacterial sensors provide another promising bio-prospecting tool, allowing the measurement of mobile heavy metals in soil samples to ppb levels (Tibazarwa et al., 2001; Rensing and Maier, 2003). This technique, which is highlighted in Case Study 3, was initially developed for ecotoxicological testing of heavy metal polluted sites in Europe and the US to distinguish between mobile and bioavailable, and non-mobile and non-bioavailable fractions of heavy metals in soils and sediments (Rensing and Maier, 2003). Metal specific bacterial sensors have been developed for a number of metals and metalloids such as As, Cu, Ni, Co, Zn, Pb, Hg and Cd, and could be tested as exploration tools in bio-prospecting (Rensing and Maier, 2003). Such biosensors are able to distinguish between mobile transported heavy metals and *in-situ* background concentrations much better than by wet chemical methods (Rensing and Maier, 2003). This is likely to be of specific interest to geochemical exploration.

Antibody-based or immuno-assays offer an alternative approach for metal ion detection in natural samples. An immuno-assay makes use of the binding between an antigen and its homologous antibody in order to quantify the specific antigen or

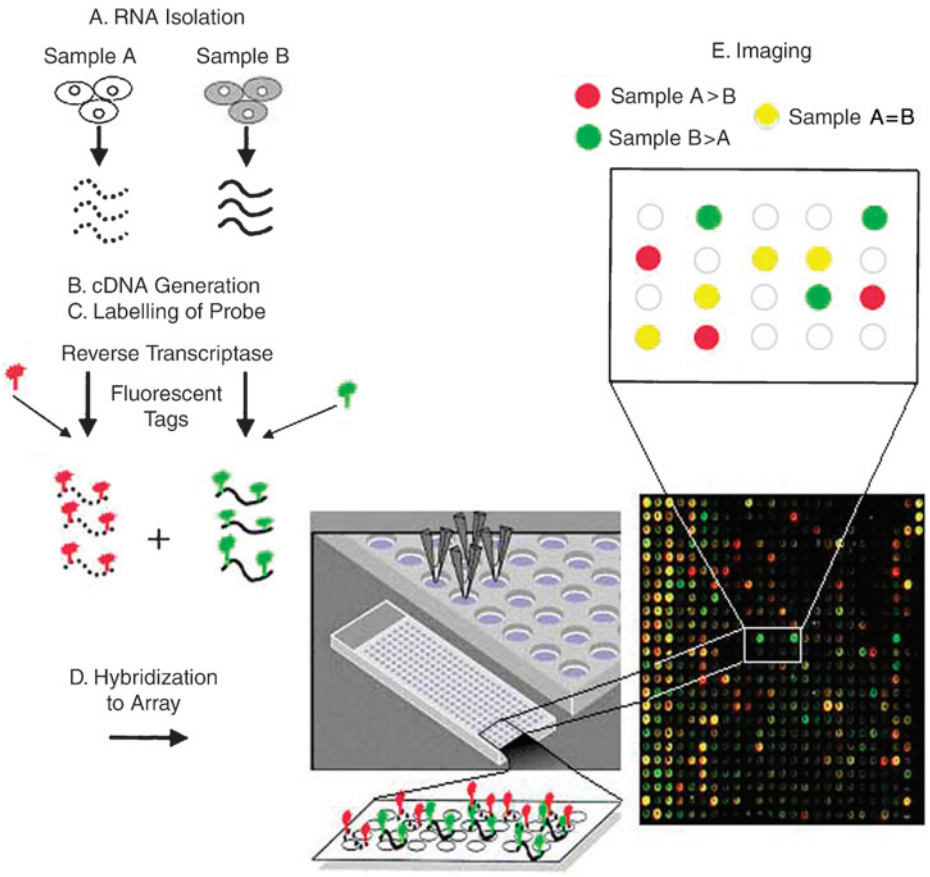

Fig. 12-10. The use of DNA microarrays in differential gene expression analysis (adapted from Albelda and Sheppard, 2000). Comparative hybridization experiment involves isolation of messenger RNA (mRNA) from two separate samples, e.g. bacteria grown in media without and amended with different concentrations of heavy metals. The mRNA from each sample is treated with reverse transcriptase and labelled with a distinct fluorescent tag. The two pools of labelled RNA are mixed, hybridized to the DNA microarray containing a full set of thousands or tens of thousands of DNA sequences based on genome or complementary DNA (cDNA) sequences, and washed. The microarray is scanned using a specialized imager, and the colour of each spot is determined. In this example, genes expressed dominantly in Sample A would be red in colour, genes expressed dominantly in Sample B would be green and those genes expressed equally in both samples would be yellow.

antibody in a sample (Blake et al., 1998; Johnson, 2003). A number of antibodies for heavy metal complexes have been developed. Methods to test for Hg, Cd, Co, Pb and U have been successfully applied in ecotoxicological studies and may be transferable to exploration purposes (Blake et al., 1998; Johnson, 2003).

CONCLUSIONS

This chapter has highlighted the use of emerging molecular biology techniques in the field of exploration geomicrobiology. Exploration geomicrobiology deals with microbially mediated processes that control the dispersion and re-concentration of economically important major and trace elements (Fig. 12-1). A number of novel molecular tools and techniques allow study of complex microbial consortia in soil materials and biogeochemical mineral transformations driven by these microbiota, such as trace metal dissolution or precipitation. The case studies show how these classical and molecular methods have been used to establish the role of microbiota in the cycling of Au in the Australian regolith; how the presence of Zn in soils influences the composition of the natural bacterial communities; and how biosensors may provide a quick and reliable tool for mineral exploration.

By identifying biogeochemical processes that lead to the dispersion and accumulation of trace elements in soil, and quantifying the reaction kinetics of these processes in different materials, this new field of research will help mineral explorers to develop predictive biogeochemical modelling tools (Fig. 12-1). Ultimately, it will be possible to incorporate appropriate data into numerical geochemical models to predict dispersion, transport and concentration of trace elements in and around mineralized zones. Identification of the microbiota and microbial processes that control the solubilization, transport and precipitation of trace metals in the soil should lead to the discovery of indicator micro-organisms and the development of microbial biosensors for detecting concealed mineralization. However, for this to become reality molecular microbial biosensor methods must first demonstrate their full potential as well as their cost competitiveness and benefit over existing methods.

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Chapter 13

A LOOK TO THE FUTURE

INTRODUCTION

In the mid-20th century, Professor Harry Warren and his associates did much to raise the credibility of biogeochemistry in mineral exploration from a level of, as he eloquently put it, 'general disbelief, through benevolent skepticism to general acceptance … and that, when used properly, biogeochemistry can be used as a viable exploration tool' (Warren and Delavault, 1950; Warren et al., 1968). During the half century since that pronouncement, science has steadily progressed and a vast amount of new data has been acquired from thousands of locations around the world. However, the world of plants is sufficiently complex that the current level of development has been able to look only at the tip of the iceberg. There remains that immense area of unknown and under-explored processes that present stimulating challenges to the next generation of researchers in this field. This chapter examines where the next generation of advances might be heading.

HYPERSPECTRAL IMAGERY IN RELATION TO BIOGEOCHEMISTRY

In order to understand the signals and images obtained from hyperspectral imagery of the forests some 'ground-truthing' of the chemistry of treetops is required. This aspect of trace element biogeochemistry is examined as a future use of plant chemistry in recognizing signatures that might be related to concealed mineral deposits.

Hyperspectral Imagery (HSI) is a term used to describe the imagery derived from subdividing the electromagnetic spectrum into very narrow bandwidths. Much of this imagery is obtained from sensors aboard satellites or aircraft. The narrow bandwidths, typically in the order of tens of nanometers, may be combined with or subtracted from each other in various ways to form images useful for detailed terrain analysis.

The sensors used for HSI obtain vast quantities of data. Some signals can very clearly be related to obvious terrain features – e.g., forests, bogs, clearings or water bodies. Other signals are more subtle and from libraries of accumulated signals they can be tuned to recognize such geological phenomena as alteration zones around mineral deposits. In forested areas the ground surface signal from alteration zones may be completely obscured by the forest canopy. It follows, therefore, that if the subtle signature from the chemistry of the forest canopy can be recognized from HSI,

there becomes the possibility of geochemically mapping the Earth using airborne sensors. However, in order to do this, various data 'training sets' must be established by providing ground-truthing of the chemistry of the forest canopy. This involves sample collection (i.e., treetops, or perhaps tissues from the ground that can be related to the canopy signature) and chemical analysis in order to geochemically map an area and relate the data to spectral images obtained from remote sensing instrumentation.

The Greater Victoria Watershed District (GVWD), on Vancouver Island, is one of several designated test sites across North America that have been established to conduct reflectance studies using NASA's AVIRIS (Airborne Visible/Infrared Imaging Spectrometer). Preliminary biogeochemical work from this area (Dunn, unpublished) examined the relationship between HSI and the chemistry of Douglas-fir bark from 45 trees within an area of 100 km^2 in the GVWD. The rationale for this orientation study was that prior studies, elsewhere, indicated a moderately good relationship between the chemistry of the bark and that of foliage from the canopy, although absolute concentrations of elements are substantially different. Locally, the bark was found to contain unusually high concentrations of As and Pb, with strong background-to-anomaly contrast. Investigation of the data involved focusing on the spectral wavelengths for As and Pb using ENVI (a remote sensing exploitation software platform). It was concluded that although there appeared to be a diffuse positive relationship, results were inconclusive because of the lack of resolution at 10 nm (nanometres).

Goodenough et al. (2003) and McDonald et al. (2003) reported results of a study to compare the chemistry of foliage from the tops of Douglas-fir, collected from a helicopter at approximately the same time that a flight took place to collect AVIRIS data. The AVIRIS system was mounted onboard NASA's high altitude ER-2 aircraft, and both HSI data and canopy samples were acquired over the GVWD in the summer of 2001. With 20 m spatial resolution, the HSI sensor has the capability of capturing 224 contiguous spectral channels at approximately 10 nm intervals in the visible to near-infrared portion (410–2450 nm) of the electromagnetic spectrum (Green et al., 1998). A total of six AVIRIS scenes were acquired to assess the canopy chemistry in the GVWD. A digital 1 m orthophotograph, based on 1:15,000 scale aerial orthophotographs, was used to assess the spectral components of each hyperspectral pixel (McDonald et al., 2003).

Preliminary results of the data analysis demonstrated that the hyperspectral reflectance data were capable of mapping nitrogen, total chlorophyll and moisture contents of the foliage (McDonald et al., 2003). However, with the limit of the spectral resolution at 10 nm, to date this has proved insufficiently precise to reliably map the elemental content of the foliage. It appears that a spectral resolution of <1 nm (and preferably 0.1 nm) would be required to focus on spectral wavelengths of individual elements, using a wavelength for each element that gives optimum contrast to the background spectrum.

At this time, it appears that hyperspectral data have insufficient resolution for individual element geochemical mapping of forested regions. Only major constituents

(e.g., N, moisture and chlorophyll) can be mapped. There is, however, potential for future development of remote geochemical mapping of the forests, once sub-nanometre resolution is readily available. The integration of the hyperspectral data will require substantial ground-truthing by systematically collecting suitable plant tissues for analysis, and ultimately entering the signatures into reference databases of the type currently available for the reflectance characteristics of minerals.

EXPLORATION GEOMICROBIOLOGY

Chapter 12 takes a step back from the conventional concept of biogeochemistry in mineral exploration (i.e., largely using vascular land plants) to examine the structurally most primitive but the genetically most diverse forms of life: the microorganisms (i.e., bacteria, archaea and fungi). Microorganisms play a vital role in the cycling of elements in the environment, and many important steps in the nutrient cycle are catalyzed exclusively by the microbes. They also play a key role in interfacing the inorganic world, comprising the geological substrate, and the organic world of higher order plants, by affecting the mobility, speciation and distribution of major and trace elements in the rhizosphere. The first table of in this book (Table 1-I) shows the extraordinary concentrations of metals that bacteria and fungi can accumulate. There are estimates that a single gram of soil typically contains 10 billion or more bacterial cells and that the bacterial population of the world is about 5×10^{30}. Consequently, this astronomical population plays a fundamental role in biogeochemical processes and an improved understanding of this part of the biogeochemical cycle can be used to advantage in refining biogeochemical exploration methods in general. Most bacteria are 0.5–5 μm in length, which is the common range in the size of heavy metal phases within plant structures (Chapter 2), and raises the question as to whether this is simply a coincidence, or is it a physicochemical constraint, or is there a direct link to the interaction between bacteria and the metal phases found within plant structures?

In order to obtain an improved understanding of biogeochemical processes, the role of microorganisms needs to be considered. The contribution of Chapter 12 by Frank Reith and Steve Rogers from Australia provides new ideas on how biogeochemical processes, that were not until recently given consideration by the exploration community, help to understand the cycling of pathfinder elements in the environment, and how the use of microbial signatures (biomarkers) for mineral exploration is worthy of future consideration.

FORENSIC BIOGEOCHEMISTRY

Although of indirect relevance to mineral exploration, the role of biogeochemistry in investigating past mining practices has come to the fore in recent years. There have been situations where mining companies, accused of improper practices by watchdog

agencies, have required the expertise of the biogeochemist to resolve issues concerning possible environmental contamination. Although for legal reasons it is inappropriate to specify details, through examination of foliar and twig tissues it has been possible to exonerate companies from certain accusations concerning environmental contamination. From chemical analysis of tree tissues it has proved possible to ascertain whether tree mortality was consistent with pollution or with death by drowning of roots that can occur following periods of natural water ponding. As more substantial biogeochemical databases are accumulated, the role of biogeochemistry in forensic investigations is likely to see an increase.

PLANT MINERALOGY

There remains much to be learnt about the mineral phases within plants. Proton microprobe analysis has been applied successfully to map the distribution of elements within plant tissues. Using this technique Morrison et al. (1981) were able to show the locations of Co, Mn and K in leaf tissue of the Co-hyperaccumulating species *Haumaniastrum robertii* from West Africa, and recent studies with state-of-the-art analytical instrumentation have given greater insight into the distribution and speciation of some elements in plants. Electron microscopy permits the examination of crystalline phases, and there is scope for modern instruments to add substantially to the quite limited knowledge of the $<2\,\mu$m-sized discrete phases within which base and heavy metals mostly seem to be concentrated. This knowledge will be largely of academic interest, but it has practical applications by assisting in the understanding of metals in plants and may help to define more precisely what and when samples should be collected and analysed to optimize results from a biogeochemical survey for minerals.

CHEMICAL ANALYSIS

Over the past 30 years analytical methods have permitted ever-deeper insight into the composition of plant materials. Of particular importance in generating a vast amount of multi-element data have been first INAA, and more recently commercialization of ICP-MS. Thirty years ago a 'multi-element package' typically comprised analytical data for about a dozen elements. The ICP-ES broadened this suite, but most trace elements returned values in dry vegetation that were below detection limits. A great advance came in the form of INAA, which permitted simultaneous determination of more than 30 elements at trace levels with excellent accuracy and precision. In the last 10 years, ICP-MS has broadened this range to more than 60 elements, with even the 'basic' packages generating the same high levels of precision for 35 or more elements, including determinations for some elements (e.g., Au) to sub-ppb levels. Costs are typically only a few tens of dollars, and for a few extra

dollars 'enhanced' packages provide data for another 20–30 elements with the similar levels of sensitivity.

High-resolution ICP-MS is now available for determining even lower levels of more than 60 elements. For some of the REE determinations, routine detection levels are as low as 0.01 ppb using 1 g of dry vegetation dissolved in acids. For the exploration biogeochemist, ICP-MS and HR-ICP-MS are the analytical instruments that currently generate data for almost all practical requirements. However, sample dissolution requires additional research to establish the optimum methods for improving data quality at ultratrace levels. Closed-vessel microwave digestion promises to be a useful technique that is not yet widely available from commercial laboratories that are generating biogeochemical data for the exploration and environmental industries. Development of these techniques should improve the precision on determinations for some elements – notably the precious metals.

Partial leaches, such as those described for the halogen elements on the accompanying CD, warrant further investigation to determine if they will generate biogeochemical data that better reflect the labile phases of elements released from concealed mineralization.

From a research perspective the synchrotron promises to provide important new insight into the location, migration and speciation of trace elements. Armed with information of this sort, techniques of when and what to collect can be refined. For example, the data on Se speciation in *Astragalus* (Chapter 6) clearly demonstrate that organoselenium is concentrated in young leaf tissue whereas it is preserved as a selenate in mature leaves, and there is very little Se in stems. This information can assist the field geologist in collecting the most suitable sample medium, and the chemist in optimizing an appropriate extraction procedure.

Isotopes in plant tissues are being examined at several institutes, but their role in biogeochemical exploration for minerals is poorly established and remains at the research stage.

CONCLUDING REMARKS

Collaboration between biogeochemists and geophysicists can assist in establishing whether certain geophysical signatures are from barren sulphides or from sulphides rich in valuable base and/or precious metals. Greater liaison with mathematicians and statisticians should help the biogeochemist to extract subtle structure in datasets that may prove to be of value in relating patterns to concealed mineralization. Judicious use of neural-net software might assist in revealing subtle trends in mobile pathfinder elements and their relationships to poorly-defined signatures of the valuable, yet less mobile metals.

In his latter years Alexander Kovalevsky was working on methods to predict from biogeochemical patterns (especially of Mo and Ag), the depth to mineralization and an estimation of ore grade, and he claimed success in doing so. These bold

predictions, estimated from sampling different tissues in a 'non-barrier' plant, he defined as 'biogeochemical logging'. This quantification of depth to mineralization is an exciting concept, but it does not appear to have been tested outside of Siberia. Clearly, this warrants further investigation.

Biogeochemical methods comprise another tool that explorationists have at their disposal. Data should be interpreted in conjunction with all other available geological, geochemical and geophysical information, because the technique is not a panacea and in some environments there may be little or no biogeochemical response to mineralization and so it may not be the best tool to use. However, the 'case history' examples demonstrate that a number of mines have been developed long after the recognition of a biogeochemical signature, attesting to the value of the method.

Increasingly, it appears that biogeochemical data exhibit subtle trends that can assist in elucidating faults, structural trends, stratigraphic relationships and lithologies, all of which may combine to indicate a geological setting suitable for the emplacement of mineral deposits. Such trends, coupled with subtle enrichments of elements typically associated with specific styles of mineralization, can provide focus for further exploration activities. Pattern recognition of element distribution patterns and their spatial relationships is a significant factor in the successful application of biogeochemistry to mineral exploration. There is now sufficient knowledge of the application and usefulness of biogeochemical methods for the thoughtful explorationist to consider using biogeochemistry as an integral part of a comprehensive mineral exploration program. Frequently, vegetation chemistry can provide information on the substrate that cannot be obtained by other means.

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BOTANICAL INDEX*

*Species in bold font are those from the Eden Project (ch. 9)

SUBJECT INDEX

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Except for Table 5-I-D, Tables are cross referenced to Tables bearing the same numbers in the text.

1. SURVEY DATA

Table 5-I-D. Balsam Fir (*Abies balsamea*). Data listings (INAA and ICP-ES on ash) for 499 twig samples (5 years of growth). Samples collected during a regional survey of southeastern Cape Breton Island in the mid-1990s. A full report on this study was published by the Geological Survey of Canada as Open File 2758.

Reference: Dunn, C.E., S.W. Adcock and W.A. Spirito (1994b), Reconnaissance biogeochemical survey of southeastern Cape Breton Island, Nova Scotia: Part 2 – Balsam fir twigs (Parts of NTS 11F,G,J,K), Geological Survey of Canada, Open File 2758, approx. 150 pp.

2. CONTROL DATA

Table 6-1-D. Acacia and litter from Western Australia. Comparison of samples analyzed as dry tissue, and as ash. For each element the first column shows the analysis of dry tissue; the second column is analysis of ash, normalized to a dry weight basis; the third column shows the analysis of the ash. Samples prepared in Canada (C. Dunn); data courtesy of Dr. Ravi Anand and Dr. Matthias Cornelius, CSIRO, Perth.

Table 6-II-D. Control V6. Data for 19 samples (each 10 g) of dry tissue by INAA, obtained over several years.

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Table 6-IV-D. Control V6. Data for 240 samples (each 0.5 g) of ashed tissue analyzed by ICP-ES (aqua regia digestion), obtained between 1992 and 1998.

Table 6-V-D. Control V6. Data for 500 samples (each 1 g) of dry tissue analyzed by ICP-MS (nitric acid and aqua regia digestion), obtained since 2000.

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3. EDEN PROJECT DATA

Table 8-I-D. Analysis of soils by ICP-MS (aqua regia digestion)

Table 8-II-D. Multi-element analysis of dry foliage from the Warm Temperate Biome

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Table 8-IV-D1. Data sorted by elements - Ag to K

Table 8-IV-D2. Data sorted by elements - La to Zr

4. HALOGEN STUDY

1. Full report as pdf.
2. Appendix 1 - Test data - Tables (zipped)
3. Appendix 2 - Field studies - Tables (zipped)
4. Appendix 3 - Procedure for viewing embedded charts

5. ABSTRACTS

All Abstracts on Biogeochemistry and Geobotany Published in the Journal of Geochemical Exploration, since its First Issue in 1972 up to January 2007.